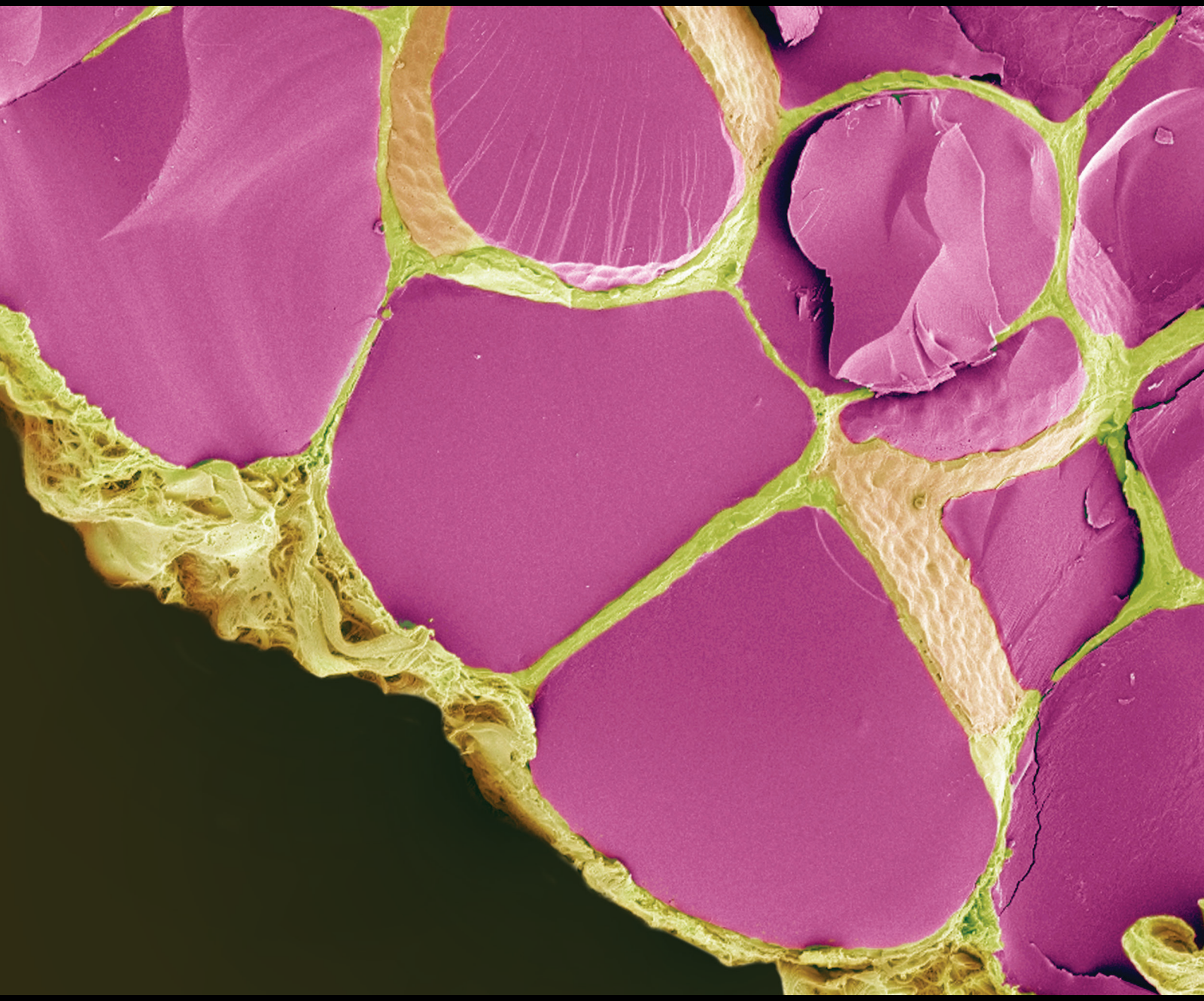
International Journal of Endocrinology

Diabetic Microvascular Complications

Lead Guest Editor: Ilias Migdalis
Guest Editors: Leszek Czupryniak, Nebojsa Lalic, David Leslie, Nikolaos Papanas, and Paul Valensi



Diabetic Microvascular Complications

International Journal of Endocrinology

Diabetic Microvascular Complications

Lead Guest Editor: Ilias Migdalis
Guest Editors: Leszek Czupryniak, Nebojsa Lalic, David Leslie, and Nikolaos Papanas

Editorial Board

Contents

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 5683287, 2 pages
https://doi.org/10.1155/2018/5683287

Editorial
Diabetic Microvascular Complications

I. Migdalis[1], **L. Czupryniak**[2], **N. Lalic**[3], **R. D. Leslie**[4], **N. Papanas**[5] and **P. Valensi**[6]

[1]*Second Medical Department and Diabetes Centre, NIMTS Hospital, 12 Monis Petraki, 11521 Athens, Greece*
[2]*Department of Diabetology and Internal Medicine, Warsaw Medical University, Warsaw, Poland*
[3]*Faculty of Medicine, University of Belgrade, Clinic for Endocrinology, Diabetes and Metabolic Diseases, CCS, Belgrade, Serbia*
[4]*Department of Diabetes, Saint Bartholomew's Hospital, University of London and Blizard Institute, EC1A 7BE London, UK*
[5]*Diabetic Foot Clinic-Diabetes Centre, Second Department of Internal Medicine, Democritus University of Thrace, 68100 Alexandroupolis, Greece*
[6]*Department of Endocrinology, Diabetology and Nutrition, Jean Verdier Hospital, AP-HP, Paris Nord University, CRNH-IdF, CINFO, 93140 Bondy, France*

Correspondence should be addressed to I. Migdalis; ilianmig@otenet.gr

Received 14 March 2018; Accepted 14 March 2018; Published 28 May 2018

Microvascular complications account for a substantial increase in morbidity and a considerable impairment in the quality of life in patients with diabetes mellitus (DM) [1–3]. The present special issue provides new research data in the field of diabetic microvascular complications.

T. Tönnies et al. in their paper entitled "Risk of Microvascular Complications and Macrovascular Risk Factors in Early-Onset Type 1 Diabetes after at Least 10 Years Duration: An Analysis of Three Population-Based Cross-Sectional Surveys in Germany between 2009 and 2016" examined patients with early-onset and long duration type 1 diabetes. They reported an increased risk of microvascular complications at 14 years after the diagnosis of type 1 DM. Obviously, there is a lot to learn and improve in our understanding of the evolution and prevention of complications in such patients.

P. Ruiz-Ocaña et al. in their paper "Decreased Retinal Thickness in Type 1 Diabetic Children with Signs of Nonproliferative Diabetic Retinopathy" evaluated retinal thickness in paediatric patients with type 1 DM in relation to nonproliferative diabetic retinopathy, glycaemic control, and DM duration. They observed decreased thickness and volumes of retina in the presence of nonproliferative diabetic retinopathy.

Y. Takamura et al. in their article "Direct Photocoagulation Guided by Merged Retinal Images for the Treatment of Focal Diabetic Macular Edema" introduced the new merged image-guided laser photocoagulation for the treatment of focal diabetic macular edema. They documented improvements in visual acuity and retinal thickness, as well as in the number of laser shots required and in the need for new treatment. These results are very promising.

D. Zhang et al. in their paper "Elevated Serum Total Bilirubin Concentrations are Negatively Associated with Diabetic Retinopathy among the Chinese Northeastern Population" showed a significant negative association between serum total bilirubin and diabetic retinopathy in Chinese patients. They suggested that total bilirubin might prove useful in the early detection of this complication.

M. Šimunović et al. studied the early development of cataract in children and adolescents with type 1 DM. In their work entitled "Cataract as Early Ocular Complication in Children and Adolescents with Type 1 Diabetes Mellitus," they found a 0.7–3.4% prevalence of cataract in children and adolescents with T1DM. Cataract development frequently occurred in as the first sign of or during the first 6 months of diagnosis of type 1 DM.

In their paper entitled "Characterization of In Vivo Retinal Lesions of Diabetic Retinopathy Using Adaptive Optics Scanning Laser Ophthalmoscopy," S. G. Karst et al. found that adaptive optics scanning laser ophthalmoscopy can provide high-resolution visualisation of diabetic retinopathy. An additional advantage was the assessment of retinal perfusion and the identification of small vascular lesions.

M. A. Ahmed et al. in their paper "Perspectives on Peripheral Neuropathy as a Consequence of Metformin-Induced Vitamin B12 Deficiency in T2DM" have summarised all contemporary results of the available and rather conflicting evidence on the relationship between vitamin B12 deficiency, the potential contribution of metformin therapy, and the development of diabetic peripheral neuropathy. There is a lot to appreciate, but, as the authors rightly point out, there is also a need for more appropriate design of further trials.

In their work "Susceptible and Prognostic Genetic Factors Associated with Diabetic Peripheral Neuropathy: A Comprehensive Literature Review," L. B. L. Prabodha et al. have reviewed the role of the genetic component (susceptibility and prognostic factors, mutations, and polymorphisms) in the development of peripheral neuropathy in type 2 DM. Identification of common genetic variants along with additional gene expression studies may enable future gene-targeted therapies, and this is eagerly awaited.

T. Didangelos et al. in their paper "A Comparative Assessment of Cardiovascular Autonomic Reflex Testing and Cardiac ^{123}I-Metaiodobenzylguanidine Imaging in Patients with Type 1 Diabetes Mellitus without Complications or Cardiovascular Risk Factors" compared the diagnostic performance of cardiovascular autonomic reflex tests with that of sympathetic innervation heart imaging with ^{123}I metaiodobenzylguanidine for cardiac autonomic neuropathy in type 1 DM. The former yielded high sensitivity but low specificity, as compared with the latter, and it also depended on the duration of type 1 DM.

L. Yazdanpanah et al. in their paper "Incidence and Risk Factors of Diabetic Foot Ulcer: A Population-Based Diabetic Foot Cohort (ADFC Study)—Two-Year Follow-Up Study" found a 2.8% average annual incidence of diabetic foot ulcers in Iran. Risk factors of ulcers were prior ulcer/amputation, insulin therapy, male gender, neuropathy, and foot deformity.

A. Gatt et al. in their work entitled "Establishing Differences in Thermographic Patterns between the Various Complications in Diabetic Foot Disease" evaluated the utility of thermography in the identification of foot temperature patterns in patients with DM. Foot temperature was significantly higher in the presence of diabetic complications. Intriguingly, higher temperature was associated with increased likelihood of neuropathy and/or peripheral arterial disease. These results add to the accumulating data on the importance of temperature measurements in the diabetic foot [4].

In their article "Oxidative Stress, Apoptosis, and Mitochondrial Function in Diabetic Nephropathy," S. Sifuentes-Franco et al. reviewed the current views on the pathogenesis of diabetic nephropathy. Chronic hyperglycaemia, oxidative stress, apoptosis, and mitochondrial dysfunction are key underlying factors. Improved insight into the precise role and the interplay of these factors is expected to offer more efficacious treatment options.

J. Labad et al. in their article "Limited Joint Mobility Progression in Type 1 Diabetes: A 15-Year Follow-Up Study" demonstrated significant reductions in the flexions of the 5th metacarpal and wrist joints at 15 years after the initial examination in patients with type 1 DM. This deterioration was more pronounced among patients with microalbuminuria.

Finally, M. J. Crespo et al. in their paper "Synergistic Effects of Dantrolene and Nimodipine on the Phenylephrine-Induced Contraction and ACh-Induced Relaxation in Aortic Rings from Diabetic Rats" examined streptozotocin-induced diabetic rats. They showed beneficial effects of dantrolene and nimodipine combination in decreasing arterial tone of their aortic rings.

There are abundant and useful new data from the research of diabetic microvascular complications. This ongoing research will enrich our understanding in the area and eventually contribute to improved therapeutic modalities in the near future [5].

I. Migdalis
L. Czupryniak
N. Lalic
R. D. Leslie
N. Papanas
P. Valensi

References

[1] D. G. Armstrong, A. J. M. Boulton, and S. A. Bus, "Diabetic foot ulcers and their recurrence," *New England Journal of Medicine*, vol. 376, no. 24, pp. 2367–2375, 2017.

[2] I. Migdalis, D. Leslie, N. Papanas, P. Valensi, and H. Vlassara, "Diabetes mellitus," *International Journal of Endocrinology*, vol. 2014, Article ID 108419, 6 pages, 2014.

[3] American Diabetes Association, "10. Microvascular complications and foot care: standards of medical care in diabetes—2018," *Diabetes Care*, vol. 41, Supplement 1, pp. S105–S118, 2017.

[4] R. G. Sibbald, A. Mufti, and D. G. Armstrong, "Infrared skin thermometry: an underutilized cost-effective tool for routine wound care practice and patient high-risk diabetic foot self-monitoring," *Advances in Skin & Wound Care*, vol. 28, no. 1, pp. 37–44, 2015.

[5] C. R. L. Cardoso, N. C. Leite, C. B. M. Moram, and G. F. Salles, "Long-term visit-to-visit glycemic variability as predictor of micro- and macrovascular complications in patients with type 2 diabetes: The Rio de Janeiro Type 2 Diabetes Cohort Study," *Cardiovascular Diabetology*, vol. 17, no. 1, p. 33, 2018.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 7492946, 12 pages
https://doi.org/10.1155/2018/7492946

Research Article

Characterization of *In Vivo* Retinal Lesions of Diabetic Retinopathy Using Adaptive Optics Scanning Laser Ophthalmoscopy

Sonja G. Karst,[1,2] **Jan Lammer**,[1,2] **Salma H. Radwan**,[1,3] **Hanna Kwak**,[1] **Paolo S. Silva**,[1,4] **Stephen A. Burns**,[5] **Lloyd Paul Aiello**,[1,4] **and Jennifer K. Sun**[1,4]

[1]*Beetham Eye Institute, Joslin Diabetes Center, Boston, MA, USA*
[2]*Department of Ophthalmology and Optometry, Medical University of Vienna, Vienna, Austria*
[3]*Department of Ophthalmology, Cairo University, Cairo, Egypt*
[4]*Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
[5]*School of Optometry, Indiana University, Bloomington, IN, USA*

Correspondence should be addressed to Jennifer K. Sun; jennifer.sun@joslin.harvard.edu

Received 30 August 2017; Accepted 16 January 2018; Published 2 May 2018

Academic Editor: Nikolaos Papanas

Purpose. To characterize hallmark diabetic retinopathy (DR) lesions utilizing adaptive optics scanning laser ophthalmoscopy (AOSLO) and to compare AOSLO findings with those on standard imaging techniques. *Methods.* Cross-sectional study including 35 eyes of 34 study participants. AOSLO confocal and multiply scattered light (MSL) imaging were performed in eyes with DR. Color fundus photographs (CF), infrared images of the macula (Spectralis, Heidelberg), and Spectralis spectral domain optical coherence tomography SDOCT B-scans of each lesion were obtained and registered to corresponding AOSLO images. *Main Outcome Measures.* Individual lesion characterization by AOSLO imaging. AOSLO appearance was compared with CF and SDOCT imaging. *Results.* Characterized lesions encompassed 52 microaneurysms (MA), 20 intraretinal microvascular abnormalities (IRMA), 7 neovascularization (NV), 11 hard exudates (HE), 5 dot/blot hemorrhages (HEM), 4 cotton wool spots (CWS), and 14 intraretinal cysts. AOSLO allowed assessment of perfusion in vascular lesions and enabled the identification of vascular lesions that could not be visualized on CF or SDOCT. *Conclusions.* AOSLO imaging provides detailed, noninvasive *in vivo* visualization of DR lesions enhancing the assessment of morphological characteristics. These unique AOSLO attributes may enable new insights into the pathological changes of DR in response to disease onset, development, regression, and response to therapy.

1. Introduction

Diabetic retinopathy (DR) is characterized by hallmark retinal lesions including microaneurysms (MA), hard exudates (HE), cotton wool spots (CWS), intraretinal hemorrhages, and retinal neovascularization (NV) which are present in over 77–90% of individuals after 15 or more years of diabetes [1–3]. The distribution and extent of these lesions determine DR severity and predict the risk of worsening DR [4–8]. Thus, crucial clinical management decisions including recommendations for follow-up and treatment are dependent on the ability to accurately assess DR lesions over time.

Color fundus photography is the standard method by which DR severity is assessed for clinical and research purposes [1]. Alternative imaging methods such as spectral domain optical coherence tomography (SDOCT), scanning laser ophthalmoscopy (SLO), and fluorescein angiography (FA) allow evaluation of specific aspects of retinal pathology such as neural retinal layer thickening, disorganization or disruption, and vascular leakage, respectively, and have also

been utilized to assess individual DR lesions in detail [9–14]. However, all these imaging modalities are limited by a lateral resolution of approximately 10–15 μm and are thus unable to resolve structural details at the cellular level. Although there are numerous histological studies of DR lesions at the cellular level in human postmortem tissues, similarly detailed *in vivo* evaluation has been limited [15–17].

The adaptive optics (AO) systems allow ultrahigh resolution assessment of the human retina *in vivo* [18–23]. AO technology compensates for ocular wave front errors primarily induced by the cornea and lens and allows correction of >90% of the optical aberrations within an individual eye, thus providing a theoretical lateral resolution limit of 1.4 μm for large pupils and short wavelength light [24]. AOSLO can also capture video output, allowing dynamic visualization of intravascular blood cell flow in vessels down to the capillary level [25–28].

In this study, we characterized vascular and nonvascular hallmark DR lesions using a custom-built AOSLO with a lateral resolution of approximately 2.5 μm. Whereas previous reports have focused on the overall capillary network, MA, and intraretinal microvascular abnormalities in the diabetic eye, this manuscript also evaluates confocal and multiply scattered light imaging findings for the following additional diabetic lesions that have not been systematically assessed using AOSLO: retinal neovascularization, hard exudates, hemorrhages, cotton wool spots, and intraretinal cysts [28–32]. We document the longitudinal history of selected individual lesions as well as the response of particular lesions to antivascular endothelial growth factor (VEGF) therapy. In addition, we systematically describe the AOSLO characteristics in static and dynamic (video) assessments for each lesion type recorded with two AOSLO acquisition modes: confocal imaging and aperture offset imaging. Advantages and disadvantages of the AOSLO technique in relation to traditional color fundus photography and SDOCT are also presented.

2. Methods

The study was approved by the Institutional Review Board of the Joslin Diabetes Center, and all study procedures adhered to the tenets of the Declaration of Helsinki. Prior to study inclusion, informed consent was obtained from all subjects.

Subjects were eligible for the study if they met the following inclusion criteria: age 18 years or older, diagnosis of type 1 or type 2 diabetes mellitus as defined by the American Diabetes Association, optical media clear enough to obtain good quality images, and stable central fixation [33]. Participants with substantial macular pathology attributable to nondiabetic eye disease, such as age-related macular degeneration, retinal vein occlusion, uveitis, and Irvine Gass syndrome were excluded from participation.

All participants received a comprehensive dilated ophthalmologic examination followed by retinal imaging including SDOCT (Spectralis Heidelberg Engineering, Germany), ETDRS 7 standard field color stereoscopic fundus photography (Carl Zeiss Meditec Inc., Dublin, CA) or ultrawide field retinal imaging (Optos PLC, Scotland, United Kingdom), and AOSLO (Boston Micromachines Corp., Cambridge, MA). AOSLO imaging and SDOCT imaging were performed at each visit on the same day. For SDOCT imaging, cubic ($20^\circ \times 20^\circ$ field, 49 B-scans, 16 frames ART mean, and high resolution setting) and detailed ($15^\circ \times 5^\circ$ field, 24 B-scans, 25 frames ART mean, and high resolution setting) macular volume scan patterns centered on the fovea were performed. Details of the AOSLO imaging are provided below. The axial length of each study eye was determined using an IOL Master (Carl Zeiss Meditec, Dublin, CA) in order to subsequently convert angular to metric coordinates on the AOSLO images.

The AOSLO used in this study was a double pass, single deformable mirror version of the Indiana system that has been previously described [34, 35]. AOSLO images were acquired confocally, using a standardized protocol that obtained images focused at the following planes: the lesion of interest, posteriorly at the photoreceptor level and anteriorly at the nerve fiber layer. In addition, a multiply scattered light (MSL) or pinhole aperture offset technique as recently described was utilized to image each DR lesion (Figures 1(a) and 1(b)) [36, 37]. With this technique, images are generated from spatial variations of multiply scattered light leading to higher contrast, especially of vessel walls and erythrocytes, as the specular component of the image is reduced by the offset aperture [38]. Aperture size and displacement (ranging from 25 μm to 500 μm for size and 0 μm to 350 μm for displacement) were adjusted for individual lesions and eyes to obtain the best quality image possible and depth of focus ranged between 80 μm and 150 μm. However, a 500 μm aperture which was displaced by 300 μm (~5 Airy disk diameters) perpendicular to the targeted lesion was used to acquire most images. The displacement was adjusted using a computer-controlled, motorized positioning stage allowing a positioning accuracy of approximately 1 μm. AOSLO image acquisition sessions ranged between 15 and 60 minutes depending on the number of lesions and size of area scanned.

Image processing was performed using a customized Matlab platform (MatLab, The MathWorks, Natick, MA) and took approximately 30 min per image. Sinusoidal distortion artifacts were corrected utilizing a polynomial dewarping algorithm [35]. After manual selection of 5–50 frames from each video block, automated image alignment and averaging were performed. Individual diabetic retinal lesions were characterized by size, shape, and appearance from the averaged images, and perfusion status was assessed on AOSLO videos viewed in ImageJ (NIH, Bethesda, Maryland). Individual lesions were registered to widefield SDOCT IR images or color fundus photographs by manually or semi automatically montaging 3–20 adjacent AOSLO images and then identifying comparable vessel landmarks on each image set with guidance from recorded AOSLO navigation coordinates. Once registration was completed, corresponding SDOCT B-scans were assessed in order to determine key features of each lesion's appearance on SDOCT such as visibility, presence of associated hyperreflectivity, and location within or anterior to the neural retinal layers. Figure 2 contains examples of 6 lesions obtained using the different imaging modalities in this study: color and IR photographs, SDOCT B-scans, and AOSLO images.

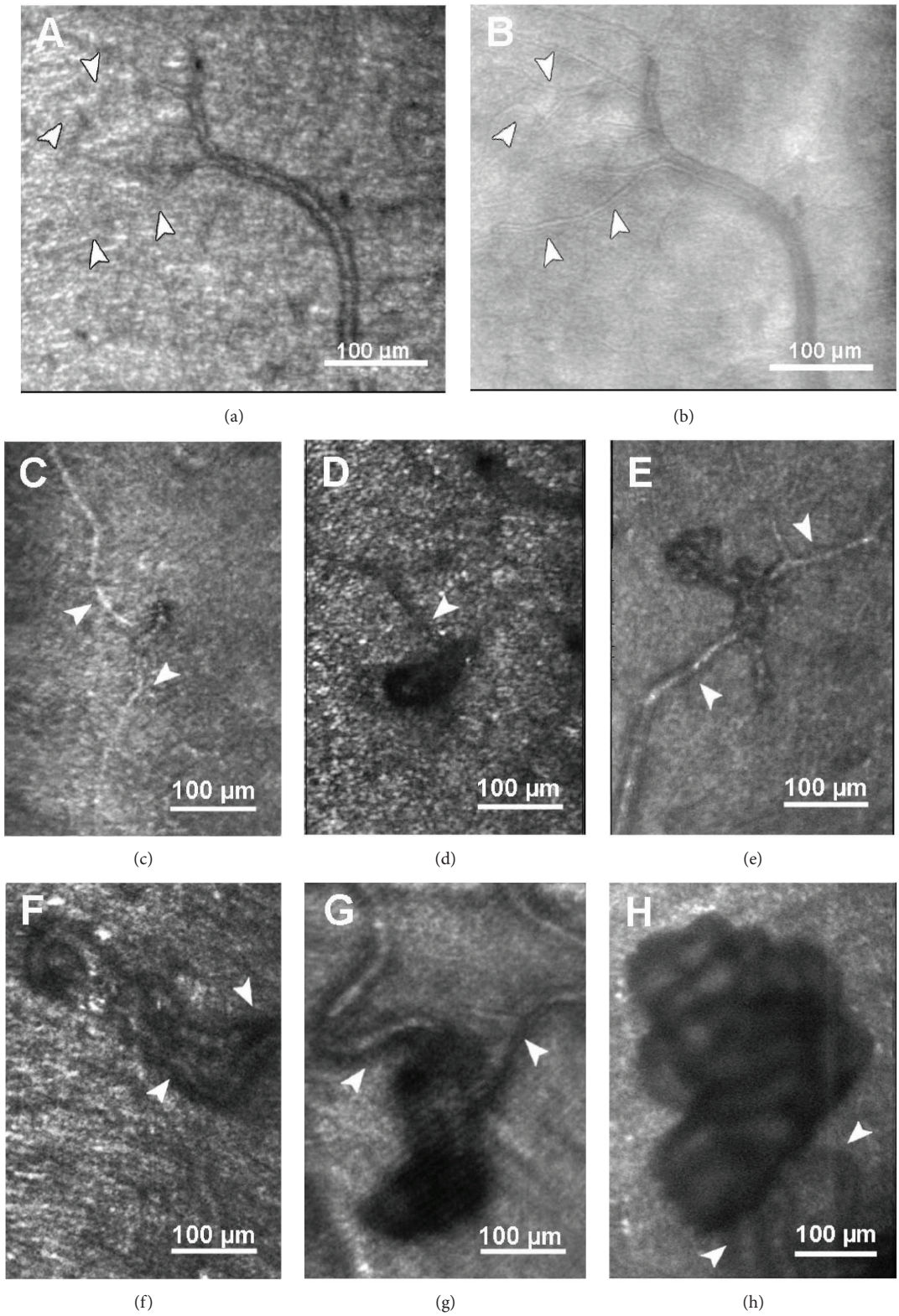


Figure 1: (a) Example of an arteriole in confocal and multiply scattered light (MSL) and (b) AOSLO imaging. Finer details of the vessel walls are visualized with MSL AOSLO imaging, which increases the sensitivity to scattered light reflected from vasculature and erythrocytes. (c–f) Intraretinal microvascular abnormalities (IRMA). (g, h) Neovascular proliferations (NVE) growing anteriorly to the RNFL. Arrowheads indicate feeder/draining vessels. In (d), the AOSLO image is focused at the photoreceptor level and displays a regular photoreceptor mosaic surrounding the blurred shadow of an IRMA. In (g) and (h), the 3-dimensional structure of the NVE results in clear focus on some vascular loops and blurry vessel formations in other areas of the NVE.

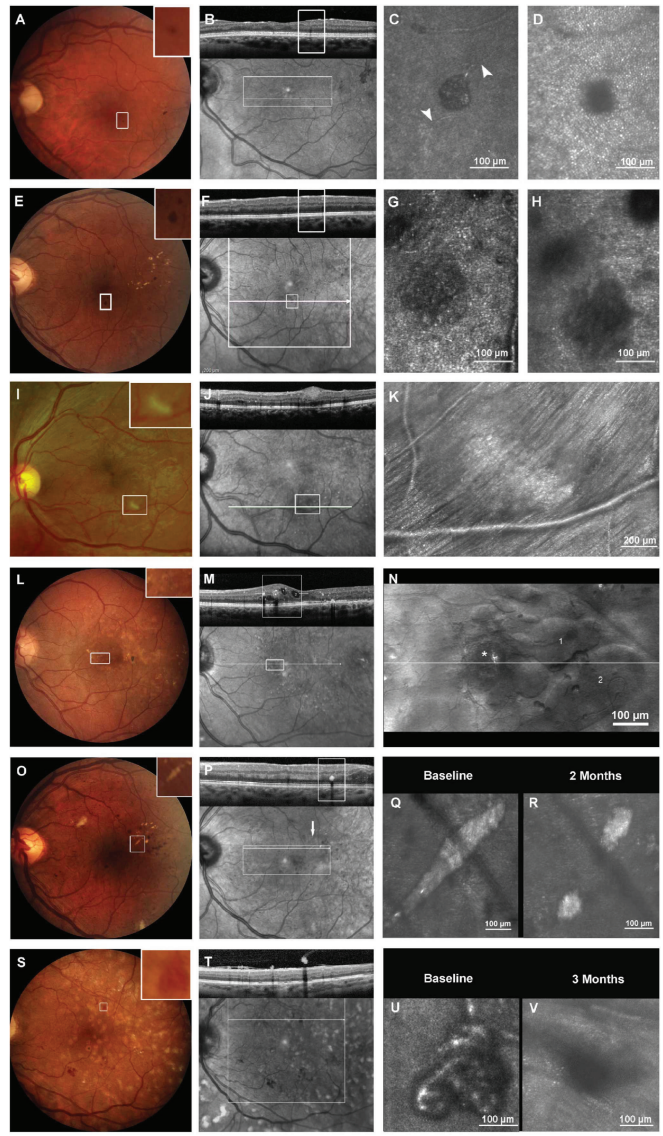


Figure 2: Multimodal imaging (color fundus photo, OCT, IR image, and AOSLO) of six hallmark lesions: a microaneurysm, MA (A–D), a blot hemorrhage (E–H), a cotton wool spot, CWS (I–K), intraretinal cysts (L–N), hard exudate, HE (O–R), and neovascular proliferation, NV (S–V). (A) Microaneurysm visible as small red dot in 30° color fundus photo (CF) centered on the macula. (B) In the corresponding B-scan the inner plexiform layer, MA is visible primarily by its shadowing effect on the outer retinal layers. (C) In the AOSLO image focused at the vascular level, the MA is visible as a well-defined saccular bulge within the capillary network. Feeding and draining vessels (arrowheads) are discernable. (D) When focused at the photoreceptor plane, shadowing from the MA is present surrounded by a clearly imaged photoreceptor mosaic. (E) Small blot hemorrhage easily visible in 30° color fundus photo and Spectralis IR image (F) but not clearly identifiable in the corresponding cross-sectional SDOCT B-scan. G Outlines of the hemorrhage are blurred even in the AOSLO image focused at the hemorrhage plane. (H) When focused on the photoreceptor plane, there is shadowing of the photoreceptor mosaic by the anteriorly located blot hemorrhage. (I) CWS clearly visible in 30° Optos color fundus image and Spectralis IR image (J). In the corresponding SDOCT B-scan, the CWS appears as a nodular thickening of the retinal nerve fiber layer (RNFL) compressing the inner plexiform and inner nuclear layer. Notice the faint shadowing effect on the outer retinal layers. (K) Montage of 17 AOSLO images focused at the RNFL level to cover an area of $3^\circ \times 4.5^\circ$. The CWS appears hyperreflective with a less clearly delineated nerve fiber striation pattern compared to adjacent areas. Nerve fiber bundles adjacent to the CWS are pushed aside at the lower right border of the lesion. (L) DME is not evident on the 30° color fundus and Heidelberg Spectralis IR (M) photographs, but is clearly visible on the montaged MSL AOSLO images (N) and corresponding SDOCT B-scan. The white boxes in (L) and (M) correspond to the area imaged on AOSLO (N). An asterisk marks a microaneurysm and [1, 2] indicates corresponding cysts in the AOSLO (N) and SDOCT (M) images. (O) The HE is clearly visible on color fundus photography (O) OCT and IR image (P). In confocal AOSLO images (Q), the HE appears as a hyperreflective granular structure with an internal honeycomb-like pattern. Two months after intravitreal ranibizumab injections for center involved diabetic macular edema, the HE decreased in size to reveal an intact photoreceptor pattern. (S) Fundus photograph of neovascularization elsewhere (NVE) that is clearly visible on OCT and IR image (T). (U) Corresponding NVE imaged with AOSLO before treatment (U) and 3 months after administration of intravitreal ranibizumab (V). AOSLO imaging reveals the persistence of an involuted neovascular formation.

3. Results

DR lesions were imaged in 35 eyes of 34 participants (16 females, mean age 41 ± 12.5 years). The mean duration of DM was 24 ± 8 years (28 type 1 DM), and mean HbA1c was 8 ± 2%. DR severity grading of the 35 eyes that were included was as follows: 2 mild, 9 moderate, 8 severe nonproliferative DR, and 16 proliferative DR.

The lesions that were evaluated included microaneurysms (MA, $N = 52$), intraretinal microvascular abnormalities (IRMA, $N = 20$), retinal neovascularization (NV, $N = 7$), hemorrhages (HEM $N = 5$), hard exudates (HE, $N = 11$), cotton wool spots (CWS, $N = 4$), and intraretinal cysts ($N = 14$) (Table 1). In the following sections, the main AOSLO characteristics are systematically described. A more detailed description observed for each lesion type using static confocal imaging, MSL imaging, and dynamic (video) assessment as well as a thorough comparison to other imaging modalities is available at the journal's website.

3.1. Vascular Lesions. Small vascular lesions like MA, IRMA, and NV that were sometimes hard to detect or distinguish in IR images or fundus photos could be clearly identified in AOSLO images. In confocal images, vessel walls of these vascular lesions were markedly thickened and appeared darker compared to vessel walls of normal intraretinal capillaries. In some MA, focal areas of granular hyperreflectivity were present along their wall in 35% ($n = 18$) or within their lumen (46% $n = 24$) (Figures 3(c)–3(f)). Wall hyperreflectivity present on AOSLO imaging was not always present in SDOCT images (31%) and vice versa (59%). SDOCT intraluminal hyperreflectivity was observed in 17 MA (57%). Only 9 of these 17 MA (53%) showed intraluminal hyperreflectivity in corresponding AOSLO images.

MSL imaging technique revealed more sharply defined vessel walls than confocal imaging, so perfused and nonperfused vascular channels could be clearly identified (Figures 1(a) and 1(b)). This distinction was particularly evident in imaging areas of fibrosis within patches of NV (Figure 4). These structural findings were complemented by dynamic assessment, as blood cell flow was clearly visible in all perfused vascular lesions. Though blood flow could not be quantified, it appeared qualitatively slower (often markedly so) in some MA or regressing neovascular tissue, particularly in the eyes that had undergone treatment with antivascular endothelial growth factor (VEGF) therapy or panretinal photocoagulation (Videos 5 and 6).

3.2. Nonvascular Lesions. Hemorrhages presented with distinct border and homogenously hyporeflective internal appearance. Although HEM could not be differentiated from MA in color fundus photos or IR images, they could be easily distinguished from perfused MAs on AOSLO due to the hemorrhage's lack of blood flow, hyperreflective foci, and/or adjacent feeder vessels. Hemorrhages were not visible in SDOCT.

Hard exudates were visible in SDOCT, IR images, and color fundus photography. In confocal AOSLO images, HE appeared as irregularly shaped, grainy-appearing hyperreflective patches with dark borders (Figure 2, Q) casting a shadow on the photoreceptor mosaic. With high resolution AOSLO imaging, changes in HE size after anti-VEGF treatment could be measured more accurately than on standard color fundus photography or on SDOCT B-scans (Figure 5). In addition, AOSLO imaging showed that the underlying photoreceptor mosaic remained intact and was gradually revealed as the HE were resolved (Figure 2, R).

Cotton wool spots were visible in all imaging modalities applied whereas confocal AOSLO images revealed more details than MSL images. In confocal AOSLO, CWS appeared hyperreflective in comparison to the surrounding retinal tissue (Figure 2, K). Within each CWS, the RNFL striation pattern was less distinct and boundaries between the RNFL bundles could not always be clearly identified. Individual RNFL bundles within each CWS were wider in diameter than the nerve fiber bundles outside but immediately adjacent to the CWS, which appeared compressed and displaced at the border of the lesions.

Intraretinal cysts were difficult to visualize on fundus photographs or IR images but were clearly visualized on SDOCT images. Though intraretinal cysts could not be identified in confocal AOSLO images, the MSL imaging technique allowed clear delineation of cyst boundaries (Figure 2, N) and wall structures. Lateral cyst dimensions and proximity to different retinal structures could be well defined due to the ability to precisely discern cyst wall boundaries.

4. Discussion

This study provides the first detailed, systematic description of multiple vascular and nonvascular retinal lesions of diabetic retinopathy as imaged using noninvasive confocal and multiply scattered light AOSLO technology. In comparison to the previous studies of AOSLO which have evaluated either more global features of the diabetic capillary network or limited their focus to specific vascular lesions such as MAs, this investigation provides a broad survey that directly compares the appearance of a diverse set of diabetic pathologies on AOSLO imaging to that on standard fundus photography and SDOCT scans [30–32, 37].

Small lesions of clinical importance, including neovascularization and microaneurysms, were readily detectable on AOSLO even when they were not visualized using SDOCT or standard color fundus photographs. AOSLO also allowed longitudinal monitoring of structural changes at the cellular level over time, including retinal anatomic response following therapeutic intervention. Vascular perfusion was often detectable with AOSLO even when the lesion itself was undetectable by other imaging modalities or when a vascular lesion appeared entirely fibrotic and nonperfused on standard retinal photographs. Thus, AOSLO promises earlier detection and more precise determination of structural changes in the diabetic eye both over time and in response to treatment than that currently available with other standard imaging modalities. The high sensitivity of AOSLO to detect intraretinal lesions such as MAs and hemorrhages could be a reason for altered photoreceptor counts in diabetic patients

Table 1: Diabetic retinopathy lesion characteristics on AOSLO and SDOCT imaging modalities.

Lesion type	Size range (μm)	Appearance on AOSLO imaging		Increased reflectivity	Longitudinal follow-up	Shadowing of cones	Detectable in SDOCT (%)	Blood flow visible on AOSLO imaging
		Confocal	Multiply scattered light					
Microaneurysms ($n = 52$)	46–168	Round/oval lesions with dark, thickened vessel walls	Better defined vessel walls than in confocal imaging	46% intraluminal 35% vessel wall	No	Yes	58	Feeder vessels and intraluminal blood flow
IRMA ($n = 20$)	69–360	Distinct convoluted vessel formation	Better defined vessel walls than in confocal imaging	No	No	Yes	90	Feeder vessels and intraluminal blood flow
NV ($n = 7$)	283–1406	Distinct convoluted vessel formation	Sharply defined vessel walls, distinction to fibrotic tissue, perfused and nonperfused vessels	No	Yes, under treatment of 0.3 ranibizumab	Yes	100	Feeder vessels and intraluminal blood flow, nonperfused vascular channels
Hemorrhages ($n = 5$)	52–234	Dark homogenous patches	Dark homogenous patches, same information as in confocal imaging	No	No	Yes	No	n/a
CWS ($n = 4$)	432–954	RNFL striation pattern disrupted, hyperreflective, fluffy	Less RNFL reflectivity	Yes	No	No	100	n/a
HE ($n = 11$)	27–745	Highly reflective distinct granular patches	Highly reflective distinct granular patches, same information as in confocal imaging	Yes	Yes, under treatment of 0.3 ranibizumab	Yes	91	n/a
Cysts ($n = 14$)	72–1086	Blurred dark shadows	Clear delineation of cyst boundaries and wall structures	No	No	Inconsistent	100	n/a

AOSLO: adaptive optics scanning laser ophthalmoscopy; SDOCT: spectral domain optical coherence tomography; IRMA: intraretinal microvascular abnormalities; NV: neovascularization; CWS: cotton wool spot; RNFL: retinal nerve fiber layer; HE: hard exudates.

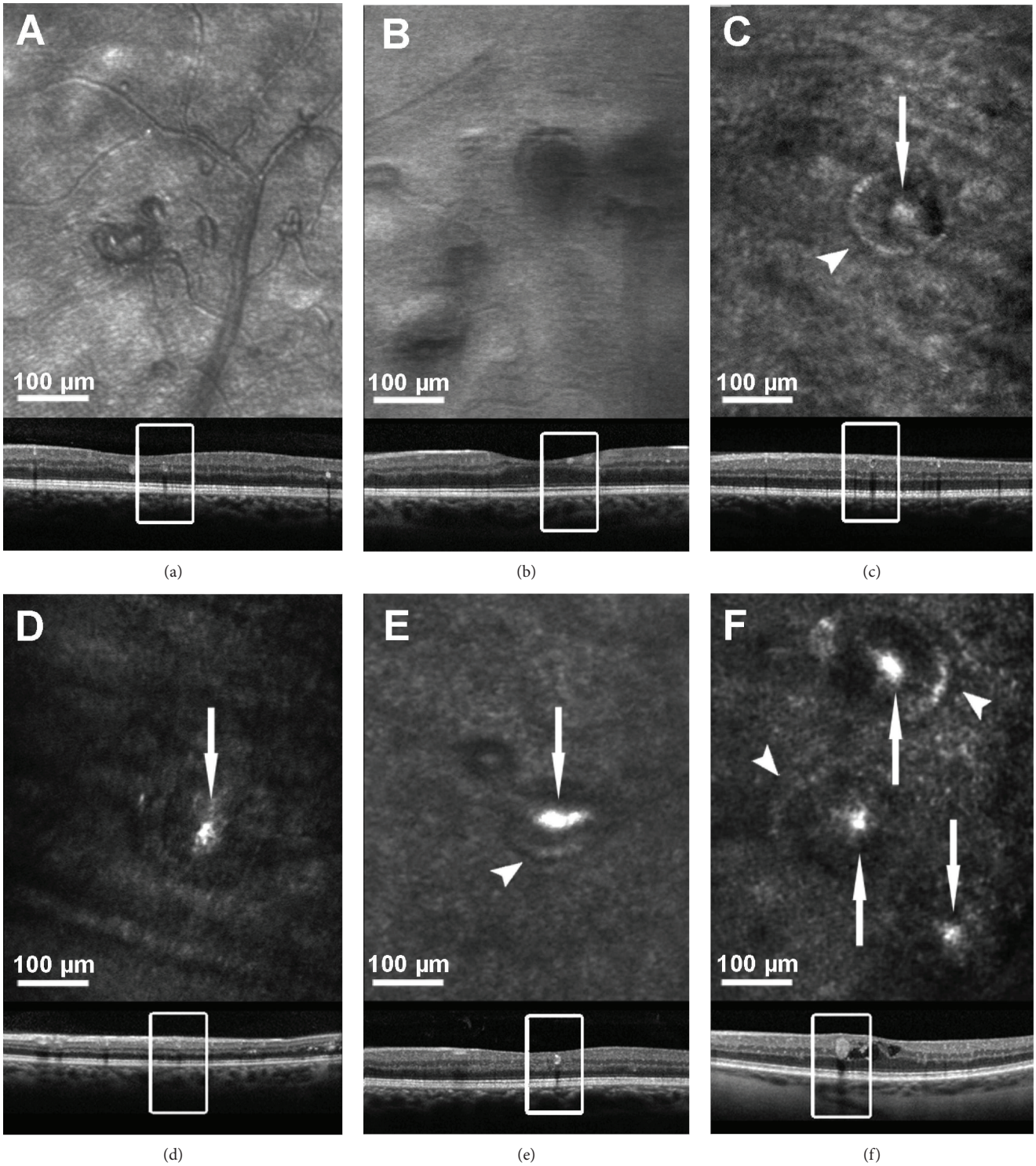


Figure 3: Six examples of microaneurysms (MA) on AOSLO. The rectangles indicate the MA location in the corresponding SDOCT B-scans. Some MAs have hyperreflective vessel walls (arrowhead) or intraluminal hyperreflective structures (arrow) in AOSLO images (c–f). Using MSL AOSLO imaging (a, b), MA vessel walls are more clearly visible compared to confocal AOSLO imaging (c–f).

due to shadowing artifacts from early diabetic lesions in subclinical DR [26].

A major advantage of AOSLO imaging is the ability to visualize intraluminal red blood cell flow in a detailed and dynamic fashion in combination with ultra-high resolution details of blood vessel walls. AOSLO videos can readily distinguish perfused MAs and NV from nonperfused lesions and can even distinguish areas of perfusion and nonperfusion within a single lesion. Although SDOCT and OCT angiography (OCTA) can localize NV location relative to the

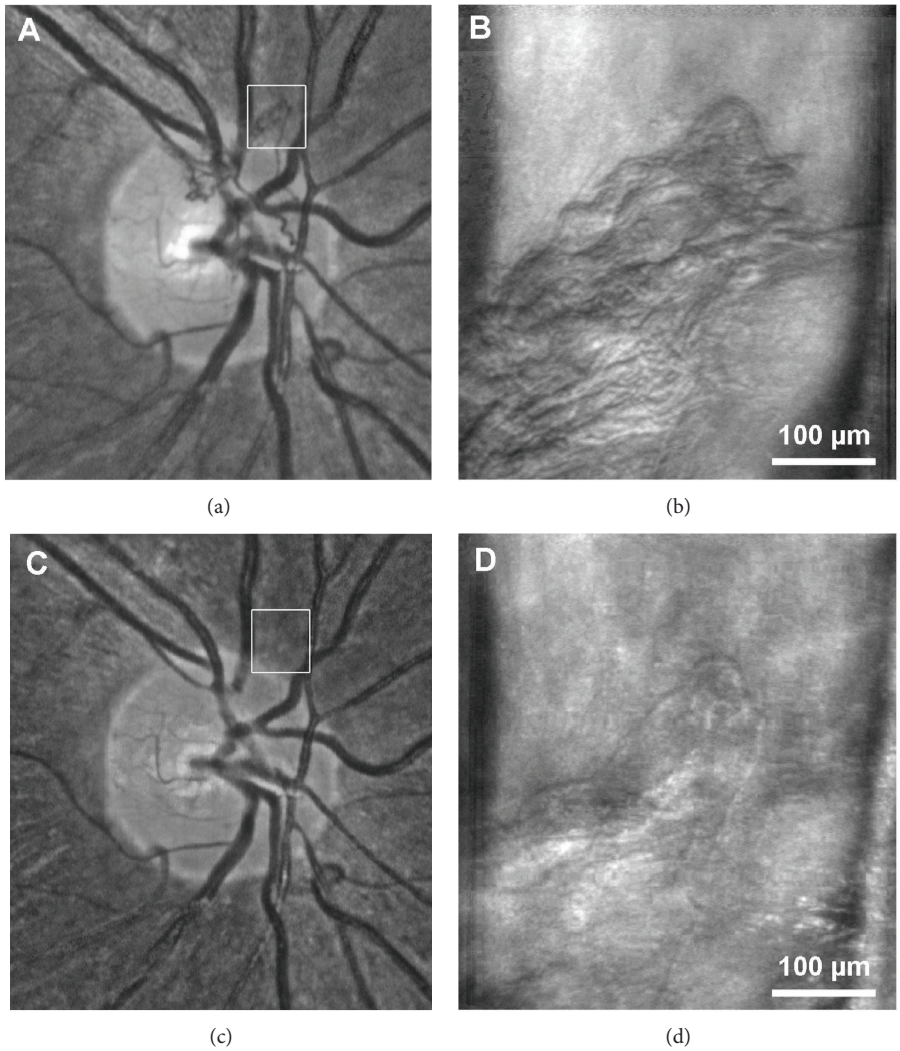


Figure 4: Response of proliferative diabetic retinopathy to anti-VEGF therapy on AOSLO. (a) Fundus photograph of neovascularization at the optic disc (NVD). (b) MSL AOSLO image of the area highlighted by the white box in (a). (c, d) Same NVD 4 weeks after administration of intravitreal ranibizumab. The NVD can no longer be identified in the infrared image; however, AOSLO imaging reveals the persistence of an involuted neovascular formation.

posterior hyaloid and retinal surface, blood flow assessment in OCTA is currently limited to certain blood flow velocities [14, 39, 40]. Areas of slow blood flow such as in MA or fibrotic NV may be missed. In contrary, red blood cell flow can be visualized independently of its velocity in AOSLO videos. A comparison of both imaging techniques was not within the scope of our study because OCTA images were not acquired in our patients. However, the ability to longitudinally evaluate perfusion changes of vascular DR lesions in the human eye may prove valuable in predicting the functional impact of antiangiogenic therapies on MAs, NV, and capillary occlusion [32].

The technique of multiply scattered light through decentration of the pinhole aperture, a recently introduced AOSLO imaging method, further enhances image quality of vessel walls and erythrocytes [36, 37]. Imaging of retinal vascular lesions including MAs, IRMA, and neovascularization is substantively improved by the use of this MSL technique due to improved visualization of vascular walls and individual blood cell flow. The MSL method also dramatically improves the ability to identify intraretinal cyst boundaries in the eyes with diabetic macular edema as compared with standard AOSLO confocal imaging. However, the MSL technique does not appear to offer substantial advantages over standard AOSLO confocal imaging in the evaluation of intraretinal hemorrhages, hard exudates, or cotton wool spots.

Hyperreflectivity on AOSLO images was observed in diverse lesion types and may have multiple etiologies. Hard exudates were brightly hyperreflective on AOSLO images, likely demonstrating high reflectance from lipid deposits. Cotton wool spots were also hyperreflective in comparison to the surrounding tissue, possibly resulting from edema of the nerve fiber layer and the accumulation of mitochondria, neurofilaments, and endoplasmic reticulum in enlarged axons [41, 42]. In addition, AOSLO hyperreflectivity was

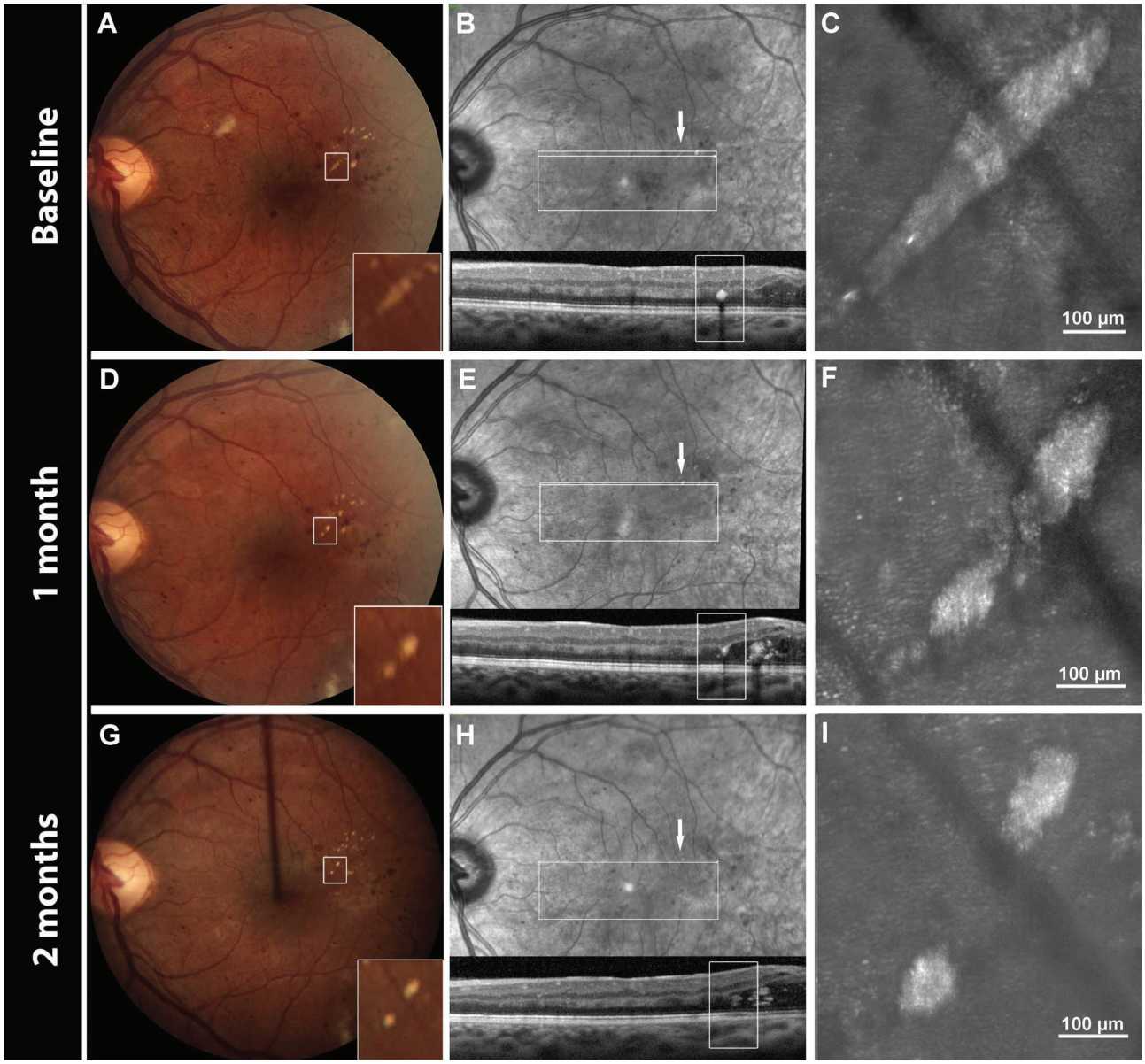


Figure 5: Multimodal imaging of a resolving hard exudate (HE) over a 2-month period. Intravitreal ranibizumab injections for center-involved diabetic macular edema were performed at the baseline and 1-month visits. The HE is clearly visible on color fundus photography (A, D, G) and IR images (B, E, H). Its location within the outer nuclear layer can be determined in cross-sectional SDOCT B-scans (B, E, H). Confocal AOSLO images (C, F, I) of the HE.

variably present within vascular walls and lumens of some MAs. Dubow et al. previously described intraluminal hypofluorescent areas imaged with AOSLO FA that might correspond to the static hyperreflectivity we observed in AOSLO images [30]. In both imaging methods, these intraluminal areas of different reflectivity indicate missing red blood cell flow which is consistent with intraluminal clotting. The histopathologic correlate of this AOSLO hyperreflectivity remains uncertain. Stitt et al. found degenerate lipid containing macrophages in some trypsin digest histologic preparations of MAs [43]. Clotting within MA lumens with basement membrane-like matrix and lipid-containing macrophages has also been described [43]. It is possible that AOSLO hyperreflectivity within MAs and along their walls might represent the presence of these lipid-containing macrophages.

Interestingly, hyperreflectivity of the MA wall on AOSLO images was not consistently correlated with the hyperreflective "ring sign" described by Horii et al. on SDOCT [44]. Similarly, AOSLO hyperreflectivity within MAs was not related to hyperreflectivity of the MA lumen on corresponding SDOCT images. These differences might arise from the different wavelengths used in these two imaging techniques or the use of a transverse cross-sectional scanning plane of SDOCT as opposed to the en-face scanning of AOSLO. Since individual SDOCT B-scans are discontinuous, there are gaps between each line scan that are not evaluated and individual lesions may not always be scanned perpendicularly. In

contrast, with AOSLO en-face scanning, the axial scanning position is continuously variable in order to acquire a high level of detail covering the entire extent of the MA.

Hard exudates appear as irregularly shaped hyperreflective lesions independent of blood vessels on AOSLO imaging. Bolz et al. postulated that hyperreflective foci in SDOCT images of the eyes with DR are precursors of HE that accumulate in the OPL before becoming visible as HE in color fundus photographs [45]. The irregular granular structure of HEs in AOSLO is consistent with the hypothesis that HE consist of many small elements. However, small hyperreflective lesions consistent with HE precursors were not routinely identified on AOSLO images, perhaps due to interference from background reflectivity of the photoreceptors.

A general limitation of AOSLO imaging is that it can be difficult to determine the precise anteroposterior location of the image focal plane when this plane is located between the nerve fiber layer and photoreceptor layer. Thus, the anteroposterior extent of pathologies such as intraretinal cysts is better detected in cross-sectional SDOCT scans. Limitations specific to this study are the variable number of different intraretinal lesions imaged per eye which were selected in order to provide a broad range of pathology. We also did not compare AOSLO imaging to fluorescein angiography (FA) since AOSLO allows much higher resolution imaging in comparison to standard FA, and it is already well documented that AOSLO MSL imaging is similarly sensitive to AOSLO FA for the detection of perfused vasculature without the need for invasive use of contrast dyes. AOSLO MSL imaging also provides additional information about nonperfused vascular lesions and vascular wall structures that may not be evident on FA [27].

In summary, the ability to noninvasively visualize the hallmark retinal lesions of diabetic retinopathy in the human eye at a lateral resolution of 2.5 μm using AOSLO has provided a level of characterization previously impossible. Longitudinal evaluation of individual lesions over time is now feasible, and with variable axial scanning, the three-dimensional characteristics of individual lesions become readily evident. Furthermore, utilizing high-resolution video sequences, AOSLO imaging enables assessment of individual blood cell flow within the retinal vasculature. These AOSLO attributes allow unprecedented evaluation of the retina and the pathologic changes induced by diabetes, potentially facilitating novel insights into the development, regression, and response to therapy of diabetic eye disease.

Disclosure

The funding organizations had no role in the design or conduct of this research.

Conflicts of Interest

Jennifer K. Sun received nonfinancial support from Boston Micromachines. No conflicting relationship exists for any other author.

Acknowledgments

This study was supported by NEI R01 EY024702-01, NEI R44 EY016295, and P30EY019008; JDRF 17-2011-359 and 3-SRA-2014-264-M-R; NIDDK 5 P30 DK036836-24 P&F grant; Eleanor Chesterman Beatson Childcare Ambassador Program Foundation grant; and Massachusetts Lions Eye Research Fund Inc.

Supplementary Materials

Supplementary 1. Video 1: example of an MA with perfusion visible throughout all portions of the lesion on confocal AOSLO imaging. Blood flow through the surrounding capillary network is also visible.

Supplementary 2. Video 2: a nonperfused MA imaged with the MSL technique. No blood flow is visible within the centrally located vascular lesion despite clearly visible flow through the surrounding vessels.

Supplementary 3. Video 3: the central, bean-shaped MA in this video demonstrates apparent deformability of the superior lesion wall with movement of blood cells adjacent to the wall. In contrast, the inferior wall of the MA is fixed and does not appear to be flexible in response to the passage of blood cells through the inferior portion of the MA.

Supplementary 4. Video 4: an example of a perfused IRMA on MSL imaging.

Supplementary 5. Video 5: video of retinal NV demonstrating adjacent vascular channels within the same area of NV that are perfused (located superiorly) and nonperfused (located inferiorly).

Supplementary 6. Video 6: area of retinal NV before (left panel) and after (right panel) treatment with intravitreal anti-VEGF therapy. After anti-VEGF treatment, many neovascular channels appear to have resolved entirely and others are substantially decreased in caliber. In contrast, the larger, nonneovascular vessels present along the left and bottom right edges of the videos do not appear to have changed noticeably in caliber or appearance.

References

[1] Early Treatment Diabetic Retinopathy Study Research Group, "Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie house classification," *Ophthalmology*, vol. 98, no. 5, pp. 786–806, 1991.

[2] R. Klein, B. E. Klein, S. E. Moss, M. D. Davis, and D. L. DeMets, "The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years," *Archives of Ophthalmology*, vol. 102, no. 4, pp. 527–532, 1984.

[3] M. Porta, G. Curletto, D. Cipullo et al., "Estimating the delay between onset and diagnosis of type 2 diabetes from the time course of retinopathy prevalence," *Diabetes Care*, vol. 37, no. 6, pp. 1668–1674, 2014.

[4] R. N. Frank, "Diabetic retinopathy," *The New England Journal of Medicine*, vol. 350, no. 1, pp. 48–58, 2004.

[5] P. S. Silva, J. D. Cavallerano, J. K. Sun, A. Z. Soliman, L. M. Aiello, and L. P. Aiello, "Peripheral lesions identified by mydriatic ultrawide field imaging: distribution and potential impact on diabetic retinopathy severity," *Ophthalmology*, vol. 120, no. 12, pp. 2587–2595, 2013.

[6] P. S. Silva, J. D. Cavallerano, D. Tolls et al., "Potential efficiency benefits of nonmydriatic ultrawide field retinal imaging in an ocular telehealth diabetic retinopathy program," *Diabetes Care*, vol. 37, no. 1, pp. 50–55, 2014.

[7] J. D. Cavallerano, P. S. Silva, A. M. Tolson et al., "Imager evaluation of diabetic retinopathy at the time of imaging in a telemedicine program," *Diabetes Care*, vol. 35, no. 3, pp. 482–484, 2012.

[8] Early Treatment Diabetic Retinopathy Study Research Group, "Fundus photographic risk factors for progression of diabetic retinopathy," *Ophthalmology*, vol. 98, no. 5, pp. 823–833, 1991.

[9] D. F. Kiernan, W. F. Mieler, and S. M. Hariprasad, "Spectral-domain optical coherence tomography: a comparison of modern high-resolution retinal imaging systems," *American Journal of Ophthalmology*, vol. 149, no. 1, pp. 18–31.e2, 2010.

[10] M. Adhi and J. S. Duker, "Optical coherence tomography–current and future applications," *Current Opinion in Ophthalmology*, vol. 24, no. 3, pp. 213–221, 2013.

[11] R. H. Webb, G. W. Hughes, and F. C. Delori, "Confocal scanning laser ophthalmoscope," *Applied Optics*, vol. 26, no. 8, pp. 1492–1499, 1987.

[12] H. Wang, J. Chhablani, W. R. Freeman et al., "Characterization of diabetic microaneurysms by simultaneous fluorescein angiography and spectral-domain optical coherence tomography," *American Journal of Ophthalmology*, vol. 153, no. 5, pp. 861–867.e1, 2012.

[13] M. M. K. Muqit and P. E. Stanga, "Fourier-domain optical coherence tomography evaluation of retinal and optic nerve head neovascularisation in proliferative diabetic retinopathy," *British Journal of Ophthalmology*, vol. 98, no. 1, pp. 65–72, 2014.

[14] H. Cho, A. A. Alwassia, C. V. Regiatieri et al., "Retinal neovascularization secondary to proliferative diabetic retinopathy characterized by spectral domain optical coherence tomography," *Retina*, vol. 33, no. 3, pp. 542–547, 2013.

[15] D. G. Cogan, D. Toussaint, and T. Kuwabara, "Retinal vascular patterns. IV. Diabetic retinopathy," *Archives of Ophthalmology*, vol. 66, no. 3, pp. 366–378, 1961.

[16] D. Toussaint, "Histological aspects of diabetic retinopathy," *Bulletin de la Societe Belge D'ophtalmologie*, vol. 129, pp. 428–444, 1961.

[17] D. Toussaint, D. G. Cogan, and T. Kuwabara, "Extravascular lesions of diabetic retinopathy," *Archives of Ophthalmology*, vol. 67, no. 1, pp. 42–47, 1962.

[18] A. Roorda, F. Romero-Borja, W. Donnelly III, H. Queener, T. Hebert, and M. Campbell, "Adaptive optics scanning laser ophthalmoscopy," *Optics Express*, vol. 10, no. 9, pp. 405–412, 2002.

[19] J. Liang, D. R. Williams, and D. T. Miller, "Supernormal vision and high-resolution retinal imaging through adaptive optics," *Journal of the Optical Society of America A*, vol. 14, no. 11, pp. 2884–2892, 1997.

[20] D. T. Miller, O. P. Kocaoglu, Q. Wang, and S. Lee, "Adaptive optics and the eye (super resolution OCT)," *Eye*, vol. 25, no. 3, pp. 321–330, 2011.

[21] R. Zawadzki, S. Choi, and S. Jones, "Adaptive optics–optical coherence tomography: optimizing visualization of microscopic retinal structures in three dimensions," *Journal of the Optical Society of America A*, vol. 24, no. 5, pp. 1373–1383, 2007.

[22] P. Godara, A. M. Dubis, A. Roorda, J. L. Duncan, and J. Carroll, "Adaptive optics retinal imaging: emerging clinical applications," *Optometry and Vision Science*, vol. 87, no. 12, pp. 930–941, 2010.

[23] F. Romero-Borja, K. Venkateswaran, A. Roorda, and T. Hebert, "Optical slicing of human retinal tissue *in vivo* with the adaptive optics scanning laser ophthalmoscope," *Applied Optics*, vol. 44, no. 19, pp. 4032–4040, 2005.

[24] Y. Zhang and A. Roorda, "Evaluating the lateral resolution of the adaptive optics scanning laser ophthalmoscope," *Journal of Biomedical Optics*, vol. 11, no. 1, article 14002, 2006.

[25] J. Carroll, D. B. Kay, D. Scoles, A. Dubra, and M. Lombardo, "Adaptive optics retinal imaging - clinical opportunities and challenges," *Current Eye Research*, vol. 38, no. 7, pp. 709–721, 2013.

[26] S. A. Burns, A. E. Elsner, T. Y. Chui et al., "*In vivo* adaptive optics microvascular imaging in diabetic patients without clinically severe diabetic retinopathy," *Biomedical Optics Express*, vol. 5, no. 3, pp. 961–974, 2014.

[27] C. TYP, M. Dubow, A. Pinhas et al., "Comparison of adaptive optics scanning light ophthalmoscopic fluorescein angiography and offset pinhole imaging," *Biomedical Optics Express*, vol. 5, no. 4, pp. 1173–1189, 2014.

[28] J. Tam and K. Dhamdhere, "Subclinical capillary changes in non-proliferative diabetic retinopathy," *Optometry and Vision Science*, vol. 89, no. 5, pp. 1–21, 2012.

[29] T. Y. Chui, D. A. Vannasdale, and S. A. Burns, "The use of forward scatter to improve retinal vascular imaging with an adaptive optics scanning laser ophthalmoscope," *Biomedical Optics Express*, vol. 3, no. 10, pp. 2537–2549, 2012.

[30] M. Dubow, A. Pinhas, N. Shah et al., "Classification of human retinal microaneurysms using adaptive optics scanning light ophthalmoscope fluorescein angiography," *Investigative Ophthalmology & Visual Science*, vol. 55, no. 3, pp. 1299–1309, 2014.

[31] T. Y. P. Chui, S. Mo, B. Krawitz et al., "Human retinal microvascular imaging using adaptive optics scanning light ophthalmoscopy," *International Journal of Retina and Vitreous*, vol. 2, no. 1, p. 11, 2016.

[32] T. Yuen, P. Chui, A. Pinhas et al., "Longitudinal imaging of microvascular remodelling in proliferative diabetic retinopathy using adaptive optics scanning light ophthalmoscopy," *Ophthalmic & Physiological Optics*, vol. 36, pp. 290–302, 2016.

[33] American Diabetes Association, "Standards of medical care in diabetes—2012," *Diabetes Care*, vol. 35, Suppl 1, pp. S11–S63, 2012.

[34] R. H. Webb, M. J. Albanese, Y. Zhou, T. Bifano, and S. A. Burns, "Stroke amplifier for deformable mirrors," *Applied Optics*, vol. 43, no. 28, pp. 5330–5333, 2004.

[35] S. A. Burns, R. Tumbar, A. E. Elsner, D. Ferguson, and D. X. Hammer, "Large-field-of-view, modular, stabilized, adaptive-optics-based scanning laser ophthalmoscope," *Journal of the Optical Society of America A*, vol. 24, no. 5, pp. 1313–1326, 2007.

[36] T. Y. Chui, T. J. Gast, and S. A. Burns, "Imaging of vascular wall fine structure in the human retina using adaptive optics

scanning laser ophthalmoscopy," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 10, pp. 7115–7124, 2013.

[37] T. Y. P. Chui, D. A. VanNasdale, and S. A. Burns, "The use of forward scatter to improve retinal vascular imaging with an adaptive optics scanning laser ophthalmoscope," *Biomedical Optics Express*, vol. 3, no. 10, pp. 2537–2549, 2012.

[38] A. E. Elsner, A. Weber, M. C. Cheney, D. A. VanNasdale, and M. Miura, "Imaging polarimetry in patients with neovascular age-related macular degeneration," *Journal of the Optical Society of America A*, vol. 24, no. 5, pp. 1468–1480, 2007.

[39] T. E. de Carlo, M. A. Bonini Filho, C. R. Baumal et al., "Evaluation of preretinal neovascularization in proliferative diabetic retinopathy using optical coherence tomography angiography," *Ophthalmic surgery, lasers and imaging retina*, vol. 47, no. 2, pp. 115–119, 2016.

[40] K. V. Chalam and K. Sambhav, "Optical coherence tomography angiography in retinal diseases," *Journal of Ophthalmic & Vision Research*, vol. 11, no. 1, pp. 84–92, 2016.

[41] H. Inomata, H. Ikui, and K. Kimura, "Pathogenesis of cotton-wool spots. 1. Fine structure of cytoid bodies," *Nihon Ganka Gakkai Zasshi*, vol. 71, no. 9, pp. 1352–1364, 1967.

[42] J. R. Wolter, "The cytoid body reaction of the human retina," *Transactions of the American Ophthalmological Society*, vol. 65, pp. 106–127, 1967.

[43] A. W. Stitt, T. A. Gardiner, and D. B. Archer, "Histological and ultrastructural investigation of retinal microaneurysm development in diabetic patients," *British Journal of Ophthalmology*, vol. 79, no. 4, pp. 362–367, 1995.

[44] T. Horii, T. Murakami, K. Nishijima, A. Sakamoto, M. Ota, and N. Yoshimura, "Optical coherence tomographic characteristics of microaneurysms in diabetic retinopathy," *American Journal of Ophthalmology*, vol. 150, no. 6, pp. 840–848.e1, 2010.

[45] M. Bolz, U. Schmidt-Erfurth, G. Deak, G. Mylonas, K. Kriechbaum, and C. Scholda, "Optical coherence tomographic hyperreflective foci: a morphologic sign of lipid extravasation in diabetic macular edema," *Ophthalmology*, vol. 116, no. 5, pp. 914–920, 2009.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 1897058, 5 pages
https://doi.org/10.1155/2018/1897058

Research Article

Limited Joint Mobility Progression in Type 1 Diabetes: A 15-Year Follow-Up Study

Javier Labad,[1,2] **Antoni Rozadilla**,[3] **Paula Garcia-Sancho**,[1] **Joan M. Nolla**,[3,4,5] **and Eduard Montanya**[1,4,5,6]

[1]*Endocrine Unit, Hospital Universitari Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain*
[2]*Parc Tauli Hospital Universitari, I3PT, Universitat Autònoma, CIBERSAM, Barcelona, Spain*
[3]*Rheumatology Section, Hospital Universitari Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain*
[4]*Institut d'Investigació Biomedical de Bellvitge (IDIBELL), Barcelona, Spain*
[5]*Department of Clinical Sciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain*
[6]*CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain*

Correspondence should be addressed to Javier Labad; jlabad@tauli.cat and Eduard Montanya; montanya@ub.edu

Received 8 September 2017; Revised 28 December 2017; Accepted 3 January 2018; Published 2 May 2018

Academic Editor: Ilias Migdalis

Objective. To assess the evolution of joint mobility over a period of 15 years in type 1 diabetic patients and healthy controls and to determine whether microalbuminuria is associated with a different evolution of joint mobility. *Methods.* Joint mobility of hand and wrist was determined in 63 patients with type 1 diabetes and 63 healthy subjects. Fifteen years later, 37 (58.7%) diabetic patients and 16 (25.4%) healthy subjects were studied again. Joint mobility was assessed with the Prayer sign and by measuring the angle of maximal flexion of the fifth and third metacarpophalangeal (MCP) joints and wrist. Patients with diabetes were visited 2–4 times every year with regular assessment of glycated hemoglobin ($HbA1_c$), urinary albumin excretion (UAE), and ophthalmoscopy. *Results.* Fifteen years after the initial exam, diabetic patients showed reduced flexion of the fifth MCP joint (82.6 ± 5.8 versus 76.0 ± 6.4 degrees, $p < 0.001$) and wrist (75.9 ± 8.1 versus 73.2 ± 7.4 degrees, $p = 0.015$) compared to baseline examination. Joint mobility did not change significantly in healthy subjects. Patients with microalbuminuria showed greater reduction in hand joint mobility than diabetic patients with normal UAE or than healthy subjects ($p < 0.001$). *Conclusions.* In type 1 diabetic patients, the severity of LJM progresses with time, and the progression is enhanced in patients with microalbuminuria.

1. Introduction

Limited joint mobility (LJM), a nonpainful contracture of finger joints, is the most common hand abnormality in diabetes, but it has received little attention in clinical research and care [1–3]. LJM usually begins at the fifth interphalangeal joints and extends radially, affecting other interphalangeal and metacarpophalangeal joints. Long-term glycemic control influences the onset of LJM, and the incidence of LJM is greater in patients with a poor metabolic control [4, 5]. Although some studies have shown that improvement in standards of glycemic control and diabetes care has reduced the prevalence of LJM [6, 7], the Diabetes Control and Complications Trial (DCCT) could not show a consistent association between intensive insulin therapy and the elements of cheiroarthropathy [5]. The prevalence of LJM has been reported to increase with diabetes duration [5, 8]. However, age may be a confounding factor in the increased prevalence of LJM with longer diabetes duration since joint mobility deteriorates with aging [9, 10].

It has been shown that in elderly patients, diabetes adds a negative effect on LJM [9]. However, an association between LJM with diabetes duration as age increases has not been established [9]. Moreover, the few prospective studies that have determined joint mobility in diabetic subjects have not included a control group of healthy subjects

[11–13]. Thus, it is not well established whether the evolution of LJM with aging is different in patients with diabetes and in the general population.

Cross-sectional studies have reported an association between LJM and microvascular complications [5, 7, 14–19], and the presence of LJM is considered a useful clinical tool to identify patients at increased risk for developing diabetic microvascular complications. However, prospective studies have yielded controversial results. In adults, the role of LJM in the prediction of microvascular diabetic complications has not been confirmed [11, 12], whereas in children, LJM is associated with an increased risk of microalbuminuria [13]. In a previous study [14], we found that hand joint mobility was limited in type 1 diabetic patients compared with control subjects and that LJM was associated with microalbuminuria. In the current follow-up study, performed 15 years later after the initial evaluation, we aimed to determine whether the evolution of joint mobility is different in diabetic and nondiabetic subjects and whether it is modified in the presence of albuminuria.

2. Methods

2.1. Subjects. In a previous study, we measured joint mobility in 63 patients with type 1 diabetes recruited from the outpatient clinic and in 63 age- and sex-matched healthy subjects [14]. The inclusion/exclusion criteria were described in the baseline study [14]. For the current study, the cohorts of diabetic patients and healthy subjects were contacted 15 years later and invited to participate in this follow-up study. Thirty-seven type 1 diabetic patients (58.7% of the initial cohort) and 16 healthy subjects (25.4% of the initial cohort) were accepted to participate and were included in the study. The clinical characteristics of patients and control subjects included in the follow-up were not significantly different from those of the initial study.

2.2. Joint Mobility. Joint mobility was assessed by the same rheumatologist (AR) that performed the initial assessment and using the same methodology. He was unaware of the metabolic control of patients, presence of diabetic complications, and individual values of the baseline joint mobility assessment. Joint mobility was determined qualitatively with the Prayer sign and quantitatively by measuring the maximal flexion of the fifth and third metacarpophalangeal (5MCP, 3MCP) joints and wrist, as previously described [14]. The maximal extension of the 5MCP and 3MCP joints was not recorded since the values, in both diabetic and control subjects, were inconsistent with those of baseline examination. In brief, the Prayer sign was defined as positive when subjects were unable to oppose palmar surfaces at any interphalangeal or MCP joint. To measure the angle of active maximal flexion of the fifth MCP joint, the fourth finger was fixed on a flat surface and the subject was asked to actively perform the flexion of the fifth finger at the level of the MCP joint. The angle of maximal flexion was measured with a goniometer and expressed in degrees using the zero method. To measure the mobility of the third MCP joint, the second finger was fixed. The arm and forearm were fixed in complete extension to measure the angles of active maximal flexion and extension of the wrist. Both hands were evaluated in all subjects, and the mean value between left and right measurements was used for statistical calculations.

Table 1: Clinical characteristics of diabetic patients ($n = 37$).

	Baseline	Follow-up
Age (years)	27.4 (12.5)	42.9 (12.3)
Female sex	19 (51.4)	19 (51.4)
Age at diabetes diagnosis (years)	19.8 (10.6)	19.8 (10.6)
Duration of diabetes (years)	7.7 (6.1)	22.6 (5.9)
BMI (kg/m2)	23.0 (3.0)	26.6 (4.0)
Systolic blood pressure (mmHg)	118.8 (14.6)	126.6 (19.5)
Diastolic blood pressure (mmHg)	71.1 (9.1)	72.1 (10.9)
HbA_1 (%)	11.2 (1.7)	
HbA_{1c} (%)		8.1 (1.5)[a]
Insulin dose (U/kg)	0.75 (0.18)	0.83 (0.17)
Smoking	5 (13.5)	8 (21.6)
Hypertension	6 (16.2)	9 (24.3)
Dyslipidemia	2 (5.4)	8 (21.6)
Retinopathy	13 (35.1)	18 (48.6)
Microalbuminuria	7 (18.9)	16 (43.2)[b]

Values are mean (SD) or number of patients (%). BMI: body mass index; [a]mean HbA1c values of the last 10 years of follow-up; [b]two patients showed macroalbuminuria at the end of follow-up.

2.3. Metabolic Control and Microvascular Complications. Medical records of the 15-year follow-up period were reviewed. Five years after the baseline study, the method to evaluate glycated hemoglobin was changed from HbA_1 to the more specific HbA_{1c}. Therefore, the metabolic control of the follow-up period was calculated as the mean HbA_{1c} value of the final 10 years. Diabetic retinopathy was assessed by direct ophthalmoscopy through dilated pupils. Urinary albumin excretion (UAE) was determined in 24 hour sterile urine. Albuminuria was defined as UAE greater than 30 mg/24 h in two consecutive samples or in two of three consecutive samples.

2.4. Statistical Analysis. Data were analysed using SPSS 15.0. Nonparametric tests Wilcoxon and Kruskal-Wallis were used to compare continuous data between groups. Kruskal-Wallis test was used to analyse differences in flexion among control group, diabetic patients with normoalbuminuria, and diabetic patients with microalbuminuria. Post hoc analysis between two groups was performed with the Wilcoxon test. Fisher exact test was used to compare categorical data between groups. The relationship between continuous variables was assessed with Pearson's correlation coefficient. A p value <0.05 (two-tailed) was considered to be significant.

3. Results

Clinical characteristics of diabetic patients at baseline and at follow-up are shown in Table 1. Nondiabetic control subjects

Table 2: Joint mobility in diabetic patients and healthy control subjects.

Joint mobility (degrees)	Patients with diabetes ($n = 37$)		Control group ($n = 16$)	
	Baseline	Follow-up	Baseline	Follow-up
Fifth metacarpophalangeal flexion	82.6 (5.8)††	76.0 (6.4)**	86.0 (6.5)	85.8 (6.7)
Third metacarpophalangeal flexion	86.1 (3.8)*	85.2 (4.4)	89.8 (5.8)	88.0 (5.8)
Wrist flexion	75.9 (8.1)†	73.2 (7.4)*	76.3 (8.5)	76.5 (9.2)
Positive Prayer sign, n (%)	18 (48.6)*	18 (48.6)*	2 (12.5)	2 (12.5)

Values are mean (SD), except for Prayer sign (number and percentage) $^{*}p < 0.05$, $^{**}p < 0.01$ between patients and controls at each time point (Wilcoxon test for independent samples or Chi-square test [Prayer sign comparison]). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$ between baseline and follow-up in patients or controls (Wilcoxon test for paired samples).

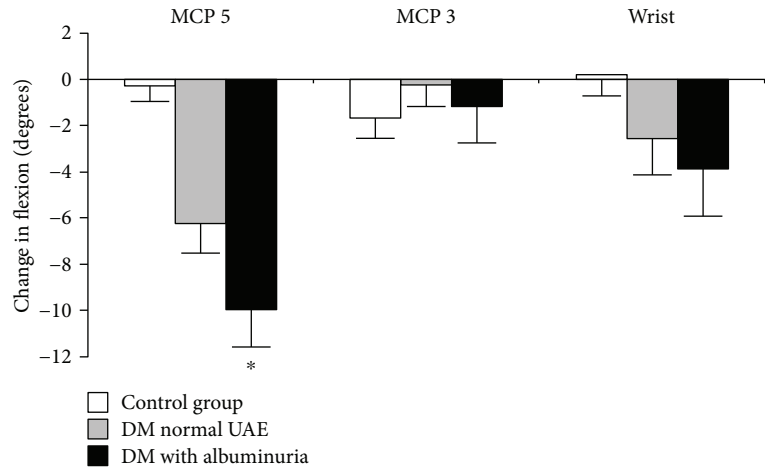


Figure 1: Change in hand joint flexion in diabetic patients (DM) with ($n = 16$) and without ($n = 21$) albuminuria and in healthy subjects ($n = 16$) over 15 years. DM: diabetes mellitus; MCP: metacarpophalangeal. Error bars represent ±1 SE. $^{*}p < 0.001$ between groups (Kruskal-Wallis comparison). Post hoc between-group comparisons: control group versus diabetic patients without microalbuminuria, $p = 0.001$; control group versus diabetic patients with microalbuminuria, $p < 0.001$; diabetic patients without microalbuminuria versus diabetic patients with microalbuminuria $p = 0.059$.

had a similar sex distribution (43.8% male patients) than diabetic patients but were slightly older (33.8 ± 10.9 versus 27.4 ± 12.5 years at baseline). Diabetic patients showed LJM both at baseline and at follow-up compared with control subjects (Table 2). Joint mobility deteriorated with time in diabetic patients that showed reduced flexion of 5MCP joint and wrist at the end of follow-up compared to baseline (Table 2). In contrast, no significant differences were detected between baseline and follow-up in control healthy subjects. The prevalence of Prayer sign was the same at baseline and at follow-up examination and in the range of what has been described in diabetic and in nondiabetic subjects [20, 21].

Patients with albuminuria showed greater reductions in joint mobility after 15 years of follow-up than diabetic patients with normal UAE or than healthy subjects (Figure 1). In contrast, the presence of retinopathy was not associated with greater reductions in joint mobility. Changes in joint mobility were not associated with age, smoking, hypertension, dyslipidemia, and duration of diabetes or mean HbA_{1c} levels. There were gender differences in the reduction in wrist joint mobility, with a greater reduction in male patients (males: -7.1 ± 7.2 versus females 0.8 ± 7.0, $p = 0.005$). There were no significant differences in HbA_{1c} levels between patients with or without a Prayer positive sign.

4. Discussion

In this study, we show that, over a period of 15 years, joint mobility deteriorated significantly in patients with type 1 diabetes but not in healthy control subjects. Moreover, diabetic patients with albuminuria showed a more severe deterioration of joint mobility than those with normal UAE or than control nondiabetic subjects. The changes of joint mobility did not correlate with age, duration of diabetes, or metabolic control and were not modified by the presence of diabetic retinopathy.

To the best of our knowledge, this is the first study prospectively comparing the evolution of joint mobility in diabetic patients and nondiabetic subjects. The high prevalence of LJM in type 1 diabetes is well established [5], but longitudinal studies of joint mobility are scarce, and it is not known whether the evolution is different in diabetic patients and the general population. The presence of a control group of nondiabetic subjects with similar age is essential for this

analysis, since age is associated with LJM [9]. In our initial study [14], we found that joint mobility was reduced in patients with type 1 diabetes compared with healthy controls. We now show that 15 years later, joint mobility deteriorated significantly in diabetic patients but did not change significantly in control subjects, indicating that the progression of limited joint mobility was specifically associated with the presence of diabetes and was not due to aging.

Type 1 diabetic patients with albuminuria showed a higher reduction in joint mobility after 15 years of follow-up compared with diabetic subjects with normal UAE and with control healthy subjects. In our initial cross-sectional study, we found that LJM was associated with microalbuminuria [14], an observation that has been subsequently confirmed by Amin et al. in a large longitudinal study [13]. We have now found that the presence of albuminuria is associated with a more severe progression of LJM. A plausible biological link between microalbuminuria and LMJ has been proposed based on a common role of advanced glycation end products in the development of LJM and diabetic complications including albuminuria [20–22]. However, other prospective studies have failed to show a relationship between microalbuminuria and LJM [11, 12]. Differences in the characteristics of diabetic patients and in the duration of follow-up may account for the discrepancy in the results. The shorter follow-up period of previous studies may have been insufficient to detect the association between microalbuminuria and poor evolution of LJM.

Our study has some limitations. First, the sample size was reduced from our initial study. Dropout rates may be accounted by the difficulty in contacting the subjects after this long follow-up period of 15 years and in particular the control subjects. Nevertheless, diabetic and control groups remained well matched in age and sex, and more than half of the initial diabetic population participated in the follow-up evaluation. Second, the extension mobility of the joints could not be evaluated. Extension of the 5MCP and 3MCP was already significantly reduced in diabetic subjects at baseline, and we may speculate that the follow-up extension measurements would have shown similar evolution than the joint flexion. Third, we focused on the diabetic microvascular complications that had been analysed in the baseline study, retinopathy, and in particular albuminuria, and we did not assess diabetic neuropathy. Reduced joint mobility in the hand has been associated with a decline in mobility in the ankle and is considered a risk factor for food ulceration in diabetic patients [21, 23]. However, diabetic neuropathy has not been consistently related to LJM [24]. Finally, the evaluation was not fully blinded regarding the presence of diabetes.

In summary, the present study shows the progressive character of LJM in type 1 diabetic patients and underscores the relationship between albuminuria and LJM. Periodic evaluation of joint mobility should be considered in patients with type 1 diabetes, particularly when albuminuria is present.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM) and CIBER de Salud Mental (CIBERSAM) is an initiative from Instituto de Salud Carlos III. Javier Labad received an Intensification of Research Activity Grant (Programme I3SNS- INT 11/323) from Instituto de Salud Carlos III.

References

[1] A. L. Rosenbloom, J. H. Silverstein, D. C. Lezotte, W. J. Riley, and N. K. Maclaren, "Limited joint mobility in diabetes mellitus of childhood: natural history and relationship to growth impairment," *The Journal of Pediatrics*, vol. 101, no. 5, pp. 874–878, 1982.

[2] A. Grgic, A. L. Rosenbloom, F. T. Weber, B. Giordano, J. I. Malone, and J. J. Shuster, "Joint contracture—common manifestation of childhood diabetes mellitus," *The Journal of Pediatrics*, vol. 88, no. 4, Part 1, pp. 584–588, 1976.

[3] C. F. Clarke, A. T. Piesowicz, and G. S. Spathis, "Limited joint mobility in children and adolescents with insulin dependent diabetes mellitus," *Annals of the Rheumatic Diseases*, vol. 49, no. 4, pp. 236-237, 1990.

[4] J. H. Silverstein, G. Gordon, B. H. Pollock, and A. L. Rosenbloom, "Long-term glycemic control influences the onset of limited joint mobility in type 1 diabetes," *The Journal of Pediatrics*, vol. 132, no. 6, pp. 944–947, 1998.

[5] M. E. Larkin, A. Barnie, B. H. Braffett et al., "Musculoskeletal complications in type 1 diabetes," *Diabetes Care*, vol. 37, no. 7, pp. 1863–1869, 2014.

[6] J. R. Infante, A. L. Rosenbloom, J. H. Silverstein, L. Garzarella, and B. H. Pollock, "Changes in frequency and severity of limited joint mobility in children with type 1 diabetes mellitus between 1976-78 and 1998," *The Journal of Pediatrics*, vol. 138, no. 1, pp. 33–37, 2001.

[7] J. R. Lindsay, L. Kennedy, A. B. Atkinson et al., "Reduced prevalence of limited joint mobility in type 1 diabetes in a U.K. clinic population over a 20-year period," *Diabetes Care*, vol. 28, no. 3, pp. 658–661, 2005.

[8] N. Papanas and E. Maltezos, "The diabetic hand: a forgotten complication?," *Journal of Diabetes and its Complications*, vol. 24, no. 3, pp. 154–162, 2011.

[9] Z. Smahel and A. Klimova, "The influence of age and exercise on the mobility of hand joints: 1: metacarpophalangeal joints of the three-phalangeal fingers," *Acta Chirurgiae Plasticae*, vol. 46, no. 3, pp. 81–88, 2004.

[10] M. Abate, C. Schiavone, P. Pelotti, and V. Salini, "Limited joint mobility (LJM) in elderly subjects with type II diabetes mellitus," *Archives of Gerontology and Geriatrics*, vol. 53, no. 2, pp. 135–140, 2011.

[11] D. R. McCance, G. Crowe, M. J. Quinn, M. Smye, and L. Kennedy, "Incidence of microvascular complications in type 1 diabetic subjects with limited joint mobility: a 10-year prospective study," *Diabetic Medicine*, vol. 10, no. 9, pp. 807–810, 1993.

[12] P. E. T. Arkkila, I. M. Kantola, J. S. A. Viikari, T. Rönnemaa, and M. A. Vähätalo, "Limited joint mobility is associated with the presence but does not predict the development of microvascular complications in type 1 diabetes," *Diabetic Medicine*, vol. 13, no. 9, pp. 828–833, 1996.

[13] R. Amin, T. K. Bahu, B. Widmer, R. N. Dalton, and D. B. Dunger, "Longitudinal relation between limited joint mobility, height, insulin-like growth factor 1 levels, and risk of developing microalbuminuria: the Oxford regional prospective study," *Archives of Disease in Childhood*, vol. 90, no. 10, pp. 1039–1044, 2005.

[14] E. Montana, A. Rozadilla, J. M. Nolla, N. Gomez, D. R. Escofet, and J. Soler, "Microalbuminuria is associated with limited joint mobility in type I diabetes mellitus," *Annals of the Rheumatic Diseases*, vol. 54, no. 7, pp. 582–586, 1995.

[15] P. E. T. Arkkila, I. M. Kantol, and J. S. A. Viikari, "Limited joint mobility in type 1 diabetic patients: correlation to other diabetic complications," *Journal of Internal Medicine*, vol. 236, no. 2, pp. 215–223, 1994.

[16] S. K. Garg, H. P. Chase, G. Marshall et al., "Limited joint mobility in subjects with insulin dependent diabetes mellitus: relationship with eye and kidney complications," *Archives of Disease in Childhood*, vol. 67, no. 1, pp. 96–99, 1992.

[17] A. C. Duffin, K. C. Donaghue, M. Potter et al., "Limited joint mobility in the hands and feet of adolescents with type 1 diabetes mellitus," *Diabetic Medicine*, vol. 16, no. 2, pp. 125–130, 1999.

[18] A. L. Rosenbloom, J. H. Silverstein, D. C. Lezotte, K. Richardson, and M. McCallum, "Limited joint mobility in childhood diabetes mellitus indicates increased risk for microvascular disease," *The New England Journal of Medicine*, vol. 305, no. 4, pp. 191–194, 1981.

[19] D. Frost and W. Beischer, "Limited joint mobility in type 1 diabetic patients: associations with microangiopathy and subclinical macroangiopathy are different in men and women," *Diabetes Care*, vol. 24, no. 1, pp. 95–99, 2001.

[20] J. G. Larkin and B. M. Frier, "Limited joint mobility and Dupuytren's contracture in diabetic, hypertensive, and normal populations," *British Medical Journal*, vol. 292, no. 6534, p. 1494, 1986.

[21] D. J. Fernando and J. Vernidharan, "Limited joint mobility in Sri Lankan patients with non-insulin-dependent diabetes," *Rheumatology*, vol. 36, no. 3, pp. 374–376, 1997.

[22] A. Goldin, J. A. Beckman, A. M. Schmidt, and M. A. Creager, "Advanced glycation end products: sparking the development of diabetic vascular injury," *Circulation*, vol. 114, no. 6, pp. 597–605, 2006.

[23] S. Zimny, H. Schatz, and M. Pfohl, "The role of limited joint mobility in diabetic patients with an at-risk foot," *Diabetes Care*, vol. 27, no. 4, pp. 942–946, 2004.

[24] J. L. Lázaro-Martínez, F. J. Aragón-Sánchez, J. V. Beneit-Montesinos, M. A. González-Jurado, E. G. Morales, and D. M. Hernández, "Food biomechanics in patients with diabetes mellitus. Doubts regarding the relationship between neuropathy, food motion and deformities," *Journal of the American Podiatric Medical Association*, vol. 101, no. 3, pp. 208–214, 2011.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 1078531, 8 pages
https://doi.org/10.1155/2018/1078531

Research Article

Decreased Retinal Thickness in Type 1 Diabetic Children with Signs of Nonproliferative Diabetic Retinopathy

P. Ruiz-Ocaña,[1] **P. Espinoza Requena**,[2] **A. Alonso-Ojembarrena**,[3] **P. Alemany Márquez**,[2,4] **S. Jiménez Carmona**,[2,4] **and A. M. Lechuga-Sancho**[1,5]

[1]*Diabetes and Metabolism Unit, Department of Pediatrics, University Hospital Puerta del Mar, Cádiz, Spain*
[2]*Department of Ophthalmology, University Hospital Puerta del Mar, Cádiz, Spain*
[3]*Neonatology Unit, Department of Pediatrics, University Hospital Puerta del Mar, Cádiz, Spain*
[4]*Department of Surgery, School of Medicine, Cádiz University, Cádiz, Spain*
[5]*Department of Mother and Child Health and Radiology, School of Medicine, Cádiz University, Cádiz, Spain*

Correspondence should be addressed to A. M. Lechuga-Sancho; alfonso.lechuga@uca.es

Received 24 November 2017; Revised 14 February 2018; Accepted 1 March 2018; Published 26 April 2018

Academic Editor: Nikolaos Papanas

The retina functions as a neurovascular unit. How early vascular alterations affect neuronal layers remains controversial; early vascular failure could lead to edema increasing retinal thicknesses, but alternatively neuronal loss could lead to reduced retinal thickness. *Objective*. To evaluate retinal thickness in a cohort of pediatric patients with type 1 diabetes mellitus (PwT1DM) and to analyze differences according to the presence or absence of nonproliferative diabetic retinopathy (NPDR), poor metabolic control, and diabetes duration. *Patients and Methods*. We performed retinographies and optical coherence tomography (OCT) (TOPCON 3D1000®) to PwT1DM followed at our center and healthy controls. Measurements of the control group served to calculate reference values. *Results*. 59 PwT1DM (age 12.51 ± 2.59) and 22 healthy controls (age 10.66 ± 2.51) volunteered. Only two PwT1DM, both adolescents with poor metabolic control, presented NPRD. Both showed decreased thicknesses and retinal volumes. The odds ratio of having decreased retinal thickness when signs of NPDR were present was 11.72 (95% IC 1.16–118.28; $p = 0.036$). *Conclusions*. PwT1DM with NPDR have increased odds of decreased retinal thicknesses and volumes. Whether these changes are reversible by improving metabolic control or not remains to be elucidated.

1. Introduction

Diabetic retinopathy (DR) is one of the microvascular complications associated with T1DM, being the most frequent cause of blindness in "active population" (adults between the ages of 20 and 74) [1]. It is estimated that one-third of people with diabetes have signs of retinopathy, one-third of whom suffer from vision-threatening diabetic retinopathy (VTDR, defined by the presence of proliferative retinopathy and/or macular edema), with approximately 93 million people in the world with DR, 17 million with proliferative DR, 21 million with diabetic macular edema, and 28 million with VTDR worldwide [2].

The mechanisms involving DR development include the following: hyperglycaemia may cause sorbitol accumulation in retinal cells, increased polyol metabolism, advanced glycosylation end product (AGE) elevation in the extracellular fluid, increased protein kinase C and hexosamine pathway activity [3], upregulation of growth factors and proinflammatory cytokines, hyperactivation of the renin-angiotensin system, and exacerbated production of superoxides. The mechanism by which these cytokines contribute to vascular and neuronal apoptosis is not yet clear and may respond to excitotoxicity, oxidative stress, and/or mitochondrial dysfunction [3].

In preclinical retinopathy, no ophthalmoscopic alterations are observed, although alterations of the neurovascular unit have already been demonstrated at this point. The neurovascular unit is a histological concept explaining that, under normal conditions, endothelial cells and pericytes,

astrocytes, Müller cells, and neurons are closely related and connected, establishing the so-called blood-retinal barrier, in order to favor an adequate flow of nutrients, an ionic environment appropriate for neurological signaling, synaptic transmission, and adaptive responses that allow an adequate visual function [4].

Thus, the damage induced by DR would not be limited to an isolated angiopathy but would involve both the vascular and neuronal compartments. This statement is based on the demonstration of early subtle changes in microvascular hemodynamics [5], neuronal functionality in studies performed by electroretinography [6, 7], and the decrease in thicknesses of the nerve fiber layer of the retina [8] in these early preclinical stages of retinal involvement.

In children, although it is common to demonstrate ophthalmoscopic findings such as isolated retinal microaneurysms or small unilateral hemorrhages (all of which are signs of nonproliferative diabetic retinopathy (NPDR)), it is extremely rare to find preproliferative retinopathy, proliferative retinopathy, or macular edema, regardless of the degree of metabolic control or the time of evolution of diabetes [4], with a clear tendency in our days to the decrease in the detection of retinopathy [4, 9, 10].

To date, the younger age at which severe DR (preproliferative or proliferative) has been documented is 15 years of age, and the shortest documented duration of diabetes since its clinical onset to severe DR presentation is 5 years [11]. Besides diabetes duration and patient's age, another independent risk factor for DR development is the age at diabetes onset; those who were under 5 years of age at the start of their clinical diabetes have decreased risk of DR development [12].

Since its introduction in clinical practice, optical coherence tomography (OCT) has been used in research on diabetic retinopathy, given its ability to accurately determine retinal thickness, being a noninvasive technique. OCT enables the objective and quantitative assessment of retinal volumes and thicknesses, having excellent reproducibility in children with respect to adults [13, 14]. The main problem in the realization of OCT in children is the interpretation of the measurements when the devices lack a specific reference database in the pediatric age.

Different reference values for macular and papillary structures have been published, rendering great variability of the measurements depending on the device used (up to 26 μm in the same patient). Thus, investigators and clinicians need to calculate their own reference values for the OCT device used, since different software calculate their measurements based on different delimitations in the external segmentation line [15–19].

Previous publications have found retinal thickness alterations in both directions: on the one hand, there are reports of increased retinal thickness, both in established diabetic retinopathy [20] and in clinically significant diabetic macular edema [21–23], pointing at OCT's suitability as a screening method for the subclinical macular edema in diabetic patients [24]. On the other hand, decreased thicknesses have also been reported [8, 25], suggesting neuronal cell loss as the cause. Finally, there are also a number of studies that do not appreciate significant differences in any way [26, 27].

2. Objective

The aim of this study is to compare retinal thickness in pediatric patients with type 1 diabetes mellitus (PwT1DM) diagnosed with nonproliferative diabetic retinopathy (NPDR) with those obtained in healthy subjects.

3. Patients and Methods

We performed a transversal comparative study between the cohort of PwT1DM followed at the Children's Diabetes Unit of Puerta del Mar University Hospital (Cadiz) and healthy children and adolescents aged between 4 and 18 years. The study protocol was evaluated and accepted by our institution's ethics committee for clinical research, and written informed consent was obtained from all study subjects and/or their legal guardians.

Patients were recruited from our Diabetes Unit, and controls were recruited from patients' close relatives or friends, aiming at similar age and sex distribution in both groups. Clinical variables involving demographics, anthropometry, characteristics of the diabetic process, and degree of metabolic control were registered from their electronic medical records after informed consent was obtained. As a variable of diabetes control, the glycated hemoglobin levels, its average levels in the year before, and the mean in the whole "historical period of illness" were registered at the time of ocular exploration. In addition, the variation coefficient and standard deviation of the average levels of the historical period were determined as measures of the dispersion of these levels.

The analytical determinations were carried out by the usual methods (colorimetry and ECLIA) in analytical platforms C-711 and E-170 of Roche Diagnostics in the Clinical Analysis Service of our center.

The ophthalmological evaluation consisted of anterior chamber study, intraocular pressure, autorefractometry, and visual acuity. The retina was explored by conventional retinography and OCT, using the 3D OCT-1000 spectral domain device (TOPCON, Japan) for macular and papillary study. The study map used was the grid proposed by the Early Treatment Diabetic Retinopathy Study (ETDRS), composed of three concentric circles of 1, 3, and 6 mm in diameter corresponding to the fovea, inner ring, and outer ring, respectively. Each ring is subdivided into superior, nasal, inferior, and temporal depending on its location with respect to the midline as shown in Figure 1.

3.1. Exclusion Criteria. Patients with other types of diabetes different from type 1 were not included, as well as patients with less than 12 months from diabetes clinical onset at the time of the study. Those presenting limitations for undergoing the exploration or for the interpretation of the results, such as subjects unable to collaborate adequately to complete the ocular explorations, those presenting a refractive error > 5.5 diopters (D) or astigmatism greater than 3 D, those with a visual acuity (VA, Snellen Visual Acuity Scale) of less than 0.7 with a difference of vision greater than one line of the optotype between the AV of both eyes, and those with intraocular pressure (IOP) greater than 21 mmHg or

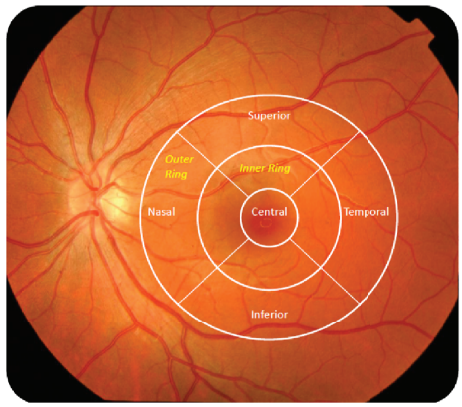


Figure 1: Outline of the macular regions according to the ETDRS's grid.

those presenting other conditions, such as strabismus, amblyopia, or the presence of pathology that may cause changes in retinal thickness such as chorioretinal scars, myelin fibers, or papillary druses, were also excluded.

Finally, we discarded those OCT reports with a signal strength lower than 41, since this strength is considered the reliability limit of the exploration.

We used the statistical packages IBM © PASW STATISTICS © (IBM Corporation, Somers, NY, USA) in its version 18 and GraphPad Prism 6.0 for the statistical analysis of the data. We express the qualitative variables as number and percentage and the quantitative variables as average and standard deviation. First, we performed a descriptive study and used the data from controls to calculate the reference values (in the form of thickness and volume percentiles) of each of the macular and papillary sectors of the grid. Subsequently, we calculated the same percentiles for the T1D patients and compared these with the controls' using Student's t-test for independent samples, previously evaluating the equality of variances with the Levene test and establishing a level of significance of 95%, determining the difference with a p value under 0.05 as significant.

For the variables expressed as a scale, a study of bivariate correlations was performed using Pearson coefficients in the study of parametric correlations and Spearman coefficients in the study of nonparametric correlations. It was determined as significant with a level less than or equal to 0.05 (bilateral), and it was considered relevant when presenting a value of R greater than or equal to 0.4.

Finally, we calculated the odds ratio of presenting decreased thicknesses as a function of having signs of NPDR or not, having greater or less than 5 years of diabetes duration, having a mean HbA1c level of greater or less than 8.5%, or having diabetic clinical onset presentation before or after the 5 years of age (with a CI of 95%) and tested for significance using the Z statistic.

4. Results

64 PwT1DM and 36 controls volunteered. In 3 PwT1DM and 9 controls, it was not possible to complete the different exploratory techniques due to poor collaboration. Two more PwT1DM and 5 more controls met the exclusion criteria after having undergone exploration (increased intraocular pressure, strabismus, and astigmatism) and were not included in the analysis. Finally, our sample consisted of 59 PwT1DM (age 12.51 ± 2.59 years, ranged from 7.24 to 16.93 years) and 22 healthy controls (age 10.66 ± 2.51 years, ranged from 6.41 to 16.17 years).

Table 1: Anthropometric characteristics of the total population.

	Controls	PwT1DM
Subjects (N)	22	59
Decimal age (years)	10.66 ± 2.51	12.51 ± 2.58
Height (Z-score)	−0.46 ± 1.12	−0.12 ± 1.09
Weight (Z-score)	−0.28 ± 1.08	−0.14 ± 0.89
Body mass index (kg/m^2)	18.57 ± 4.11	19.63 ± 3.37
Body mass index (Z-score)	−0.05 ± 0.99	−0.09 ± 0.79
Waterlow index (%)	96.604 ± 15.88	98.353 ± 14.65
Systolic blood pressure (mmHg)	110.26 ± 14.24	111.17 ± 10.32
Systolic blood pressure (Z-score)	0.07 ± 1.12	0.40 ± 0.98
Diastolic blood pressure (mmHg)	61.83 ± 7.74	63.40 ± 7.90
Diastolic blood pressure (Z-score)	0.12 ± 0.62	0.07 ± 0.669

Table 2: Clinical characteristics of the PwT1DM study population.

	PwT1DM
Subjects (N)	59
Average age (years) at diabetic onset	6.05 ± 3.41 (0.8–13.7)
Time (years) of disease evolution	6.45 ± 3.04 (1–13.2)
Multiple dose insulin therapy (N/%)	43/72.9%
Continuous infusion of subcutaneous insulin (insulin pump) therapy (N/%)	16/27.1%

The clinical, demographical, and anthropometrical characteristics are summarized in Tables 1 and 2. As seen in Table 1, no relevant differences were found in any of the anthropometrical parameters between PwT1DM and controls. As shown in Table 2, our PwT1DM have a relatively young average age at diabetes clinical onset, and the mean duration of diabetes since the onset to the study is over 5 years. Most of our PwT1DM were under multiple insulin dose regimen with long-acting analogues as basal insulin and ultrafast analogues as rapid-acting insulin for boluses.

Our sample's degree of diabetes metabolic control is summarized in Table 3. The glycosylated hemoglobin (HbA1c) values were analyzed both at the time of exploration and during the previous year, as well as during the evolution of the disease from debut to examination.

After careful examination, 3 OCT reports were discarded for not meeting the minimum quality, 25 were considered unusable for being off-centered, 4 were excluded due to partial signal absence (blinks), and two more reports presented artifacts, and only the data from undisturbed sectors were taken into account in these two. Hence, our final total consisted of 139 OCT reports of appropriate quality. Discarding off-centered scans increases the internal validity of our data.

Table 3: HbA1c levels and variability over time in the PwT1DM group.

HbA1c (%)	N	Average	Median	Min–Max
On the date of exploration	59	7.93 ± 1.05	7.50	6.20–14.60
Year prior to exploration	59	7.97 ± 1.25	7.70	6.32–14.10
Historical period of clinical follow-up	58	7.93 ± 0.78	7.88	6.10–12.80

Table 4: Retinal thickness and volume reference values for each ETDRS sector, calculated with measurements from healthy controls.

Retinal sector	Percentiles							$N = 40$
	3	10	25	50	75	90	97	Average ± SD
Foveolar thickness (μm)	165	172.5	181	192	205	222	230	194.1 ± 17.9
Total macular volume (mm^3)	7.07	7.26	7.42	7.60	7.76	8.06	8.20	7.62 ± 0.29
Macular central thickness (μm)	194.3	209	217.2	229	245	255	267.3	230.4 ± 18.7
Superior inner thickness (μm)	272.9	287	295	302	311	318	322.3	301.8 ± 12.9
Temporal inner thickness (μm)	265.3	275	280	289	298	304	310.3	288.3 ± 12
Inferior inner thickness (μm)	269	283	289.2	297	306.7	312.5	321	297.1 ± 12.8
Nasal inner thickness (μm)	282	289	296	304	312	320	328.9	304 ± 11.7
Superior outer thickness (μm)	241.5	247.3	256	263	272	282.7	295	264.8 ± 15.8
Temporal outer thickness (μm)	213.6	233	238	249	256	263.6	273	247.8 ± 13
Inferior outer thickness (μm)	238	241	249	256.5	263	274	288.3	257.6 ± 12.6
Nasal outer thickness (μm)	253.7	263.5	271	280	287	297	307.6	279.8 ± 12.8
Central macular volume (mm^3)	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.18 ± 0.01
Superior inner volume (mm^3)	0.44	0.45	0.46	0.47	0.49	0.50	0.51	0.48 ± 0.08
Temporal inner volume (mm^3)	0.41	0.43	0.44	0.45	0.47	0.48	0.48	0.45 ± 0.03
Inferior inner volume (mm^3)	0.42	0.45	0.46	0.47	0.48	0.49	0.5	0.46 ± 0.02
Nasal inner volume (mm^3)	0.44	0.45	0.46	0.48	0.49	0.50	0.51	0.47 ± 0.02
Superior outer volume (mm^3)	1.28	1.31	1.35	1.39	1.43	1.49	1.53	1.39 ± 0.07
Temporal outer volume (mm^3)	1.23	1.24	1.28	1.32	1.36	1.41	1.50	1.32 ± 0.07
Inferior outer volume (mm^3)	1.26	1.28	1.32	1.36	1.38	1.45	1.52	1.36 ± 0.06
Nasal outer volume (mm^3)	1.35	1.40	1.44	1.49	1.53	1.57	1.60	1.48 ± 0.06
Total RNFL thickness (μm)	84.5	89	92	97	101	106	112.4	97 ± 6.6
Superior RNFL thickness (μm)	102	107	113	119	126	135	143	120.1 ± 11.0
Inferior RNFL thickness (μm)	98	107	113	120	127	133	138.4	119.9 ± 10.4

Since there are no reference values for retinal thicknesses and volumes in childhood with the device we used (TOPCON 3D OCT-1000), we proceeded to calculate our own for each macular and papillary sector measurement, using the data from the group of healthy controls (Table 4). From now on, we considered "decreased retinal thickness" values below the third percentile of the reference data and "increased retinal thickness" values over the 97th percentile.

When comparing both study groups' values, we found no differences in any thickness or volumes (Table 5).

In our group of PwT1DM, we found not even one with a retinographic image compatible with subclinical macular edema or vision-threatening retinopathy. In only two cases, we found pathological images suggestive of incipient diabetic retinopathy (IDR): one patient presented microaneurysm and the second patient flame hemorrhage. Thus, we had an IDR incidence of 3.3% of the sample. The patient with microaneurysm (case 1) was a 14.8-year-old girl, with nearly 10 years of diabetes duration, obesity, and poor metabolic control all throughout the disease, with a mean annual HbA1c of 9.1% and 8.4% in the year of the study point. The patient with flame hemorrhages (case 2) was a 13.4-year-old boy, with nearly 9 years of diabetes evolution and also poor metabolic control, with mean annual HbA1c levels of 9.1% since clinical onset and the same levels in the year and at the time of the study. None of these had hypertension, neither microalbuminuria.

When comparing the thicknesses of these two patients' retinas with the reference values, we found that both patients presented at least two values below the third percentile and at least 3 between the third and 10th percentiles (Table 6).

The power of our contrast to detect the difference in the superior inner thickness (the variable with the largest difference between controls and PwT1D with NPDR) as statistically significant is 93%, assuming an alpha error of 0.05 in a bilateral contrast with 40 subjects in the control group and 2 in the PwT1D + NPDR.

However, these were not the only patients in whom we found thicknesses or volumes below the third centile. We also identified 11 more PwT1DM without retinographic images

Table 5: Comparison of retinal thicknesses and volumes between PwT1DM and healthy controls.

	Controls	PwT1DM	p
N (eyes)	40	110	
Foveolar thickness (μm)	194.1 ± 17.9	194.76 ± 16.91	NS
Total macular volume (mm^3)	7.62 ± 0.29	7.60 ± 0.27	NS
Macular central thickness (μm)	230.4 ± 18.7	230.03 ± 18.20	NS
Superior inner thickness (μm)	301.8 ± 12.9	301.20 ± 13.31	NS
Temporal inner thickness (μm)	288.31 ± 12.01	287.66 ± 11.88	NS
Inferior inner thickness (μm)	297.1 ± 12.81	296.18 ± 11.97	NS
Nasal inner thickness (μm)	304.27 ± 11.69	303.04 ± 11.36	NS
Superior outer thickness (μm)	264.78 ± 15.8	265.02 ± 16.15	NS
Temporal outer thickness (μm)	247.8 ± 12.97	247.10 ± 13.67	NS
Inferior outer thickness (μm)	257.58 ± 12.62	256.93 ± 12.05	NS
Nasal outer thickness (μm)	279.80 ± 12.81	278.98 ± 12.42	NS
Central macular volume (mm^3)	0.182 ± 0.014	0.182 ± 0.013	NS
Superior inner volume (mm^3)	0.481 ± 0.081	0.483 ± 0.098	NS
Temporal inner volume (mm^3)	0.453 ± 0.035	0.450 ± 0.034	NS
Inferior inner volume (mm^3)	0.467 ± 0.024	0.466 ± 0.018	NS
Nasal inner volume (mm^3)	0.478 ± 0.022	0.485 ± 0.092	NS
Superior outer volume (mm^3)	1.39 ± 0.071	1.39 ± 0.064	NS
Temporal outer volume (mm^3)	1.32 ± 0.070	1.32 ± 0.073	NS
Inferior outer volume (mm^3)	1.36 ± 0.061	1.36 ± 0.063	NS
Nasal outer volume (mm^3)	1.48 ± 0.062	1.48 ± 0.064	NS
Total RNFL thickness (μm)	96.86 ± 6.60	97.37 ± 7.29	NS
Superior RNFL thickness (μm)	120.09 ± 10.97	120.13 ± 11.66	NS
Inferior RNFL thickness (μm)	119.86 ± 10.37	120.08 ± 11.36	NS
Spherical equivalent (D)	0.350 ± 1.67	0.267 ± 1.60	NS

suggestive of IDR, with at least one measurement below the third centile. Hence, we performed a multiple correlation study to try to identify those factors related to an increased risk of decreased thicknesses or volumes, but we did not appreciate significant correlation between any retinal measurement and the degree of metabolic control expressed in the levels of glycosylated hemoglobin or any of the other variables analyzed (age at diabetes onset, current age, diabetes duration, sex, pubertal stage, type of therapy, etc.) (data not shown).

We calculated the odds ratio of presenting decreased retinal thicknesses as a function of acceptable (HbA1c < 7.5%) or poor metabolic control (HbA1c > 8.5%) or as a function of the age at diabetes onset and found no significant odds ($p = 0.15$ and $p = 0.36$, resp.). The time of diabetes evolution > 5 years at the moment of the study did have an increased odds ratio of presenting decreased retinal thicknesses (OR 2.18), but it missed statistical significance ($p = 0.09$). However, the odds ratio of having decreased retinal thickness when signs of NPDR were present was 11.72 (95% IC 1.16–118.28; $p = 0.036$).

5. Discussion

The variations in retinal thickness in adult PwT1DM have been the subject of study in recent years, with controversial results. Different studies find an increase in macular thickness (global or sectoral) [22, 28], hypothesizing that the cause of this increase could respond to the accumulation of fluid between the layers of the retina secondary to the loss of BHR function and early and subclinical stage of diabetic macular edema. Others, however, find decreased thicknesses [8, 25] supporting the hypothesis of neuronal cell loss as the first event of diabetic retinopathy, prior to vascular damage. Finally, there are also a number of studies that do not appreciate significant differences in one way or another, stating that the time of disease evolution can play a decisive role in the findings and in relation to the pathogenic phenomena of the disease [26, 27].

Studies in children are more recent and similarly controversial; while some studies show a thickening of the retinal tissues in diabetic patients [27], others report the opposite [29]. In the literature, differences in subfoveal choroidal thickness between PwT1DM and healthy controls are not observed [27], neither in the retinal measurements nor in the fiber layer and nor in ganglion cells when comparing PwT1DM1 without diabetic retinopathy and healthy controls [26].

Before the Diabetes Control and Complications Trial (DCCT) of 1993 [30], a DR prevalence up to 41-42% in the United States [31] and Australia [32] and even 46% in some regions of Europe [33] had been reported in adolescents. The findings of the DCCT showed that intensive therapy in children between the ages of 13 and 17 years reduced

Table 6: Comparative study between IDR PwT1DM and control with retinal findings.

Retinal sector	Average ± SD controls	Values Case 1	Percentiles Case 1	Values Case 2	Percentiles Case 2
Foveolar thickness (μm)	194.1 ± 17.9	194	p50–75	206	p75–90
Total macular volume (mm^3)	7.62 ± 0.29	7.5	p25–50	6.94	<p3
Macular central thickness (μm)	230.4 ± 18.7	224	p25–50	227	p50–75
Superior inner thickness (μm)	301.8 ± 12.9	258	<p3	282	p3–10
Temporal inner thickness (μm)	288.3 ± 12	266	p3–10	271	p3–10
Inferior inner thickness (μm)	297.1 ± 12.8	281	p3–10	279	p3–10
Nasal inner thickness (μm)	304 ± 11.7	281	<p3	283	p3–10
Superior outer thickness (μm)	264.8 ± 15.8	267	p50–75	233	<p3
Temporal outer thickness (μm)	247.8 ± 13	241	p25–50	223	p3–10
Inferior outer thickness (μm)	257.6 ± 12.6	260	p50–75	233	<p3
Nasal outer thickness (μm)	279.8 ± 12.8	281	p50–75	255	p3–10
Central macular volume (mm^3)	0.18 ± 0.01	0.18	p50	0.18	p50
Superior inner volume (mm^3)	0.48 ± 0.08	0.45	p10	0.44	p3
Temporal inner volume (mm^3)	0.45 ± 0.03	0.42	p3–10	0.42	p3–10
Inferior inner volume (mm^3)	0.46 ± 0.02	0.44	p3–10	0.44	p3–10
Nasal inner volume (mm^3)	0.47 ± 0.02	0.45	p3–10	0.44	p3
Superior outer volume (mm^3)	1.39 ± 0.07	1.42	p50–75	1.24	<p3
Temporal outer volume (mm^3)	1.32 ± 0.07	1.29	p25–50	1.18	<p3
Inferior outer volume (mm^3)	1.36 ± 0.06	1.38	p75	1.23	<p3
Nasal outer volume (mm^3)	1.48 ± 0.06	1.49	p50	1.35	p3
Total RNFL thickness (μm)	97 ± 6.6	107	p90–97	91	p10–25
Superior RNFL thickness (μm)	120.1 ± 11.0	135	p90	97	<p3
Inferior RNFL thickness (μm)	119.9 ± 10.4	140	>p97	117	p25–50

the risk of developing DR up to 53% [30], and since then, this therapeutic option is the main one used in the pediatric age. Despite the difficulty of achieving the target HbA1c proposed as optimal (median HbA1c in 7.5%), different publications in recent years agree on the progressive decrease in the overall incidence of the onset of diabetic retinopathy (DR) in the general population and in the pediatric population in particular [34, 35].

When considered as a group, our PwT1DM did not present differences in thicknesses or volumes in any of the ETDRS sectors of the retina measured overall, but we found an incidence of 3.38% of incipient diabetic retinopathy. This incidence is lower than that previously reported by others. In 2011, Cho et al. reported, in a population similar to that of our study (adolescents between 11 and 17 years of age and a time of evolution of T1DM between 2 and 5 years), a decrease in the incidence of retinopathy from 16% (year 1990) to 7% (from 2002 onwards), with a metabolic control of the cohorts expressed in median of HbA1c with values of 8.7 and 8.2% [36]. Downie et al. also reported the data of different historical cohorts of adolescent PwT1DM from 1990 to 2009, with a DR incidence decreasing from 53% to 12%, with median HbA1c levels of 9.1% and 8.2, respectively [35].

Taking into account that the median HbA1c in our population at the date of exploration and in the previous year of exploration is below the values of these studies (Table 2), our data support the role of an improved metabolic control to decrease the incidence of DR in children.

The two cases with signs of IDR shared two risk factors associated with the development of retinopathy, such as poor metabolic control [37–42] (both had HbA1c levels over 9%) and the time of evolution of disease [43], around 10 years [11]. In addition, case 1 had a significant obesity, an independent risk factor for the onset of retinopathy [44].

The localization of the decreased thicknesses was not the same in both cases. In case 1, it affected mainly the inner ring, and in case 2, it was generalized. These observations are in accordance with those of the studies reporting decreased retinal thickness and volumes in patients with incipient diabetic retinopathy [8].

We found no relevant correlations between the different analytical, demographical, anthropometric, and ocular variables. These either were not significant or did not reach R values under 0.4. This lack of correlations could be striking although it is likely that a larger sample size could increase the power of the correlation study.

We cannot neglect commenting on other limitations of our study. The technology that we used did not facilitate the automated and individualized measurement of the different layers of the retina, which would have detected thickening or retinal thinning located in specific layers of the retina. In addition, the cross-sectional design of the study does not allow us to analyze the temporal, permanent, or

evolutionary nature of our findings or to calculate relative risks. Since different studies have shown decreased thicknesses while others have reported increased thicknesses, it is plausible to hypothesize that such changes could be evolutionary over time, and the design of our study does not allow for this differentiation. The low incidence of IDR in our sample and of reduced retinal measurements also makes it difficult to find significant associations with clinical variables; thus, multicenter studies using the same technology, as well as prospective study designs, would be desirable for the future.

In summary, taking into account the mentioned limitations, in our work, we have not observed differences between the measurements of thickness and macular volumes between PwT1DM as a group and healthy controls; we have found a low incidence of incipient diabetic retinopathy in our sample and an increased odds ratio of reduced retinal thicknesses in PwT1DM with IDR. The time of diabetes evolution also tended to increase the odds of reduced retinal thickness, but it did not reach statistical significance, probably due to the limited sample size.

We believe that our data have mainly three implications; firstly, we cannot advocate for the use of retinal thickness measurements as a DR-screening tool in children and adolescents with relatively good metabolic control of their diabetes and no ophthalmoscopic changes. Secondly, OCT scans could be of value in the study of PwT1DM and signs of incipient diabetic retinopathy to better characterize retinal changes. Lastly, our data suggest that in order to identify which changes happen first in the development of DR, it would be advisable to include the study of different retinal layers, in a prospective manner, especially in those adolescents with poor metabolic control of their diabetes.

6. Conclusions

PwT1DM with no ophthalmoscopic changes suggesting DR do not present differences in retinal thicknesses or volumes when compared to healthy controls. Adolescent PwT1DM with NPDR have an increased odds ratio of presenting decreased retinal thicknesses and volumes. Whether these changes are reversible by improving metabolic control or not remains to be elucidated.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] N. Cheung, P. Mitchell, and T. Y. Wong, "Diabetic retinopathy," *The Lancet*, vol. 376, no. 9735, pp. 124–136, 2010.

[2] J. W. Y. Yau, S. L. Rogers, R. Kawasaki et al., "Global prevalence and major risk factors of diabetic retinopathy," *Diabetes Care*, vol. 35, no. 3, pp. 556–564, 2012.

[3] J. Cunha-Vaz, L. Ribeiro, and C. Lobo, "Phenotypes and biomarkers of diabetic retinopathy," *Progress in Retinal and Eye Research*, vol. 41, pp. 90–111, 2014.

[4] D. A. Antonetti, R. Klein, and T. W. Gardner, "Diabetic retinopathy," *The New England Journal of Medicine*, vol. 366, no. 13, pp. 1227–1239, 2012.

[5] J. Kur, E. A. Newman, and T. Chan-Ling, "Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease," *Progress in Retinal and Eye Research*, vol. 31, no. 5, pp. 377–406, 2012.

[6] M. A. Bearse, A. J. Adams, Y. Han et al., "A multifocal electroretinogram model predicting the development of diabetic retinopathy," *Progress in Retinal and Eye Research*, vol. 25, no. 5, pp. 425–448, 2006.

[7] M. A. Bearse and G. Y. Ozawa, "Multifocal electroretinography in diabetic retinopathy and diabetic macular edema," *Current Diabetes Reports*, vol. 14, no. 9, p. 526, 2014.

[8] T. Oshitari, K. Hanawa, and E. Adachi-Usami, "Changes of macular and RNFL thicknesses measured by stratus OCT in patients with early stage diabetes," *Eye*, vol. 23, no. 4, pp. 884–889, 2009.

[9] P. Hovind, L. Tarnow, K. Rossing et al., "Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes," *Diabetes Care*, vol. 26, no. 4, pp. 1258–1264, 2003.

[10] J. P. Kytö, V. Harjutsalo, C. Forsblom et al., "Decline in the cumulative incidence of severe diabetic retinopathy in patients with type 1 diabetes," *Diabetes Care*, vol. 34, no. 9, pp. 2005–2007, 2011.

[11] R. Klein, B. E. Klein, S. E. Moss, M. D. Davis, and D. L. DeMets, "The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years," *Archives of Ophthalmology*, vol. 102, no. 4, pp. 520–526, 1984.

[12] M. Svensson, J. W. Eriksson, and G. Dahlquist, "Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden," *Diabetes Care*, vol. 27, no. 4, pp. 955–962, 2004.

[13] I. Altemir, V. Pueyo, N. Elía, V. Polo, J. M. Larrosa, and D. Oros, "Reproducibility of optical coherence tomography measurements in children," *American Journal of Ophthalmology*, vol. 155, no. 1, pp. 171–176.e1, 2013.

[14] H. K. Chung, Y. K. Han, S. Oh, and S. H. Kim, "Comparison of optical coherence tomography measurement reproducibility between children and adults," *PLoS One*, vol. 11, no. 1, article e0147448, 2016.

[15] S. Grover, R. K. Murthy, V. S. Brar, and K. V. Chalam, "Normative data for macular thickness by high-definition spectral-domain optical coherence tomography (Spectralis)," *American Journal of Ophthalmology*, vol. 148, no. 2, pp. 266–271, 2009.

[16] C. K. Leung, C. Y. Cheung, R. N. Weinreb et al., "Comparison of macular thickness measurements between time domain and spectral domain optical coherence tomography," *Investigative Ophthalmology & Visual Science*, vol. 49, no. 11, pp. 4893–4897, 2008.

[17] L. Pierro, M. Gagliardi, L. Iuliano, A. Ambrosi, and F. Bandello, "Retinal nerve fiber layer thickness reproducibility using seven different OCT instruments," *Investigative Ophthalmology & Visual Science*, vol. 53, no. 9, pp. 5912–5920, 2012.

[18] L. Pierro, S. M. Giatsidis, E. Mantovani, and M. Gagliardi, "Macular thickness interoperator and intraoperator reproducibility in healthy eyes using 7 optical coherence tomography

instruments," *American Journal of Ophthalmology*, vol. 150, no. 2, pp. 199–204.e1, 2010.

[19] N. M. Buchser, G. Wollstein, H. Ishikawa et al., "Comparison of retinal nerve fiber layer thickness measurement bias and imprecision across three spectral-domain optical coherence tomography devices," *Investigative Ophthalmology & Visual Science*, vol. 53, no. 7, pp. 3742–3747, 2012.

[20] W. Goebel and T. Kretzchmar-Gross, "Retinal thickness in diabetic retinopathy: a study using optical coherence tomography (OCT)," *Retina*, vol. 22, no. 6, pp. 759–767, 2002.

[21] M. R. Hee, C. A. Puliafito, C. Wong et al., "Quantitative assessment of macular edema with optical coherence tomography," *Archives of Ophthalmology*, vol. 113, no. 8, pp. 1019–1029, 1995.

[22] R. Lattanzio, R. Brancato, L. Pierro et al., "Macular thickness measured by optical coherence tomography (OCT) in diabetic patients," *European Journal of Ophthalmology*, vol. 12, no. 6, pp. 482–487, 2002.

[23] P. Massin, A. Girach, A. Erginay, and A. Gaudric, "Optical coherence tomography: a key to the future management of patients with diabetic macular oedema," *Acta Ophthalmologica Scandinavica*, vol. 84, no. 4, pp. 466–474, 2006.

[24] G. Virgili, F. Menchini, G. Casazza et al., "Optical coherence tomography (OCT) for detection of macular oedema in patients with diabetic retinopathy," *Cochrane Database of Systematic Reviews*, no. 1, article CD008081, 2015.

[25] A. Verma, P. K. Rani, R. Raman et al., "Is neuronal dysfunction an early sign of diabetic retinopathy? Microperimetry and spectral domain optical coherence tomography (SD-OCT) study in individuals with diabetes, but no diabetic retinopathy," *Eye*, vol. 23, no. 9, pp. 1824–1830, 2009.

[26] S. A. Elhabashy, N. S. Elbarbary, K. M. Nageb, and M. M. Mohammed, "Can optical coherence tomography predict early retinal microvascular pathology in type 1 diabetic adolescents without minimal diabetic retinopathy? A single-centre study," *Journal of Pediatric Endocrinology and Metabolism*, vol. 28, no. 1-2, pp. 139–146, 2015.

[27] N. Sayin, N. Kara, D. Pirhan et al., "Evaluation of subfoveal choroidal thickness in children with type 1 diabetes mellitus: an EDI-OCT study," *Seminars in Ophthalmology*, vol. 29, no. 1, pp. 27–31, 2014.

[28] D. N. Koleva-Georgieva and N. P. Sivkova, "Optical coherence tomography for the detection of early macular edema in diabetic patients with retinopathy," *Folia Medica*, vol. 52, no. 1, pp. 40–48, 2010.

[29] D. El-Fayoumi, N. M. Badr Eldine, A. F. Esmael, D. Ghalwash, and H. M. Soliman, "Retinal nerve fiber layer and ganglion cell complex thicknesses are reduced in children with type 1 diabetes with no evidence of vascular retinopathy," *Investigative Ophthalmology & Visual Science*, vol. 57, no. 13, pp. 5355–5360, 2016.

[30] The Diabetes Control and Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus," *The New England Journal of Medicine*, vol. 329, no. 14, pp. 977–986, 1993.

[31] R. Klein, B. E. Klein, and S. E. Moss, "The Wisconsin epidemiologic study of diabetic retinopathy: an update," *Australian and New Zealand Journal of Ophthalmology*, vol. 18, no. 1, pp. 19–22, 1990.

[32] M. Bonney, S. J. Hing, A. T. Fung et al., "Development and progression of diabetic retinopathy: adolescents at risk," *Diabetic Medicine*, vol. 12, no. 11, pp. 967–973, 1995.

[33] J. Stephenson, J. H. Fuller, and EUROBIAB IDDM Complications Study Group, "Microvascular and acute complications in IDDM patients: the EURODIAB IDDM Complications Study," *Diabetologia*, vol. 37, no. 3, pp. 278–285, 1994.

[34] M. M. Geloneck, B. J. Forbes, J. Shaffer, G. Ying, and G. Binenbaum, "Ocular complications in children with diabetes mellitus," *Ophthalmology*, vol. 122, no. 12, pp. 2457–2464, 2015.

[35] E. Downie, M. E. Craig, S. Hing, J. Cusumano, A. K. F. Chan, and K. C. Donaghue, "Continued reduction in the prevalence of retinopathy in adolescents with type 1 diabetes: role of insulin therapy and glycemic control," *Diabetes Care*, vol. 34, no. 11, pp. 2368–2373, 2011.

[36] Y. H. Cho, M. E. Craig, S. Hing et al., "Microvascular complications assessment in adolescents with 2- to 5-yr duration of type 1 diabetes from 1990 to 2006," *Pediatric Diabetes*, vol. 12, no. 8, pp. 682–689, 2011.

[37] J. M. Hermann, H.-P. Hammes, B. Rami-Merhar et al., "HbA_{1c} variability as an independent risk factor for diabetic retinopathy in type 1 diabetes: A German/Austrian multicenter analysis on 35,891 patients," *PLoS One*, vol. 9, no. 3, article e91137, 2014.

[38] C.-R. Hsu, Y.-T. Chen, and W. H.-H. Sheu, "Glycemic variability and diabetes retinopathy: a missing link," *Journal of Diabetes and its Complications*, vol. 29, no. 2, pp. 302–306, 2015.

[39] E. S. Kilpatrick, A. S. Rigby, and S. L. Atkin, "A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial," *Diabetes Care*, vol. 31, no. 11, pp. 2198–2202, 2008.

[40] J. Škrha, J. Šoupal, J. Škrha, and M. Prázný, "Glucose variability, HbA1c and microvascular complications," *Reviews in Endocrine and Metabolic Disorders*, vol. 17, no. 1, pp. 103–110, 2016.

[41] S. Suh and J. H. Kim, "Glycemic variability: how do we measure it and why is it important?," *Diabetes & Metabolism Journal*, vol. 39, no. 4, pp. 273–282, 2015.

[42] B. Fullerton, K. Jeitler, M. Seitz, K. Horvath, A. Berghold, and A. Siebenhofer, "Intensive glucose control versus conventional glucose control for type 1 diabetes mellitus," *Cochrane Database of Systematic Reviews*, no. 2, article CD009122, 2014.

[43] S. Salardi, M. Porta, G. Maltoni et al., "Ketoacidosis at diagnosis in childhood-onset diabetes and the risk of retinopathy 20 years later," *Journal of Diabetes and its Complications*, vol. 30, no. 1, pp. 55–60, 2016.

[44] M. Dirani, J. Xie, E. Fenwick et al., "Are obesity and anthropometry risk factors for diabetic retinopathy?: The diabetes management project," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 7, pp. 4416–4421, 2011.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 9790303, 10 pages
https://doi.org/10.1155/2018/9790303

Research Article

Synergistic Effects of Dantrolene and Nimodipine on the Phenylephrine-Induced Contraction and ACh-Induced Relaxation in Aortic Rings from Diabetic Rats

Maria J. Crespo,[1,2] **Marie Roman**,[1] **Jonathan Matias**,[2] **Myrna Morales**,[2] **Hector Torres**,[2] **and Jose Quidgley**[1]

[1]*Department of Physiology, University of Puerto Rico-School of Medicine, San Juan, PR, USA*
[2]*Department of Anesthesiology, University of Puerto Rico-School of Medicine, San Juan, PR, USA*

Correspondence should be addressed to Maria J. Crespo; maria.crespo3@upr.edu

Received 22 November 2017; Accepted 27 February 2018; Published 19 April 2018

Academic Editor: Nikolaos Papanas

Diabetics have a higher risk of developing cerebral vasospasms (CVSP) than nondiabetics. The addition of the ryanodine receptor (RyR) blocker dantrolene to standard therapies reduces vasospasms in nondiabetics. Whether diabetics with CVSP also benefit from this drug, however, is unknown. We evaluated the effects of a 30 min incubation with dantrolene (50 μM), nimodipine (50 nM), and both drugs in combination, on phenylephrine- (PHE-) induced contraction and on acetylcholine- (ACh-) induced relaxation in aortic rings from streptozotocin (STZ) diabetic rats. Age-matched, nondiabetic rats served as controls. The oxidative stress markers malondialdehyde (MDA) and 4-hydroxyalkenal (4-HAE) were also evaluated in the presence and absence of dantrolene and nimodipine. The combination of these two drugs acted synergistically to reduce the PHE-induced contraction by 80% in both diabetics and controls. In contrast, it increased the E_{max} value for ACh-induced relaxation (from $56.46 \pm 5.14\%$ to $96.21 \pm 7.50\%$; $n = 6$, $P < 0.05$), and it decreased MDA + 4-HAE values in diabetic rats only. These results suggest that the combination of dantrolene and nimodipine benefits both diabetics and nondiabetics by decreasing arterial tone synergistically.

1. Introduction

Diabetic patients have a higher incidence of vascular complications and are more prone to developing cerebral vasospasms (CVSPs) than nondiabetics [1]. Indeed, CVSPs are responsible for the majority of diabetic morbidity and mortality [2]. Although the etiology of CVSPs has not been determined, it has been associated with increased vascular tone due to persistent elevation of intracellular Ca^{2+} in vascular smooth muscle (VSM) [2, 3], which may be secondary to (1) increased extracellular Ca^{2+}entry into the cell [4] or to (2) increased release of this ion from the sarcoplasmic reticulum (SR), which is mediated by the ryanodine receptors (RyRs) [5]. Alterations in the homeostasis of multiple mediators, including nitric oxide, serotonin, and endothelin-1, have also been proposed to underlie the onset and development of CVSP [6].

Cardiovascular abnormalities in diabetes have been linked to increased oxidative stress and endothelial dysfunction [7–9]. Moreover, unlike in nondiabetics, alterations in the status of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) characterize diabetic animal models and patients [10–13] and may contribute to increased vascular tone. These findings suggest that the pathophysiology of CVSP differs between diabetics and nondiabetics. The current treatment for this condition is similar for these two groups of patients, however, and includes the use of nimodipine, nicardipine, and other voltage-dependent L-type Ca^{2+} channel antagonists [1]. Nevertheless, these drugs are not completely effective in reducing CVSPs because Ca^{2+}

Table 1: Blood glucose levels (mg/dl) of diabetic and control rats.

	Day 0	Day 1	Day 7	Day 14	Day 28
Control	166.3 ± 6.8	154.8 ± 6.6	127.8 ± 7.1	128.1 ± 4.2	143.5 ± 6.8
Diabetic	174.3 ± 13.1	451.7 ± 41.5*	417.0 ± 59.9*	446.4 ± 71.9*	445.4 ± 63.7*

Values are the means ± SEM. Rats were injected with STZ (65 mg/kg) on day 0. $n = 20$ rats per group. *$P < 0.05$, diabetics compared to age-matched controls.

Table 2: Body weight (g) of diabetic and control rats.

	Day 0	Day 1	Day 7	Day 14	Day 28
Control	180.22 ± 5.99	172.53 ± 5.24	282.3 ± 6.5	331.3 ± 4.9	360.6 ± 5.9
Diabetic	195.4 ± 3.3	199.5 ± 6.3	239.3 ± 14.3*	254.9 ± 20.0*	272.8 ± 24.8*

Values are the means ± SEM. Rats were injected with streptozotocin on day 0. $n = 20$ rats per group. *$P < 0.05$, diabetics compared to age-matched controls.

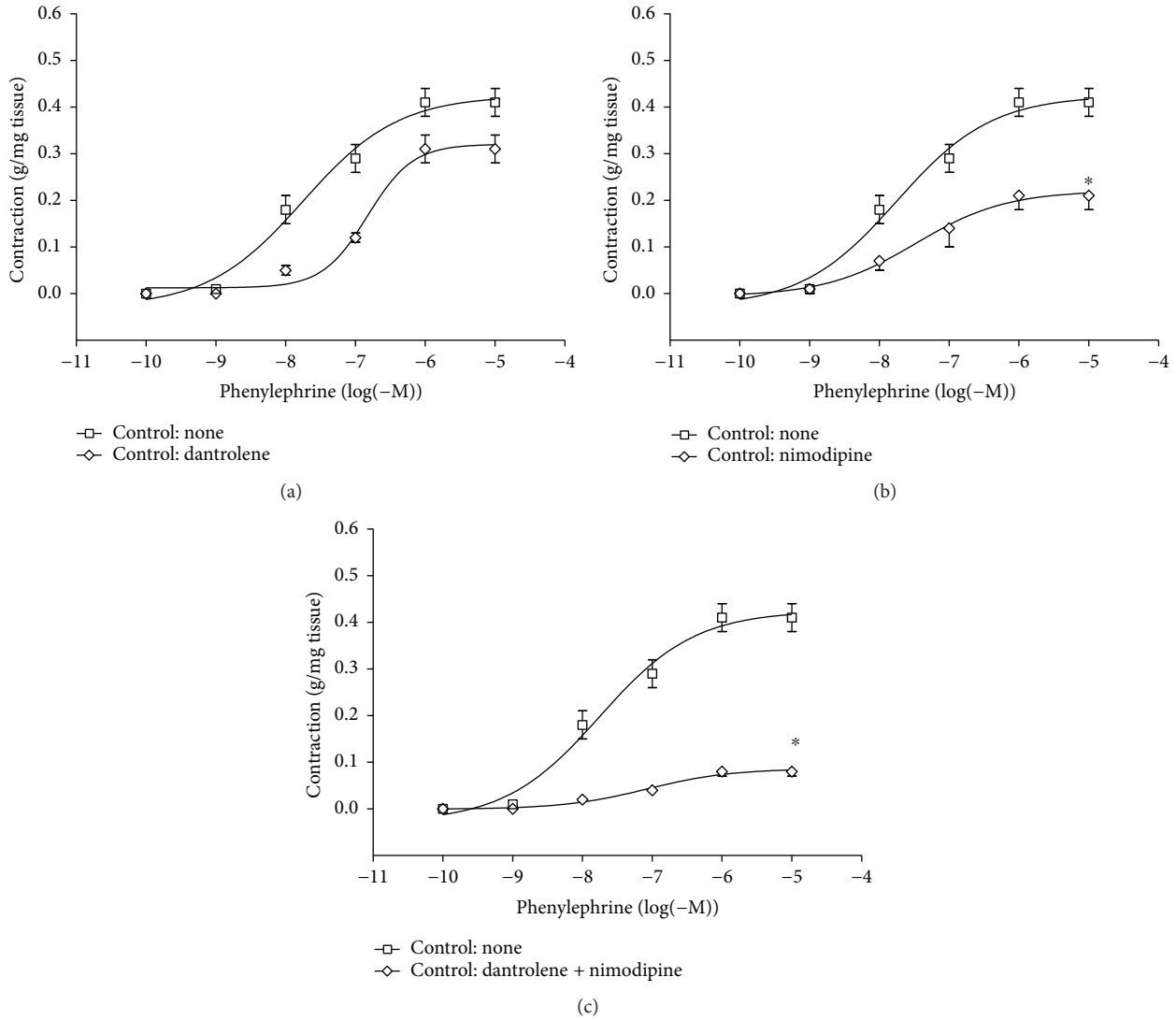


Figure 1: Cumulative concentration-response curves (from 0.1 nM to 10 μM) for the phenylephrine- (PHE-) induced contraction of aortic rings from control rats (CT) only before and after a 30 min incubation period with 50 μM dantrolene (a), with 50 nM nimodipine (b), and with both drugs in combination (c). The values shown are the means ± SEM of 6 to 9 animals per group. *$P < 0.05$, when comparing E_{max} before and after incubation with nimodipine and with dantrolene and nimodipine in combination.

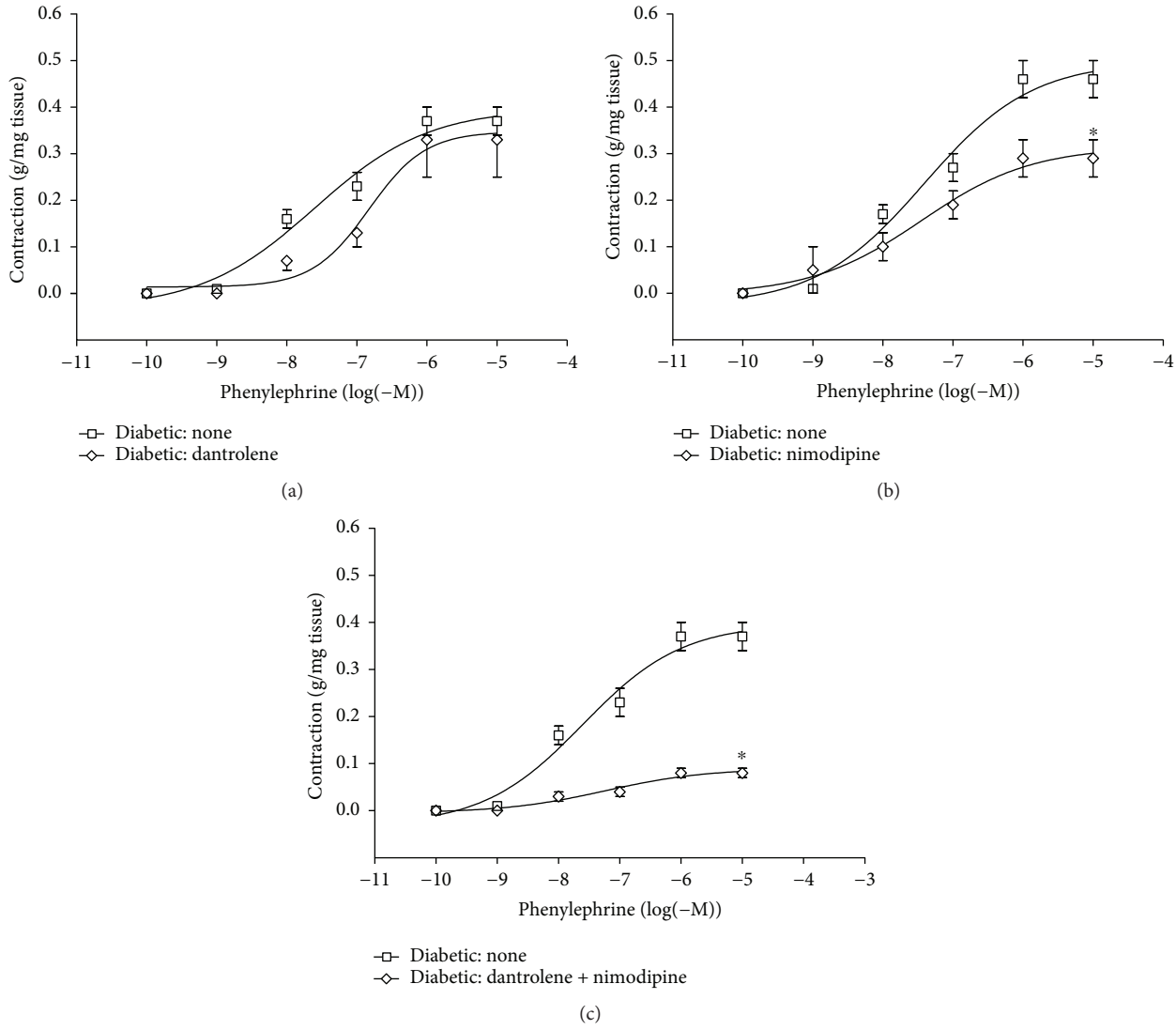


Figure 2: Cumulative concentration-response curves (from 0.1 nM to 10 μM) for the phenylephrine- (PHE-) induced contraction of aortic rings from STZ diabetic rats only before and after a 30 min incubation period with 50 μM dantrolene (a), with 50 nM nimodipine (b), and with both drugs in combination (c). The values shown are the means ± SEM of 6 to 9 animals per group. $^*P < 0.05$, when comparing E_{max} before and after incubation with nimodipine and with dantrolene and nimodipine in combination.

antagonists only block the influx of Ca^{2+} into the cytosol without interfering with the Ca^{2+} released from the SR. Therefore, cerebral infarction, neurological complications, and death rates remain elevated in patients, despite the use of Ca^{2+} antagonists [3].

Dantrolene is a peripherally acting skeletal muscle relaxant approved for the treatment of spasticity, malignant hyperthermia, and neuroleptic malignant syndrome. It depresses excitation-contraction coupling by inhibiting Ca^{2+} release from the SR throughout the blockade of the RyRs present in the VSM [14, 15]. Indeed, mRNAs from RyR1, RyR2, and RyR3 subtypes are expressed in the VSM of the thoracic aorta [15], the mesenteric arteries [15], the large cerebral arteries [16], and the cerebral microcirculation [5], indicating that the VSM from different sized vessels contains the three RyR isoforms. In nondiabetic patients, concomitant administration of the RyR blocker dantrolene with a Ca^{2+} channel antagonist improves vascular and neurologic outcomes because this combination reduces vasoconstriction more than either drug alone [17].

It is unknown, however, whether the combination of a Ca^{2+} channel blocker with dantrolene will also benefit diabetic patients because, in addition to increasing oxidative stress and inducing endothelial dysfunction, diabetes alters SR Ca^{2+} content, SR protein expression, RyR2 mRNA and RyR2 protein levels in a rat heart [18], and L-type Ca^{2+} channel regulation and expression in VSM [4]. Thus, it is crucial to determine if adding dantrolene to standard therapies with Ca^{2+} channel blockers is also beneficial to diabetic patients. In this study, we investigate the effects of dantrolene, either alone or in combination with nimodipine, on phenylephrine- (PHE-) induced contraction, and in the acetylcholine- (ACh-) induced relaxation of aortic rings from streptozocin- (STZ-) induced diabetic rats. In addition, in rabbits following

traumatic spinal cord injury, dantrolene is known to decrease malondialdehyde (MDA) levels, an indicator of lipid peroxidation, and increase reduced glutathione (GSH) [14], while in spinal cord-injured rats, nimodipine provides protection against oxidative stress [19]. We also assess the effects of dantrolene and nimodipine on oxidative stress by evaluating malondialdehyde (MDA) and 4-hydroxyalkenal (4-HAE) levels in vascular homogenates in order to determine if the antioxidant effects of these drugs observed in spinal cord injury [14, 19] occur in the vasculature as well.

2. Drugs

Dantrolene, nimodipine, phenylephrine (PHE), sodium nitroprusside (SNP), and N^G-nitro-L-arginine (L-NAME) were obtained from Sigma Chemical Co. (St. Louis, MO). Kits for lipid peroxidation were obtained from Percipio Biosciences (Burlingame, CA). The concentrations of nimodipine (50 nM) and dantrolene (50 μM) were selected based on comparable studies in different vascular beds from rats [20–22].

3. Materials and Methods

3.1. Experimental Animal Model. Forty male Sprague-Dawley rats (Taconic Biosciences Inc., Germantown, NY), approximately four weeks of age, were divided into the diabetic group and the nondiabetic group, with each group containing 20 animals. To induce diabetes, the rats were fasted overnight and then injected IP with streptozotocin (STZ, 65 mg/kg) dissolved in a 0.1 M citrate buffer (pH 4.5). Nondiabetic animals, which were used as controls, were only injected (IP) with the citrate buffer solution. Hyperglycemia was verified 24 h after the STZ injection with a TRUEtrack blood glucose monitoring system (NIPRO Diagnostics, Fort Lauderdale, FL). Blood glucose levels were monitored once a week in all animals. All the experiments were performed at four weeks following diabetes induction. The diabetic rats never received insulin supplementation. All animals were housed in a temperature-controlled room on a 12 h light/dark cycle. Water and food (Harlan Rodent Diet, 18% protein) were provided ad libitum. All procedures involving the animals were approved by the Institutional Animal Care and Use Committee (protocol number 2590115) and adhered to the *Guide and Care for the Use of Laboratory Animals* published in 2011 by the National Institutes of Health (USA).

3.2. Tissue Preparation for Isometric Tension Studies. To evaluate endothelial-dependent relaxation, we followed the methods of Quidgley et al. [23]. Briefly, rats were anesthetized with a combination of ketamine (50 mg/kg, IP) and xylazine (4 mg/kg, IP). After full anesthesia, aortic rings of approximately 5 mm in length were obtained from the proximal segment of each aorta and used for the contraction and relaxation studies. The connective tissue adjacent to the aortic adventitia was carefully removed, avoiding damages to the smooth muscle and the endothelium. The entire preparation was mounted in a two-hook, 50 ml organ chamber (Radnoti Co., Monrovia, CA) and bathed in Krebs' bicarbonate solution (aerated with a mixture of 95% O_2 and 5% CO_2 at 37°C). The rings were suspended horizontally with a resting tension of 2.3 g and connected to a FT03C Grass transducer. Once the optimal tension was reached, the rings were subjected to a 1 h equilibration. The signal was analyzed with a data acquisition card (National Instruments, Austin, TX; PC-LPM-16/PnP), and changes in isometric tension were recorded with LabView software (National Instruments).

Table 3: Effects of a 30 min incubation period with 50 μM dantrolene (D), 50 nM nimodipine (N), and both drugs in combination (D + N) on EC_{50} and E_{max} values for PHE-induced contraction in control and diabetic rats.

Condition	E_{max} (g/mg tissue)	EC_{50} (nM)
Control none	0.41 ± 0.03	43.2 ± 8.11
Control + D	0.31 ± 0.03	118.72 ± 17.27*
Control + N	0.19 ± 0.03*	40.74 ± 10.20
Control D + N	0.08 ± 0.01*	123.11 ± 34.15*
Diabetic none	0.46 ± 0.04	50.83 ± 7.78
Diabetic + D	0.33 ± 0.08	118.73 ± 16.47**
Diabetic + N	0.29 ± 0.04**	48.71 ± 16.78
Diabetic D + N	0.08 ± 0.01**	98.06 ± 34.35**

Values shown are the means ± SEM of an average of 6 to 9 animals per group.
*$P < 0.05$, when comparing treated and untreated aortic rings of controls.
**$P < 0.05$, when comparing treated and untreated aortic rings of diabetics.

3.3. Measurement of Aortic Ring Contraction and Relaxation. To determine the effect of 50 μM dantrolene, 50 nM nimodipine, and the combination of these drugs on the endothelial-dependent relaxation, aortic rings were precontracted with phenylephrine (PHE, 1.0 μM), following the protocol described previously [23, 24]. When the maximal contraction reached a plateau, cumulative concentration-response curves (from 0.1 nM to 10 μM) for ACh were generated. After the completion of the curves, the rings were washed and stabilized. Additional concentration-response curves were then performed after a 30 min incubation period with the drugs. In each experiment, the relaxation was expressed as a percentage of the relaxation relative to the maximal contraction induced by 1.0 μM of PHE. The maximal relaxation achieved (E_{max}) and the concentrations inducing 50% of maximal relaxation (EC_{50}) for ACh were determined before and after incubation with dantrolene, nimodipine, and these drugs in combination through the mathematical analysis of the concentration-response curves, which were then compared. Sodium nitroprusside (SNP, 1.0 μM) was also used to fully relax aortic rings after the completion of each protocol to assess endothelial-independent relaxation.

To evaluate the role of nitric oxide (NO) in ACh-induced relaxation after incubation with dantrolene, nimodipine, or these drugs in combination, the aortic rings were incubated with 1 mM N^G-nitro-L-arginine (L-NAME), an inhibitor of NOS, concomitantly with the drugs. In these experiments, the rings were precontracted with 1.0 μM of PHE, and a concentration-response curve for ACh was generated before

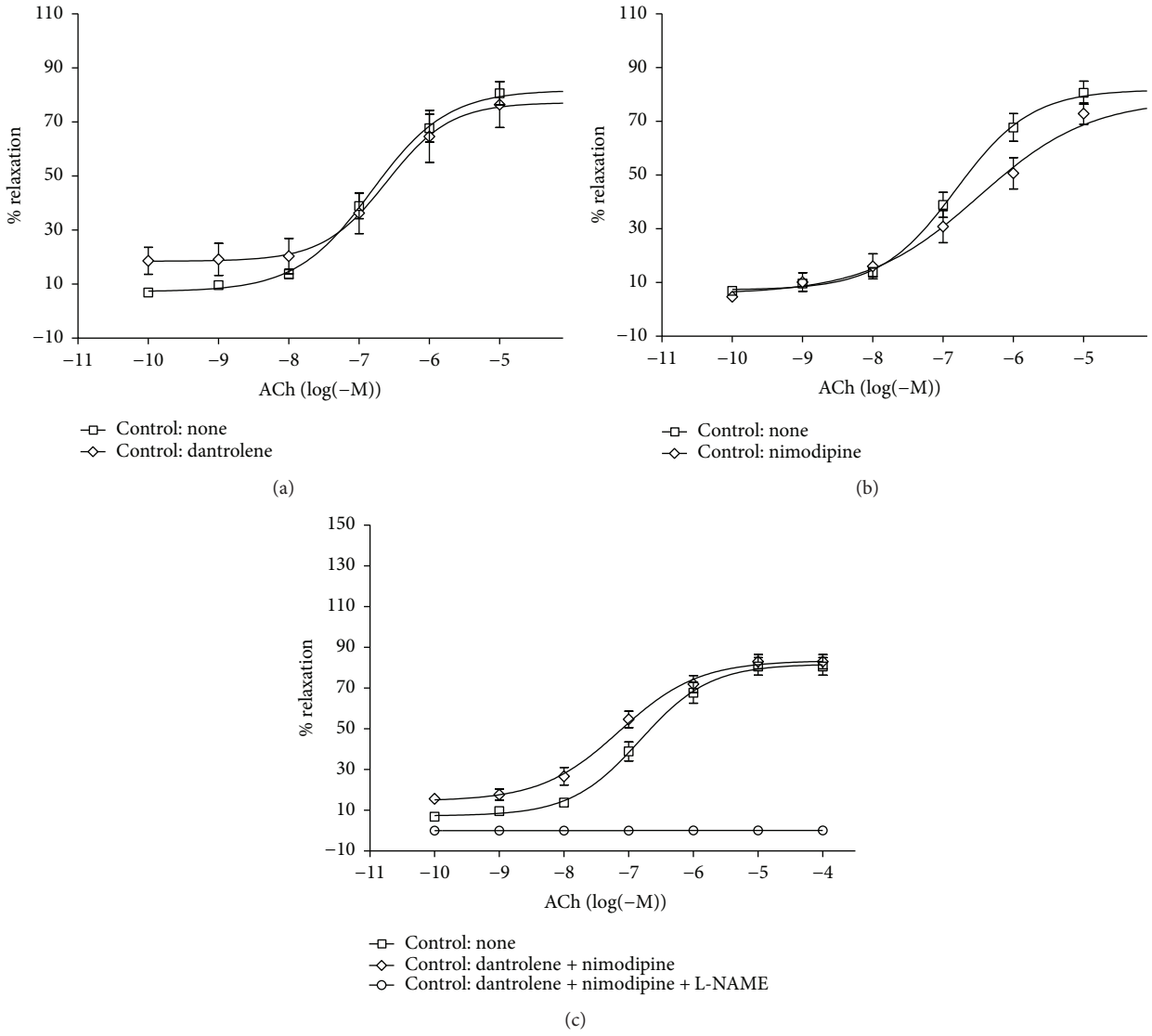


Figure 3: Cumulative concentration-response curves for the acetylcholine- (ACh-) induced relaxation of aortic rings from control rats (CT) only after a 30 min incubation period with 50 μM dantrolene (a), with 50 nM nimodipine (b), and with both drugs in combination (c). Aortic rings were precontracted with 0.1 μM phenylephrine (PHE) before the addition of cumulative concentrations of ACh. The values shown are the means ± SEM of 6 to 9 animals per group. Note that the addition of 1 mM L-NAME to the incubation bath inhibited the ACh-induced relaxation of aortic rings from dantrolene and dantrolene + nimodipine-treated control rats.

and after incubation with L-NAME and each drug, either alone or in combination.

The effects of nimodipine, dantrolene, and its combination on the PHE-induced contraction were tested by analyzing the cumulative concentration-response curves generated by PHE (0.1 nM to 10.0 μM) in aortic rings before and after incubation with the drugs. The maximal contraction (E_{max}) and the concentration inducing 50% of maximal contraction (EC_{50}) for PHE were also determined through the analysis of the concentration-response curves for each individual group.

3.4. Measurement of Malondialdehyde and 4-Hydroxyalkenal Levels. The effects of dantrolene and nimodipine on lipid peroxidation, a marker of oxidative stress, were evaluated following the methods of Quidgley et al. [23], by measuring vascular malondialdehyde (MDA) and 4-hydroxyalkenal (4-HAE) levels before and after a 30 min incubation period with dantrolene (50 μM), nimodipine (50 nM), and these drugs in combination using a commercial kit (Bioxytech LPO-586; Oxis Research, Portland, OR). Briefly, homogenized tissues were centrifuged at 4°C for 10 min, and the supernatant was collected and stored at −80°C. N-Methyl-2-phenylindole and methanesulfonic acid were added to each sample and incubated at 45°C for 60 min. Samples were then centrifuged, and the supernatant was transferred to a cuvette. The absorbance of each sample was measured at 586 nm using a SpectraMax M3 Microplate Reader (Molecular Devices, Silicon Valley, CA). The MDA and 4-HAE concentrations of each sample were

compared to the standard curve generated under the same conditions and were normalized by protein concentration, as determined by Bradford's Assay [25].

4. Statistical Analysis

Results are presented as the mean ± SEM using statistical software (GraphPad Prism 5.03, GraphPad Software Inc., San Diego, CA). Statistical comparisons between groups were performed using Student's *t*-test when comparing only two variables and the analysis of variance (ANOVA) when comparing more than two groups. The Student-Newman-Keuls test for post hoc analysis was used to further evaluate significant ANOVAs. Values were considered statistically significant at a P value less than 0.05.

5. Results

The general characteristics of the experimental animals are shown in Tables 1 and 2. Blood glucose levels in diabetic rats were higher than 400 mg/dl during the study period, ranging from 451.7 ± 41.5 mg/dl at 24 h following STZ administration to 445.4 ± 63.7 mg/dl after four weeks. In control animals, by contrast, glucose concentration remained within the normal range throughout the study, with a mean value of 143.5 ± 6.8 mg/dl. In addition, whereas body weight increased in both diabetic and control rats over the course of this study, it remained significantly lower in the diabetic rats at four weeks following diabetes induction (272.2 ± 24.8 g in diabetics versus 360.6 ± 5.9 g in controls; $n = 20$, $P < 0.05$).

Cumulative concentration-response curves for the PHE-induced contraction of aortic rings are shown in Figure 1 for control rats and in Figure 2 for diabetic rats. A 30 min incubation period with 50 μM dantrolene increased the EC_{50} value for PHE from 43.2 ± 8.11 to 118.72 ± 17.27 nM ($P < 0.05$) in controls (Figure 1(a), Table 3) and from 50.83 ± 7.78 to 118.73 ± 16.47 nM ($P < 0.05$) in diabetics (Figure 2(a), Table 3). Incubation with 50 nM nimodipine reduced E_{max} in controls (Figure 1(b)) by 53% ($P < 0.05$; $n = 9$) and by 37% ($P < 0.05$; $n = 9$) in diabetics (Figure 2(b)). The combination of dantrolene and nimodipine was equally effective in reducing the PHE-induced contraction by about 80% ($P < 0.05$; $n = 9$) in both controls (Figure 1(c)) and diabetics (Figure 2(c)).

Figure 3 illustrates the effect of a 30 min incubation with 50 μM dantrolene (Figure 3(a)), 50 nM nimodipine (Figure 3(b)), and both drugs in combination (Figure 3(c)) on the ACh-induced relaxation in aortic rings from control rats. Endothelial-dependent relaxation and EC_{50} and E_{max} values were not affected by the drugs when used individually or in combination (Table 4). In diabetic rats, by contrast, endothelial function was significantly improved after incubation with dantrolene and with a combination of dantrolene and nimodipine. The E_{max} values for the ACh-induced relaxation increased from 56.46 ± 5.14% before treatment to 95.71 ± 6.15% after dantrolene and to 96.21 ± 7.50% after treatment with the dantrolene-nimodipine combination ($n = 7$, $P < 0.05$). Nevertheless, EC_{50} values remained unchanged (Figures 4(a) and 4(b), Table 4).

Table 4: Effects of a 30 min incubation period with 50 μM dantrolene (D), 50 nM nimodipine (N), and both drugs in combination (D + N) on EC_{50} and E_{max} values for ACh-induced relaxation in control and diabetic rats.

Condition	E_{max} relaxation, %	EC_{50} (μM)
Control none	80.66 ± 4.32	1.548 ± 1.197
Control + D	76.42 ± 8.48	0.337 ± 0.126
Control + N	72.87 ± 3.94	0.466 ± 0.176
Control D + N	87.27 ± 3.51	0.121 ± 0.003
Diabetic none	56.46 ± 5.14	0.490 ± 0.200
Diabetic + D	95.71 ± 6.15**	0.465 ± 0.231
Diabetic + N	57.57 ± 6.30	0.950 ± 0.414
Diabetic D + N	96.21 ± 7.50**	0.115 ± 0.090

Values shown are the means ± SEM of an average of 6 to 9 animals per group.
**$P < 0.05$, when comparing treated and untreated aortic rings of diabetics.

Figure 5 depicts the effects of a 30 min incubation period with 50 μM dantrolene, 50 nM nimodipine, and both drugs in combination on the VSM relaxation elicited by 1.0 μM SNP in aortic rings from control rats (Figure 5(a)) and from diabetic rats (Figure 5(b)). The addition of SNP to aortic rings that were precontracted with 0.1 μM PHE produced similar endothelial-independent relaxation before and after incubation in both diabetics and controls, indicating that neither of these two drugs, either alone or in combination, modified the endothelial-independent relaxation in diabetic or control rats.

Figure 6 shows the effects of acute incubation with dantrolene, nimodipine, and both drugs in combination on MDA and 4-HAE levels in aortic homogenates from diabetic rats. MDA and 4-HAE levels (μmol/g protein) were reduced from 2.72 ± 0.72 before incubation to 1.21 ± 0.13 with dantrolene, to 0.59 ± 0.07 with nimodipine, and to 1.00 ± 0.16 with both drugs in combination ($n = 5$, $P < 0.05$).

6. Discussion

We assessed the effects of dantrolene alone and in combination with nimodipine on both PHE-induced contraction and ACh-induced relaxation in the vasculature of type 1 diabetic rats. We found that the combination of dantrolene and nimodipine has synergistic effects in reducing the PHE-induced contraction in both diabetic and nondiabetic rats, but dantrolene alone or in combination with nimodipine improves ACh-dependent relaxation only in diabetic rats.

To our knowledge, this study is the first to report that dantrolene improves the endothelial-dependent relaxation under hyperglycemic conditions. This improvement in relaxation is fully blocked by L-NAME, suggesting that it is NO-dependent. That dantrolene reduces the lipid peroxidation markers MDA and 4-HAE indicates that the drug may also have antioxidant properties. Similarly, after traumatic spinal cord injury in rabbits, dantrolene treatment results in a significant decrease in MDA levels in cerebrospinal fluid and augments endogenous enzymatic and nonenzymatic antioxidative defenses [14]. Following intracranial hypertension in

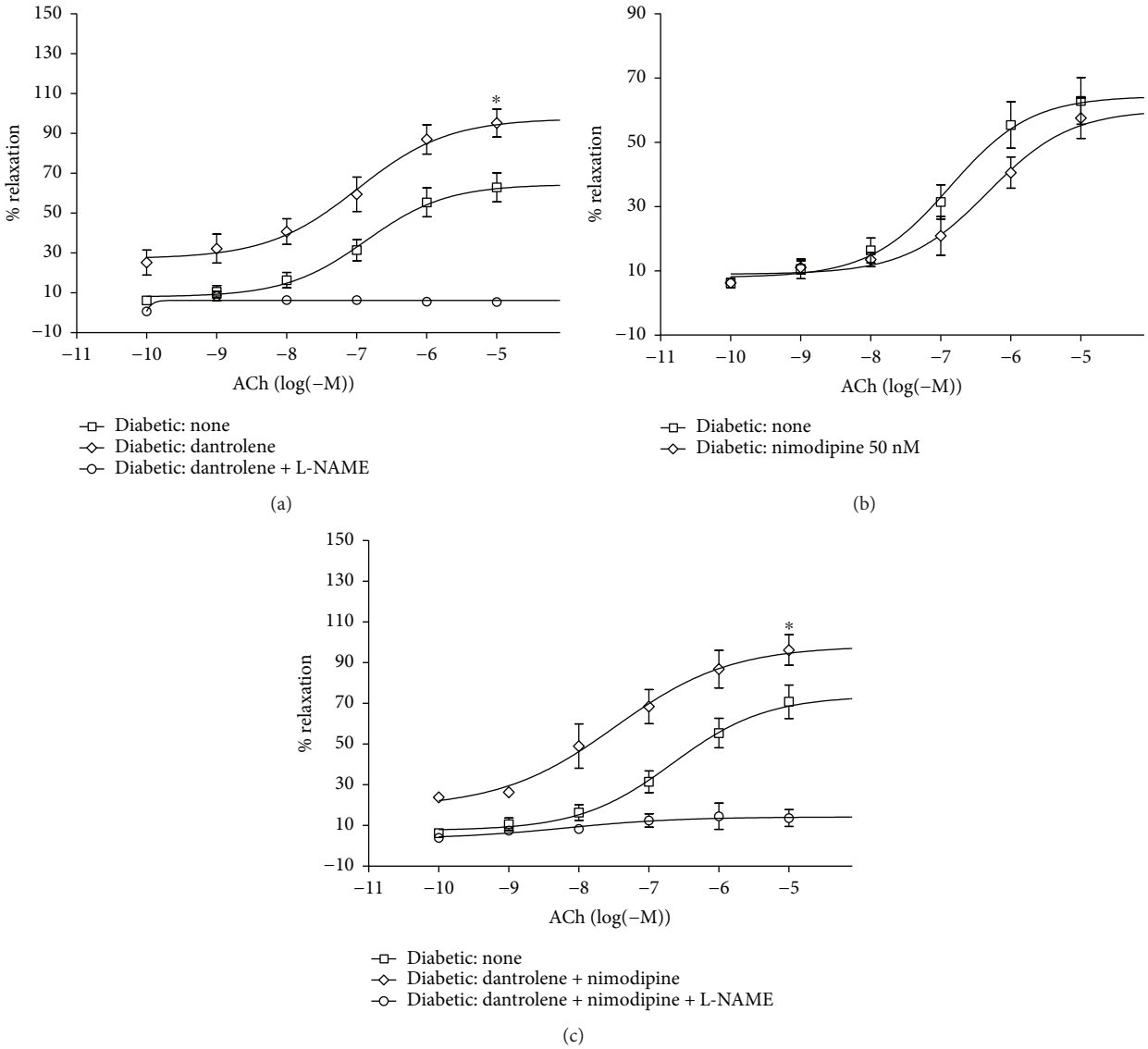


Figure 4: Cumulative concentration-response curves for the acetylcholine- (ACh-) induced relaxation of aortic rings from STZ diabetic rats after a 30 min incubation period with 50 μM dantrolene (a), with 50 nM nimodipine (b), and with both drugs in combination (c). Aortic rings were precontracted with 0.1 μM phenylephrine (PHE) before the addition of cumulative concentrations of ACh. The values shown are the means ± SEM of 6 to 9 animals per group. Note that the addition of 1 mM L-NAME to the incubation bath inhibited the ACh-induced relaxation of aortic rings from dantrolene and dantrolene + nimodipine-treated diabetic rats. $^*P < 0.05$, when comparing E_{max} before and after incubation with dantrolene and with dantrolene and nimodipine in combination.

rats, by contrast, dantrolene alone fails to prevent the myocardial dysfunction resulting from oxidative stress [26].

Oxidative stress reduction by dantrolene may underlie improvements in endothelial-dependent relaxation in diabetic rats. This finding is relevant to the current study because oxidative stress, endothelial dysfunction, and augmented vascular tone are hallmarks of vascular deterioration in both diabetic animals and patients [7–9]. Indeed, following a four-week treatment period with statins, type 1 diabetic rats show an improvement in endothelial-dependent relaxation that is secondary to decreased oxidative stress [12, 23]. This reduced oxidative stress decreases vascular remodeling and improves cardiovascular function [12, 23]. Because preexisting diabetes mellitus is independently and strongly correlated with cerebral vasospasms in diabetic patients despite intensive glycemic control [27], dantrolene may provide a promising therapeutic tool. In addition to working synergistically with Ca^{2+} channel blockers to reduce agonist-induced VSM contraction, it may decrease vascular tone by improving endothelial-dependent relaxation. Despite improved ACh-induced relaxation, neither dantrolene nor its combination with nimodipine modifies SNP-induced relaxation in either diabetics or controls, indicating that these drugs do not alter the cGMP cascade in VSM.

Unlike dantrolene, nimodipine reduces MDA and 4-HAE levels in vascular homogenates, but it fails to improve vascular relaxation in diabetic rats. The latter finding differs from that of other studies with nondiabetic rats, which

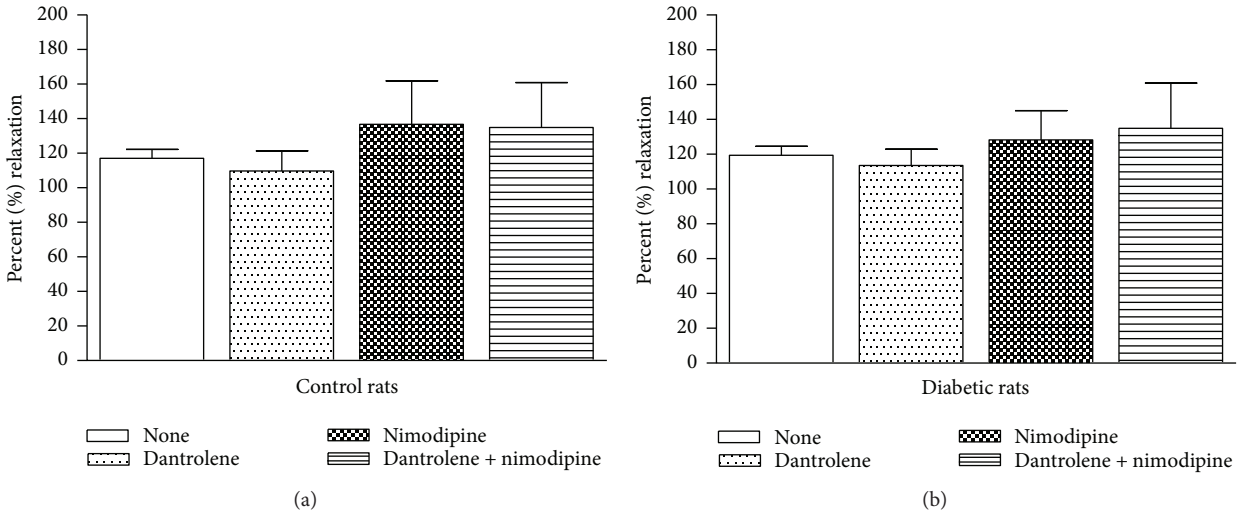


Figure 5: Effects of a 30 min incubation period with 50 μM dantrolene, with 50 nM nimodipine, and with both drugs in combination on the endothelial-independent relaxation of aortic rings from control (a) and STZ diabetic rats (b). Aortic rings were precontracted with 0.1 μM phenylephrine (PHE) before the addition of 1.0 μm sodium nitroprusside (SNP) to directly relax the vascular smooth muscle. The values shown are the means ± SEM of 6 to 9 animals per group. Note that the drugs, either alone or in combination, did not modify endothelial-independent relaxation in control or STZ diabetic rats.

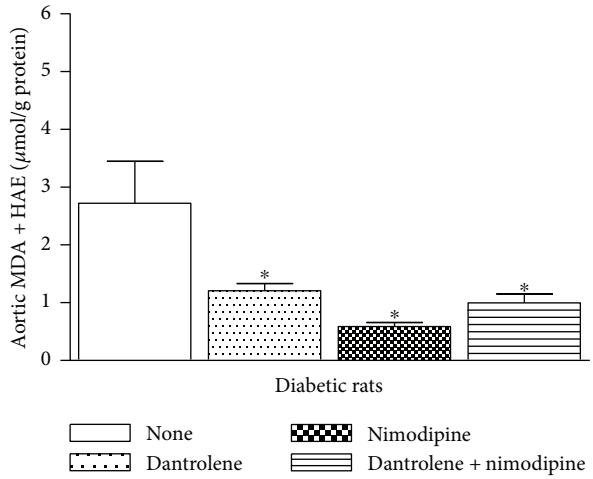


Figure 6: Effects of a 30 min incubation period with dantrolene (50 μM), with nimodipine (50 nM), and with both drugs in combination on MDA + 4-HAE levels in aortic homogenates from STZ diabetic rats. Lipid peroxidation levels were significantly reduced by dantrolene, nimodipine, and both drugs in combination. The values shown are the means ± SEM of 5 animals per group. $^*P < 0.05$, when comparing treated and untreated homogenates.

indicate that, by reducing oxidative stress, nimodipine enhances blood flow in cerebral ischemia [28]. Differences in experimental animal models, nimodipine doses, and treatment duration may partly explain these diverse findings.

At the tested dose, dantrolene alone does not reduce vascular PHE-induced contraction, but it does increase EC_{50} values in both diabetics and controls, making the vasculature less reactive to the alpha1-agonist. The mechanisms underlying this beneficial effect are unknown, although it has been proposed that the Ca^{2+} released from RyRs provides an increased pool of Ca^{2+} for the positive feedback on inositol 1,4,5-trisphosphate receptors [29]. Thus, blockade of RyRs with dantrolene may interrupt this feedback and reduce the response of agonist-induced inositol 1,4,5-trisphosphate receptors. Alterations in the kinetics of the PHE-alpha1 receptor interaction induced by dantrolene may also be responsible for the increased EC_{50} values, but specific binding studies are needed to verify this possibility.

Nimodipine significantly inhibits the PHE-induced contraction in both diabetics and controls, but the extent of the inhibition is less in diabetics (37%) than in controls (53%). The latter finding indicates that under hyperglycemic conditions, VSM regulation changes from that in controls. Similarly, the sensitivity of L-type Ca^{2+} channels to nifedipine is altered in diabetic rats, and the density of these channels is significantly less in VSM from diabetic rats than that from nondiabetic rats [2]. Thus, the modifications in Ca^{2+} homeostasis and vascular function, which result from abnormalities in L-type Ca^{2+} channels that are characteristics of diabetes, may explain, at least in part, the differences found in this study between diabetics and controls in the inhibition of the PHE-induced contraction with nimodipine.

In both diabetics and controls, the combination of dantrolene and nimodipine has synergistic effects that decrease the E_{max} of the PHE-induced contraction by approximately 80%. This synergistic effect has been previously reported in isolated basilar and femoral arteries from nondiabetic rats where the combination of these drugs significantly reduced 5-HT-induced vasoconstriction [22]. We found comparable results for diabetic rats. The mechanisms underlying the synergy between dantrolene and nimodipine in reducing the response of receptors linked to inositol 1,4,5-trisphosphate cascade are unknown. By increasing the EC_{50} value of the PHE-alpha1 receptor-mediated response, however, the combination of dantrolene and nimodipine may contribute to

reducing vascular hyper-reactivity in diabetes, even though the vascular alpha1-mediated response is increased in this condition [30, 31].

7. Limitations of the Study

One limitation of this study is that the effects of dantrolene and nimodipine were only evaluated for rings from a systemic artery (thoracic aorta), rather than for rings from the cerebral vasculature. All three members of the RyR family (RyR1, RyR2, and RyR3), however, are present in the VSM of not only the thoracic aorta [15] but also the mesenteric arteries [15], the large cerebral arteries [16], and the cerebral microcirculation [5]. Therefore, the results of the current study may offer the first step in developing a customized therapy for reducing CVSPs in diabetics. To lend greater validity to our findings, however, future experiments are needed to evaluate the effects of dantrolene on the cerebral circulation, such as measuring blood flow velocities in the middle cerebral artery of diabetic rats.

8. Conclusions

We found that the combination of dantrolene and nimodipine has synergistic effects, which significantly decrease the PHE-induced contraction in both diabetic and control rats. Moreover, this combination reduces lipid peroxidation and improves vascular function in diabetic rats. Further studies are necessary, however, to prove that the response of aortic rings to these two drugs is similar in the cerebral vasculature. If a similar response does indeed occur, the combined use of dantrolene and nimodipine may be effective in reducing CVSPs in diabetics.

Abbreviations

ACh: Acetylcholine
CVSP: Cerebral vasospasms
EC_{50}: Concentrations inducing 50% of maximal relaxation
E_{max}: Maximal relaxation achieved
eNOS: Endothelial nitric oxide synthase
4-HAE: 4-Hydroxyalkenal
iNOS: Inducible nitric oxide synthase
L-NAME: N^G-Nitro-L-arginine
MDA: Malondialdehyde
NO: Nitric oxide
PHE: Phenylephrine
RyR: Ryanodine receptor
SNP: Sodium nitroprusside
SR: Sarcoplasmic reticulum
STZ: Streptozotocin
VSM: Vascular smooth muscle.

Disclosure

Part of this work was presented as an abstract at the 3rd European Stroke Organization Conference, 2017.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

All authors have made substantial contributions to the design, data acquisition, and analysis and interpretation of results. All authors contributed to the drafting of the manuscript and give final approval of the version to be submitted.

Acknowledgments

This work was supported by Grants from the National Institute of Health (MBRS-RISE Grant R25GM061838), the NIMHD-RCMI Program (Grant G12-MD007600), and the Anesthesiology Department of the University of Puerto Rico-School of Medicine.

References

[1] M. D. Hill, "Chapter 12 - Stroke and diabetes mellitus," *Handbook of Clinical Neurology*, vol. 126, pp. 167–174, 2014.

[2] R. Wang, Y. Wu, G. Tang, L. Wu, and S. T. Hanna, "Altered L-type Ca^{2+} channel currents in vascular smooth muscle cells from experimental diabetic rats," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 278, no. 3, pp. H714–H722, 2000.

[3] S. Muehlschlegel, G. Rordorf, and J. Sims, "Effects of a single dose of dantrolene in patients with cerebral vasospasm after subarachnoid hemorrhage: a prospective pilot study," *Stroke*, vol. 42, no. 5, pp. 1301–1306, 2011.

[4] M. F. Navedo, Y. Takeda, M. Nieves-Cintrón, J. D. Molkentin, and L. F. Santana, "Elevated Ca^{2+} sparklet activity during acute hyperglycemia and diabetes in cerebral arterial smooth muscle cells," *American Journal of Physiology-Cell Physiology*, vol. 298, no. 2, pp. C211–C220, 2010.

[5] F. Dabertrand, M. T. Nelson, and J. E. Brayden, "Ryanodine receptors, calcium signaling, and regulation of vascular tone in the cerebral parenchymal microcirculation," *Microcirculation*, vol. 20, no. 4, pp. 307–316, 2013.

[6] S. Muehlschlegel, G. Rordorf, M. Bodock, and J. R. Sims, "Dantrolene mediates vasorelaxation in cerebral vasoconstriction: a case series," *Neurocritical Care*, vol. 10, no. 1, pp. 116–121, 2009.

[7] F. Giacco and M. Brownlee, "Oxidative stress and diabetic complications," *Circulation Research*, vol. 107, no. 9, pp. 1058–1070, 2010.

[8] M. Potenza, S. Gagliardi, C. Nacci, M. Carratu, and M. Montagnani, "Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets," *Current Medicinal Chemistry*, vol. 16, no. 1, pp. 94–112, 2009.

[9] H. A. R. Hadi and J. A. Suwaidi, "Endothelial dysfunction in diabetes mellitus," *Vascular Health and Risk Management*, vol. 16, no. 6, pp. 853–876, 2009.

[10] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *The International Journal of Biochemistry & Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[11] M. J. Crespo, J. Zalacaín, D. C. Dunbar, N. Cruz, and L. Arocho, "Cardiac oxidative stress is elevated at the onset of dilated cardiomyopathy in streptozotocin-diabetic rats," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 13, no. 1, pp. 64–71, 2008.

[12] M. J. Crespo and J. Quidgley, "Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats," *World Journal of Diabetes*, vol. 6, no. 10, pp. 1168–1178, 2015.

[13] K. G. Santos, D. Crispim, L. H. Canani, P. T. Ferrugem, J. L. Gross, and I. Roisenberg, "Relationship of endothelial nitric oxide synthase (eNOS) gene polymorphisms with diabetic retinopathy in Caucasians with type 2 diabetes," *Ophthalmic Genetics*, vol. 33, no. 1, pp. 23–27, 2012.

[14] A. Aslan, M. Cemek, M. E. Buyukokuroglu et al., "Dantrolene can reduce secondary damage after spinal cord injury," *European Spine Journal*, vol. 18, no. 10, pp. 1442–1451, 2009.

[15] C. B. Neylon, S. M. Richards, M. A. Larsen, A. Agrotis, and A. Bobik, "Multiple types of ryanodine receptor/Ca^{2+} release channels are expressed in vascular smooth muscle," *Biochemical and Biophysical Research Communications*, vol. 215, no. 3, pp. 814–821, 1995.

[16] T. Vaithianathan, D. Narayanan, M. T. Asuncion-Chin et al., "Subtype identification and functional characterization of ryanodine receptors in rat cerebral artery myocytes," *American Journal of Physiology-Cell Physiology*, vol. 299, no. 2, pp. C264–C278, 2010.

[17] S. Muehlschlegel, R. Carandang, W. Hall et al., "Dantrolene for cerebral vasospasm after subarachnoid haemorrhage: a randomised double blind placebo-controlled safety trial," *Journal of Neurology, Neurosurgery, & Psychiatry*, vol. 86, no. 9, pp. 1029–1035, 2015.

[18] N. Yaras, M. Ugur, S. Ozdemir et al., "Effects of diabetes on ryanodine receptor Ca release channel (RyR2) and Ca^{2+} homeostasis in rat heart," *Diabetes*, vol. 54, no. 11, pp. 3082–3088, 2005.

[19] Y.-F. Jia, H.-L. Gao, L.-J. Ma, and J. Li, "Effect of nimodipine on rat spinal cord injury," *Genetics and Molecular Research*, vol. 14, no. 1, pp. 1269–1276, 2015.

[20] K. L. Byron and C. W. Taylor, "Spontaneous Ca^{2+} spiking in a vascular smooth muscle cell line is independent of the release of intracellular Ca^{2+} stores," *Journal of Biological Chemistry*, vol. 268, no. 10, pp. 6945–6952, 1993.

[21] X. C. Ru, L. B. Qian, Q. Gao, Y. F. Li, I. C. Bruce, and Q. Xia, "Alcohol induces relaxation of rat thoracic aorta and mesenteric arterial bed," *Alcohol and Alcoholism*, vol. 43, no. 5, pp. 537–543, 2008.

[22] S. Salomone, G. Soydan, M. A. Moskowitz, and J. R. Sims, "Inhibition of cerebral vasoconstriction by dantrolene and nimodipine," *Neurocritical Care*, vol. 10, no. 1, pp. 93–102, 2009.

[23] J. Quidgley, N. Cruz, and M. J. Crespo, "Atorvastatin improves systolic function, but does not prevent the development of dilated cardiomyopathy in streptozotocin-induced diabetic rats," *Therapeutic Advances in Cardiovascular Disease*, vol. 8, no. 4, pp. 133–144, 2014.

[24] M. J. Crespo, S. Moreta, and J. González, "Cardiovascular deterioration in STZ-diabetic rats: possible role of vascular RAS," *Pharmacology*, vol. 68, no. 1, pp. 1–8, 2003.

[25] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[26] S. R. R. Hall, L. Wang, B. Milne, and M. Hong, "Mannitol but not dantrolene prevents myocardial dysfunction following intra-cranial hypertension in rats," *American Journal of Transplantation*, vol. 5, no. 12, pp. 2862–2869, 2005.

[27] T. Dumont, A. Rughani, J. Silver, and B. I. Tranmer, "Diabetes mellitus increases risk of vasospasm following aneurysmal subarachnoid hemorrhage independent of glycemic control," *Neurocritical Care*, vol. 11, no. 2, pp. 183–189, 2009.

[28] S. U. Yanpallewar, D. Hota, S. Rai, M. Kumar, and S. B. Acharya, "Nimodipine attenuates biochemical, behavioral and histopathological alterations induced by acute transient and long-term bilateral common carotid occlusion in rats," *Pharmacological Research*, vol. 49, no. 2, pp. 143–150, 2004.

[29] N. Pierobon, D. C. Renard-Rooney, L. D. Gaspers, and A. P. Thomas, "Ryanodine receptors in liver," *Journal of Biological Chemistry*, vol. 281, no. 45, pp. 34086–34095, 2006.

[30] M. J. Crespo and D. C. Dunbar, "Enalapril improves vascular and cardiac function in streptozotocin-diabetic rats," *Cellular and Molecular Biology*, vol. 49, no. 8, pp. 1311–1318, 2003.

[31] S. Setty, W. Sun, R. Martinez, H. F. Downey, and J. D. Tune, "α-Adrenoceptor-mediated coronary vasoconstriction is augmented during exercise in experimental diabetes mellitus," *Journal of Applied Physiology*, vol. 97, no. 1, pp. 431–438, 2004.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 7806980, 11 pages
https://doi.org/10.1155/2018/7806980

Research Article

Risk of Microvascular Complications and Macrovascular Risk Factors in Early-Onset Type 1 Diabetes after at Least 10 Years Duration: An Analysis of Three Population-Based Cross-Sectional Surveys in Germany between 2009 and 2016

Thaddäus Tönnies,[1,2] **Anna Stahl-Pehe**,[1,2] **Christina Baechle**,[1,2] **Katty Castillo**,[1,2] **Oliver Kuss**,[1,2] **Rhuphine Yossa**,[1,2] **Lena M. E. Lindner**,[1,2] **Reinhard W. Holl**,[2,3] **and Joachim Rosenbauer**[1,2]

[1]*Institute for Biometrics and Epidemiology, German Diabetes Centre (DDZ), Leibniz Centre for Diabetes Research, Heinrich Heine University, Auf'm Hennekamp 65, 40225 Duesseldorf, Germany*
[2]*German Centre for Diabetes Research (DZD), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany*
[3]*Institute of Epidemiology and Medical Biometry, University of Ulm, Albert-Einstein-Allee 41, 89069 Ulm, Germany*

Correspondence should be addressed to Thaddäus Tönnies; thaddaeus.toennies@ddz.uni-duesseldorf.de

Received 15 November 2017; Accepted 15 February 2018; Published 1 April 2018

Academic Editor: Nikolaos Papanas

Aims. To estimate the risk of microvascular complications and macrovascular risk factors among persons with early-onset (diagnosed at ages 0 to <5 years) and long-duration type 1 diabetes and determine temporal trends and associations with potential predictors. *Methods*. We conducted three population-based cross-sectional surveys in Germany ($N = 1789$) to obtain information on exposures and five outcomes (retinopathy, nephropathy, dyslipidemia, hypertension, and a composite endpoint combining all four outcomes). For each outcome, log-binomial spline regression was applied to estimate the risk and dose-response relationship with diabetes duration and exposures. *Results*. The risk for microvascular complications increased after 14 years since diabetes diagnosis whereas dyslipidemia and hypertension were already prevalent at 10 years. The 15-year risk (95% confidence interval) of the composite endpoint for female and male patients was 22.9% (18.8%–27.9%) and 19.2% (15.5%–23.8%), respectively. Temporal trends suggested a decreasing risk between 2009 and 2016. Glycemic control, lifestyle-related factors, and SES, but not health care-related factors, were associated with the risk of the composite endpoint. *Conclusions*. In early-onset type 1 diabetes, there exists a considerable risk of complications and comorbidities already in young ages. Future research should focus on prevention of diabetic complications in young patients and clarification of pathways of the associations found.

1. Introduction

During the past decades, many advances in routine therapy of type 1 diabetes have been achieved, for example, use of insulin pumps, glucose sensors, insulin analogues, or intensified diabetes education and psychosocial support [1]. Evidence suggests that the risk decreased for some, but not all, diabetes-related complications [2, 3]. For example, microvascular complications such as diabetic retinopathy (DR) and diabetic nephropathy (DN) still play an important role in the clinical course of type 1 diabetes. DR with pathologic changes of retinal vessels is the most frequent microvascular complication and may lead to blindness in advanced stages [4]. According to Kaplan–Meier analysis, the cumulative proportion of any DR after 40 years of diabetes duration was estimated at 84% [5]. DN with glomerular vascular alterations is a major cause of end-stage renal disease requiring dialysis or kidney transplantation [4, 6, 7]. DN is

characterized by progressive stages of proteinuria with microalbuminuria as the mildest form [4]. The crude risk of micro- and macroalbuminuria or end-stage renal disease is estimated with almost 25% and 9%, respectively, after 40 years of diabetes duration [8].

Besides microvascular complications, type 1 diabetes is still associated with an increased risk of cardiovascular disease (CVD) and associated mortality [9]. Accordingly, the prevalence of CVD risk factors is still high among patients with type 1 diabetes. For instance, Schwab et al. [10] found that 69% of persons with type 1 diabetes aged 0 to 26 years had at least one risk factor for CVD. Further, the number of risk factors increased with age.

Since the DCCT trial, the central aim of near-normal glycemic control is well established to avoid diabetes-related late complications and comorbidity [11]. Besides that, social, lifestyle-related, and health care-related factors have been associated with the risk of complications and/or CVD risk factors [12]. In turn, glycemic control has been related to psychosocial and family background in children and adolescents [12, 13].

In this study, we focus on the risk of diabetic microvascular complications and macrovascular risk factors after the onset of type 1 diabetes in preschool age and at least 10 years of diabetes duration. This patient group needs special focus since the incidence of type 1 diabetes in this age group has been predicted to further increase in many European regions [14]. Due to the early onset of type 1 diabetes, the increased risk of micro- and macrovascular diseases may occur early in life imposing a potentially large number of life years lost and years lived with disability. Additionally, the challenges of puberty may hamper self-management of type 1 diabetes, which may affect patterns of risk factors for complications. Therefore, this analysis aimed to (i) estimate the risk of microvascular complications (diabetic retinopathy, nephropathy) and macrovascular risk factors (hypertension, dyslipidemia) among persons with early-onset type 1 diabetes, (ii) determine temporal trends of these risks, and (iii) quantify the association between the risk and health care-related factors, socioeconomic status (SES), glycemic control, and lifestyle-related factors.

2. Research Design and Methods

2.1. Study Design and Data Source. We used three population-based cross-sectional baseline surveys (2009/2010, 2012/2013, and 2015/2016) of the German cohort study "Clinical Course of Type 1 Diabetes in Children, Adolescents and Young Adults with Disease Onset in Preschool Age" (type 1 diabetes study). Potential study participants with type 1 diabetes onset prior to the age of 5 years and with at least ten years diabetes duration were identified from the nationwide early-onset type 1 diabetes registry at the German Diabetes Center, Düsseldorf (Deutsches Diabetes-Zentrum, DDZ). The completeness of the registry is estimated to be 97% [15]. Standardized self-administered age-adapted questionnaires were sent to eligible type 1 diabetes patients via treating facilities having formerly reported cases to the type 1 diabetes registry. In case of participants being under 18 years of age, parents also received a questionnaire. Nonresponders were asked to fill out a short questionnaire. Further information on the type 1 diabetes study has previously been reported by Stahl et al. [16]. The studies were fully approved by the ethics committee of Düsseldorf University (reference number 3254).

2.2. Variables

2.2.1. Outcome Variables. Outcomes investigated were DR, DN, hypertension, and dyslipidemia. An outcome was considered present if the participant or the participant's parents reported that the respective outcome had ever been diagnosed by a physician. If the outcome was reported to have never been diagnosed, the outcome was considered nonpresent. To increase the statistical power, we also considered a composite endpoint evaluated as present if the participant or the participant's parents reported that at least one of the four outcomes had ever been diagnosed by a physician. The composite endpoint was evaluated as nonpresent if none of the four outcomes had ever been diagnosed by a physician.

2.2.2. Exposure Variables. Besides time trends, we investigated SES, family structure, lifestyle-related variables (body mass index (BMI), physical activity (PA)), glycemic control (HbA1c, self-monitoring of blood glucose (SMBG), and number of omitted insulin injections (OII)), and health care-related variables (continuous subcutaneous insulin injection (CSII), participation in a disease management program (DMP), and use of diabetes health card (DHC)) as exposure variables.

Time trends were investigated by comparing the prevalence of the outcomes in the three surveys (2009/2010, 2012/2013, and 2015/2016). Diabetes duration was defined as the time between type 1 diabetes diagnosis and the completion of the questionnaire and evaluated as a continuous variable. Due to the small range of onset age (0–<5 years) in our cohort, age is highly correlated with diabetes duration. Therefore, we only included diabetes duration in the analyses assuming that diabetes duration probably has a greater impact on the risk of complications than age.

We measured SES on the household level using the Winkler index, which has also been used in the German child and adolescent health-monitoring study [17]. The index combines scales for income, education, and occupation into a continuous score ranging from 3 (lowest SES) to 21 (highest SES). Family structure was defined as a dichotomous variable and distinguished between participants living with both biological parents versus all other constellations (e.g., living alone/in own apartment, with foster parents, and in a children's home).

BMI was calculated as body weight in kilograms divided by squared height in meters (kg/m^2). To account for the variability of the BMI in young ages, we calculated BMI standard deviation scores (BMI-SDS). BMI-SDS was derived based on reference data from the German Working Group Adiposity using the Lambda-Mu-Sigma method [18, 19]. In the analyses, BMI-SDS was included as a continuous variable. PA was defined according to the question "How often are you

physically active in your leisure time such that you really get to sweat or get out of breath?" We distinguished the four ordinal categories never, 1-2 times/month, 1-2 times/week, and more than twice a week.

We used three measures to assess glycemic control and self-management of glycemic control. First, the self-reported and most recently measured glycated hemoglobin (HbA1c) value in percent of total hemoglobin was evaluated as a continuous variable. In cases of different HbA1c values being reported by participants and parents, we calculated the mean of both values. SMBG referred to self-reported average daily frequency SMBG during the last three months. In the analyses, SMBG was included with four categories (0–2, 3–5, 6–8, and >8 measurements/day). The frequency of OII during the last week was used as an indicator for treatment adherence. OII was based on the self-reported frequency of insufficient or omitted insulin injections at an occasion of carbohydrate consumption during the last week.

Differences in health care were measured by three indicators. First, we distinguished participants with regard to their insulin therapy regimen using the three categories: 1–3 injections/day, at least 4 injections/day, and CSII. Second, we distinguished whether or not participants took part in a DMP, as structured model of diabetes care. In Germany, DMPs are provided by health care providers in cooperation with health insurances [20]. Third, we distinguished whether or not participants used the DHC. The DHC aims to support the monitoring of critical parameters regarding process and outcome quality in order to avoid late sequelae of diabetes. German national guidelines recommend the DHC as part of structured educational programs for persons with type 1 diabetes [21].

2.3. Statistical Analysis. Participants with missing values for all outcome variables were excluded from all analyses. For descriptive analyses, we calculated absolute frequencies and percentages for discrete variables or means and standard deviations for continuous variables for participants without complications versus with at least one complication, respectively. Joint analyses of different exposure variables included all participants with information on the respective exposure variables (complete case analysis).

For each outcome variable, the crude overall risk was estimated as the percentage of patients with the outcome. Furthermore, we conducted log-binomial regression analyses with sex and diabetes duration as independent variables for all outcome variables [22]. In addition, we conducted univariable log-binomial regression analysis (Model 1) and multivariable log-binomial regression analysis adjusting for sex and diabetes duration (Model 2) for each exposure and the composite endpoint as the dependent variable. Continuous independent variables were modelled with natural cubic splines with three equally spaced knots in order to allow nonlinear associations [23].

To illustrate dose-response relationships, we plotted the model-based predicted risk against continuous exposures for female and male patients. Using the sex-adjusted model for diabetes duration, we estimated the risk with 95% confidence intervals (CI) of the composite endpoint after a 15-year diabetes duration for males and females separately. For categorical exposures, results are presented as relative risks (RRs) and 95% CI for each category. For continuous exposures, model-based RRs are reported for the mean and the midpoint of the upper quartile, with the midpoint of the lowest quartile as reference.

3. Results

In total, 4413 ($survey_{2009/10}$: 2231; $survey_{2012/13}$: 1009; $survey_{2015/16}$: 1173) eligible persons received the questionnaires. 1875 (42%) ($survey_{2009/10}$: 839 [38%]; $survey_{2012/13}$: 452 [45%]; $survey_{2015/16}$: 584 [50%]) of these took part in the survey. Information on the composite endpoint was available for 1789 (95%) ($survey_{2009/10}$: 794 [95%]; $survey_{2012/13}$: 434 [96%]; $survey_{2015/16}$: 561 [96%]) patients. These latter patients were included in the analyses. Table 1 shows characteristics of patients with and without complications/comorbidities. The mean diabetes duration and age were 12.4 years (range: 9.9–17.7 years) and 15.4 years (range: 11.3–21.9 years), respectively.

The crude overall risks for DR, DN, hypertension, dyslipidemia, and the composite endpoint were 1.4% (95% CI: 0.8%–1.9%), 2.0% (95% CI: 1.3%–2.6%), 5.4% (95% CI: 4.3%–6.4%), 7.8% (95% CI: 6.6%–9.1%), and 14.1% (95% CI: 12.5%–15.7%), respectively. Figure 1 illustrates the risks for the single outcomes dependent on diabetes duration estimated with spline regression. Except for hypertension, girls/women are estimated to have a higher risk than boys/men. The risk of DR and DN is close to zero in patients with a diabetes duration up to 14 years in cross-sectional analysis. Thereafter, the risk increases, particularly for DR. In contrast, the risks of hypertension and dyslipidemia already show an upward trend from year ten after diabetes duration onwards. The slope for dyslipidemia risk increases slightly with diabetes duration and shows the highest risks of all outcomes considered. The slope for hypertension shows a rather linear trend until year 14 since diagnosis and flattens thereafter.

Log-binomial regression for the composite endpoint with diabetes duration and sex as independent variables estimated the 15-year risk after diabetes diagnosis for female patients at 22.9% (95% CI: 18.8%–27.9%) and male patients at 19.2% (95% CI: 15.5%–23.8%). Associations between the risk and exposure variables are shown as RRs (Table 2) and dose-response relationships (Figure 2). We assessed a time trend by comparing risks between surveys. After adjustment for sex and diabetes duration, the risk in 2012/13 and 2015/16 was 22% and 25% lower, respectively, than that in 2009/2010. Figure 2(a) suggests a slightly curved relationship between diabetes duration and the risk of at least one complication. Correspondingly, diabetes duration was associated with an increased risk independent of sex (Table 2).

Not living with the biological parents showed a tendency for an increased risk. After adjustment for diabetes duration and sex, the risk for the group with the highest SES (4th quartile) was reduced compared to the 1st quartile (Table 2). Figure 2(d) indicates a continuously decreasing risk with increasing SES in an almost linear fashion.

Table 1: Characteristics of the study population with type 1 diabetes onset in preschool age and diabetes duration of at least 10 years.

Variable (*n* missing)	Total cohort	No complication*	At least one complication†
N	1.789	1.537	252
Survey wave (0)			
2009/10	794 (44.4)	649 (42.2)	145 (57.5)
2012/13	434 (26.3)	386 (25.1)	48 (19.1)
2015/16	561 (31.4)	502 (32.7)	59 (23.4)
Female sex (0)	874 (48.9)	739 (48.1)	135 (53.6)
Age in years (0)	15.4 ± 2.0	15.2 ± 2.0	16.2 ± 2.2
Age at onset (0)	3.0 ± 1.2	2.9 ± 1.2	3.2 ± 1.1
Diabetes duration in years (0)	12.4 ± 1.7	12.3 ± 1.6	13.0 ± 2.0
Hypertension (9)	96 (5.4)	—	96 (39.51)
Dyslipidemia (17)	139 (7.8)	—	139 (59.15)
Retinopathy (16)	24 (1.4)	—	24 (10.17)
Nephropathy (14)	35 (2.0)	—	35 (14.71)
Socioeconomic status index‡ (28)	13.4 ± 4.4	13.5 ± 4.4	12.6 ± 4.4
Living with… (6)			
Both biological parents	1.382 (77.5)	1.198 (78.2)	184 (73.3)
Else	401 (22.5)	334 (21.8)	67 (26.7)
BMI-SDS (48)	0.30 ± 0.90	0.26 ± 0.89	0.54 ± 0.89
Freq. of vigorous physical activity (24)			
Never	113 (6.4)	86 (5.7)	27 (10.8)
1-2 times/month	155 (8.8)	126 (8.3)	29 (11.7)
1-2 times/week	717 (40.6)	609 (40.2)	108 (43.4)
More than 1-2 times/week	780 (44.2)	695 (45.8)	85 (34.1)
HbA1c in % (78)	8.2 ± 1.3	8.2 ± 1.3	8.6 ± 1.7
HbA1c in mmol/mol (78)	66 ± 15	66 ± 14	70 ± 18
Freq. of SMBG (35)			
0–2/day	81 (4.6)	65 (4.3)	16 (6.5)
3–5/day	853 (48.6)	718 (47.7)	135 (54.4)
6–8/day	676 (38.5)	602 (40.0)	74 (29.8)
>8/day	144 (8.2)	121 (8.0)	23 (9.3)
Freq. of omitted insulin injections (40)			
Never	520 (29.7)	450 (30.0)	70 (28.2)
1-2 times/week	789 (45.1)	675 (45.0)	114 (46.0)
3–5 times/week	309 (17.7)	268 (17.9)	41 (16.5)
(almost) 1 time/day	103 (5.9)	87 (5.8)	16 (6.5)
More than 1 time/day	28 (1.6)	21 (1.4)	7 (2.8)
Insulin therapy (18)			
1–3 injections/day	111 (6.3)	91 (6.0)	20 (8.0)
≥4 injections/day	647 (36.5)	565 (37.2)	82 (32.7)
CSII	1.013 (57.2)	864 (56.8)	149 (59.4)
Participation in DMP (19)			
No	767 (43.3)	666 (43.8)	101 (40.4)
Yes	686 (38.8)	583 (38.4)	103 (41.2)
Not known	317 (17.9)	271 (17.8)	46 (18.4)

Table 1: Continued.

Variable (*n* missing)	Total cohort	No complication*	At least one complication†
Use of diabetes health card (17)			
No	911 (51.4)	787 (51.7)	124 (49.8)
Yes	777 (43.9)	660 (43.3)	117(47.0)
Not known	84 (4.7)	76 (5.0)	8 (3.2)

Data are *N* (%) or mean ± SD. Abbreviations: BMI-SDS: body mass index standard deviation score; SMBG: self-monitoring of blood glucose; CSII: continuous subcutaneous insulin injection; DMP: disease management program; *persons who reported to have never been diagnosed with hypertension, dyslipidemia, retinopathy, or nephropathy; †persons who reported to have ever been diagnosed with at least one of hypertension, dyslipidemia, retinopathy, or nephropathy; ‡range 3–21—higher values indicate higher socioeconomic status.

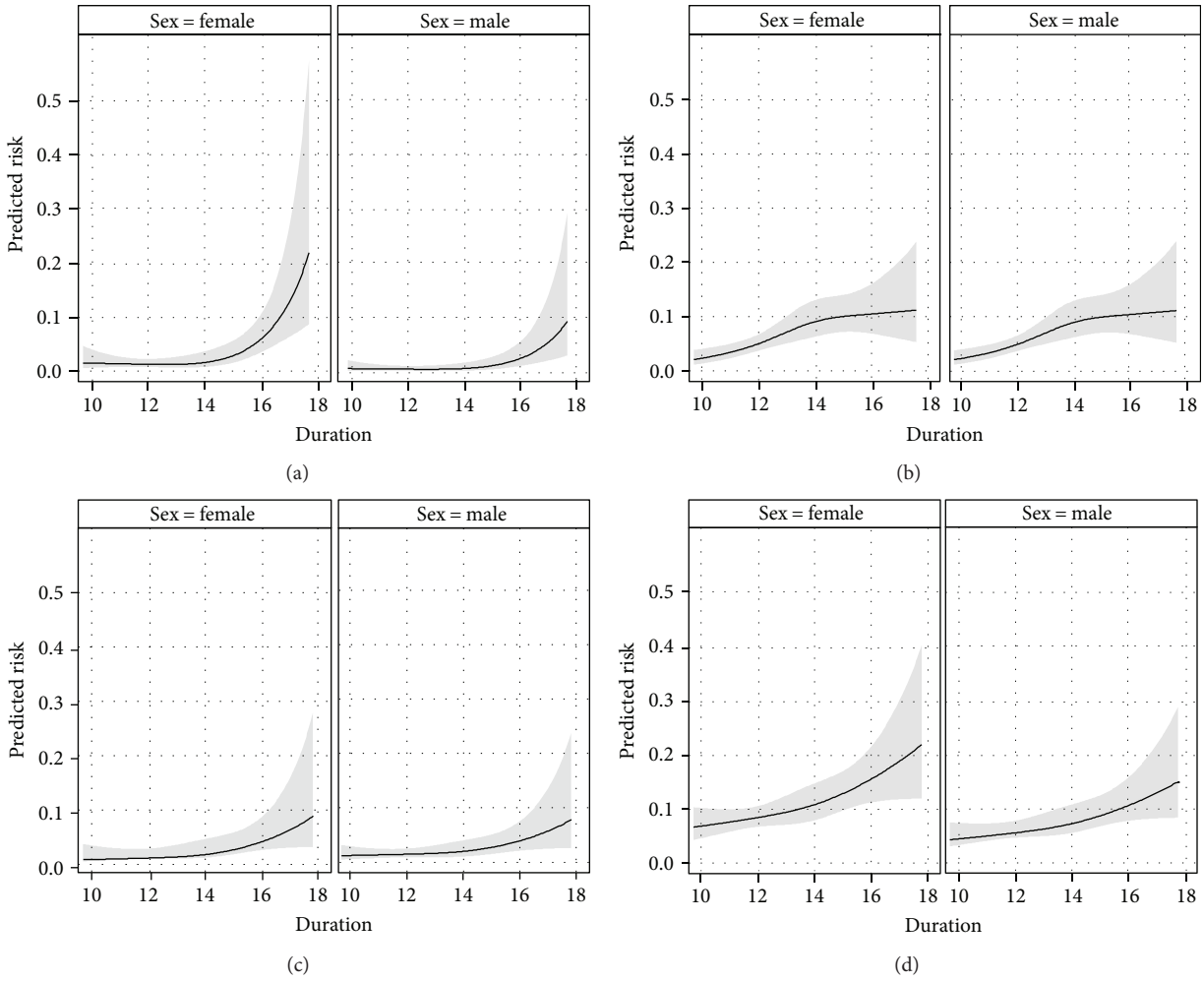


Figure 1: Risk of diabetes-related complications in relation to diabetes duration. Risk of retinopathy ((a) $N = 1773$), hypertension ((b) $N = 1780$), nephropathy ((c) $N = 1775$), and dyslipidemia ((d) $N = 1772$) with 95% confidence bands (shaded areas). The estimated risk was derived from log-binomial regression analyses with the respective outcome as the dependent variable and diabetes duration and sex as independent variables. Diabetes duration was modelled as a natural cubic spline with three equally spaced knots. Different *N* are due to missing values in the outcome variables.

BMI and PA were both associated with an increased risk (Table 2). Having a BMI in the 4th quartile versus the 1st quartile was associated with more than doubled risk. Furthermore, Figure 2(c) indicates an almost linear relationship between BMI and the risk of the composite endpoint.

A similar association was also seen for the 4th quartile of HbA1c compared to the 1st quartile (Table 2). However, this dose-response relationship (Figure 2(b)) is characterized by an exponential curve with a strongly increasing risk in higher HbA1c ranges. The RRs for SMBG and OII show no clear trends. Compared to more than 8 SMBG per day, the three categories with fewer SMBG were associated with a decreased risk in model 2. Between these lower categories, the risk decreased with increasing frequency of SMBG. Compared

Table 2: Relative risks (RRs) with 95% confidence intervals (95% CI) for the risk of have ever been diagnosed with hypertension, dyslipidemia, retinopathy or nephropathy.

Exposure*	RR from model 1 (95% CI)	RR from model 2 (95% CI)
Sex ($N = 1.789$)		
Male	1.00	1.00
Female	1.21 (0.96–1.52)	1.19 (0.95–1.49)
Survey wave ($N = 1.789$)		
2009/10	1.00	1.00
2012/13	0.61 (0.45–0.82)	0.78 (0.56–1.09)
2015/16	0.58 (0.43–0.76)	0.75 (0.54–1.03)
Diabetes duration† ($N = 1.789$)		
10.5 years	1.00	1.00
12.4 years	1.34 (1.04–1.73)	1.34 (1.03–1.72)
15.4 years	2.32 (1.67–3.23)	2.31 (1.66–3.21)
Socioeconomic status index†‡ ($N = 1.761$)		
6.5	1.00	1.00
13.0	0.67 (0.48–0.92)	0.75 (0.55–1.04)
19.0	0.57 (0.41–0.79)	0.67 (0.48–0.92)
Living with… ($N = 1.783$)		
Biological parents	1.00	1.00
Else	1.25 (0.97–1.62)	1.16 (0.90–1.50)
BMI-SDS† ($N = 1.741$)		
−1.5	1.00	1.00
0.3	1.75 (1.07–2.88)	1.72 (1.05–2.82)
2.0	2.89 (1.77–4.72)	2.64 (1.61–4.33)
Freq. of physical activity ($N = 1.765$)		
Never	2.19 (1.49–3.22)	1.76 (1.19–2.61)
1-2 times/month	1.72 (1.17–2.52)	1.45 (0.99–2.14)
1-2 times/week	1.38 (1.06–1.80)	1.30 (0.99–1.70)
More than 1-2 times/week	1.00	1.00
HbA1c† ($N = 1.711$)		
6.3% (45 mmol/mol)	1.00	1.00
8.2% (66 mmol/mol)	1.28 (1.01–1.63)	1.23 (0.97–1.57)
12.1% (109 mmol/mol)	2.67 (1.78–4.01)	2.52 (1.70–3.72)
Freq. of SMBG ($N = 1.706$)		
0–2/day	1.24 (0.69–2.20)	0.88 (0.49–1.59)
3–5/day	0.99 (0.66–1.49)	0.80 (0.53–1.21)
6–8/day	0.69 (0.45–1.06)	0.64 (0.41–0.99)
>8/day	1.00	1.00
Freq. of omitted insulin injections ($N = 1.749$)		
Never	1.00	1.00
1-2 times/week	1.07 (0.81–1.41)	1.07 (0.81–1.40)
3–5 times/week	0.99 (0.69–1.41)	0.98 (0.68–1.39)
(almost) 1 time/day	1.15 (0.70–1.90)	1.08 (0.66–1.77)
More than 1 time/day	1.86 (0.94–3.66)	1.91 (1.03–3.53)
Insulin therapy ($N = 1.771$)		
1–3 injections/day	1.23 (0.80–1.87)	1.15 (0.76–1.74)
4+ injections/day	0.86 (0.67–1.11)	0.80 (0.62–1.03)
CSII	1.00	1.00

Table 2: Continued.

Exposure*	RR from model 1 (95% CI)	RR from model 2 (95% CI)
Participation in DMP ($N = 1.770$)		
Yes	1.00	1.00
No	0.88 (0.68–1.13)	0.91 (0.71–1.17)
Do not know	0.97 (0.70–1.33)	0.95 (0.69–1.30)
Use of diabetes health card ($N = 1.772$)		
Yes	1.00	1.00
No	0.90 (0.72–1.14)	0.92 (0.73–1.15)
Do not know	0.63 (0.32–1.25)	0.55 (0.28–1.08)

Unadjusted RRs were derived from separate log-binomial regression with the composite outcome as the dependent binary variable and the respective exposure variables as the independent variable (model 1). Model 2 adjusted for sex and diabetes duration. Abbreviations: BMI-SDS: body mass index standard deviation scores; SMBG: self-monitoring of blood glucose; CSII: continuous subcutaneous insulin injection; DMP: disease management program; *different N due to missing values in exposure variables; †modelled as a natural cubic spline with three equally spaced knots—estimates are model-based RR for the mean and midpoint of the fourth quartile versus the midpoint of the first quartile; ‡range 3–21—higher values indicate higher socioeconomic position.

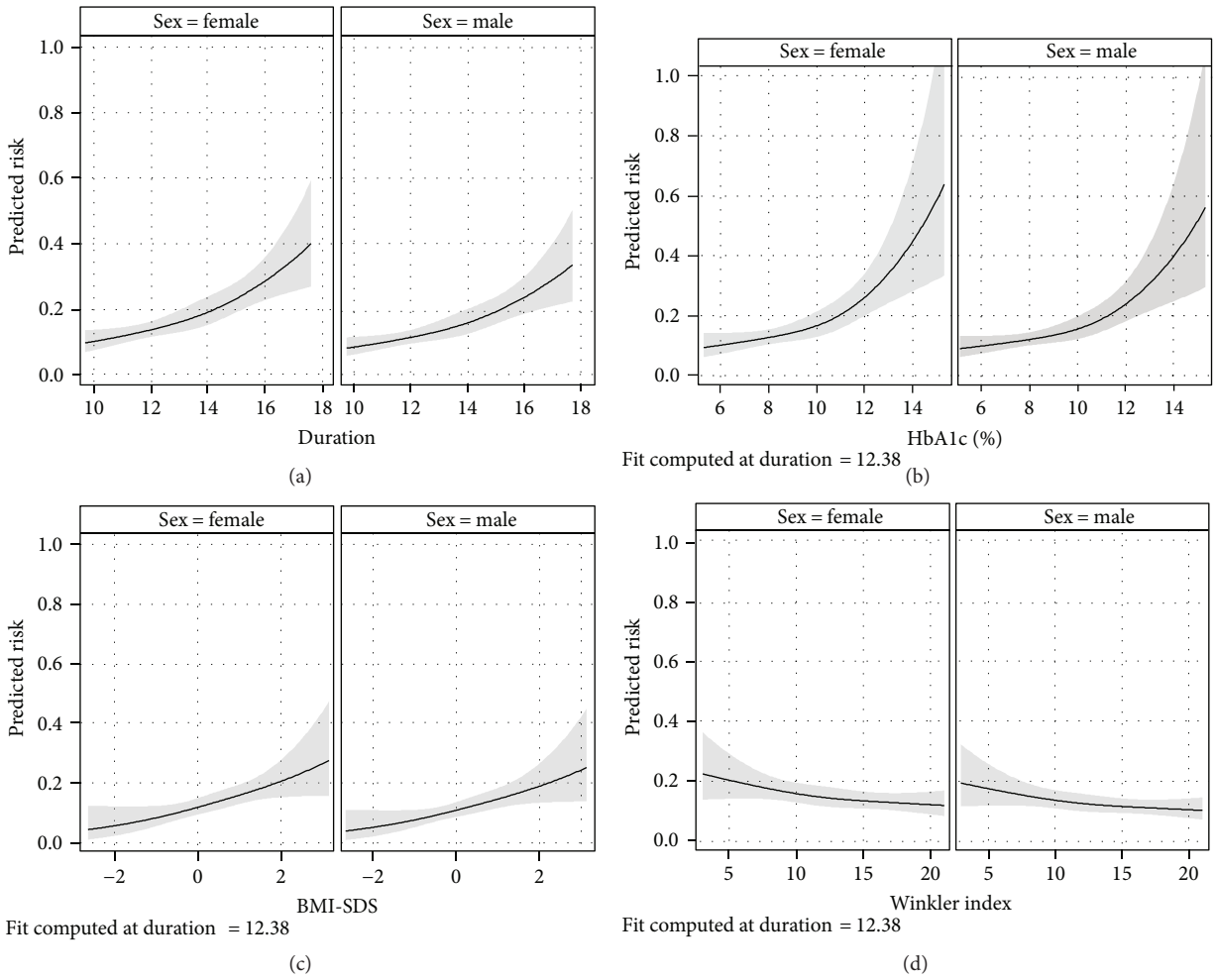


Figure 2: Risk of at least one diabetes-related complication in relation to different exposures. Risk of retinopathy, nephropathy, hypertension, or dyslipidemia (event = yes) in relation to diabetes duration ((a) $N = 1789$), HbA1c ((b) $N = 1711$), body mass index standard deviation scores ((c) $N = 1741$), and the socioeconomic status index (Winkler index) ((d) $N = 1761$) with 95% confidence bands at 12.4-year diabetes duration. The risk was estimated from separate log-binomial regression models for each exposure with the composite endpoint as the binary dependent variable and diabetes duration, sex, and the respective exposure as independent variables. Continuous variables were included as natural cubic splines with three equally spaced knots. Different N are due to missing values in the exposure variables.

to patients never omitting insulin injections, omitting insulin injection more than once a day elevated the risk 1.91-fold (Table 2).

Potentially beneficial health care-related factors (CSII, DMP, and DHC) showed no clear association with the risk of the composite endpoint. However, participants who reported that they did not know whether they used the DHC showed a tendency to a lower risk compared to DHC users (Table 2).

4. Discussion

In our analysis comprising three cross-sectional surveys of early-onset and long-duration type 1 diabetes patients, we estimated the risk of DR, DN, hypertension, or dyslipidemia at about 19% (men) and 23% (women) after 15 years of diabetes duration. Analyses of temporal trends suggested a decreasing risk of the composite endpoint over the seven-year observation period, probably reflecting intensification and improvements in diabetes care. Indicators for glycemic control and SES as well as lifestyle-related factors such as BMI and PA were strongly associated with the risk for developing at least one of the complications.

Regarding the development of DR in Germany, Hammes et al. [5] found that the risk of DR starts to increase about 10 to 15 years after the onset of type 1 diabetes and being close to zero before. This is in line with our findings. However, the crude prevalence of any DR of 27.4% is hardly comparable since the mean age and diabetes duration (31.1 and 14.5 years, resp.) were higher than in our study. The same holds for the comparison with the results from Raile et al. [8], who investigated the risk of DN in a German cohort. Their survival curves with diabetes duration as a time scale correspond to our results in Figure 1 whereas the crude risk estimates are higher, probably due to higher mean age and diabetes duration. A joint analysis of the Diabetes Control and Complications Trial and the Pittsburgh Epidemiology of Diabetes Complications Study estimates the risk of DR and DN under modern-day treatment and after a 30-year diabetes duration at approx. 50% and 20%, respectively [24]. For DR, similar risk estimates are reported from Swedish registries for young adults between 18 and 41 years of age whereas the risk for DN is estimated at approximately 10% [25]. The results show a risk increase already before 10 years since diagnosis which may suggest differences in the clinical course between countries.

With regard to dyslipidemia, our risk estimates are considerably lower than in previous studies even in comparable age groups [10, 26]. To a lower extent, also higher prevalence of hypertension were reported (e.g., Schwab et al. [10]). In these studies, the outcome assessment was based on clinical measurements. In contrast, we relied on self-reports which might have led to underreporting of dyslipidemia and hypertension due to recall bias. Diagnosis of DN and DR might be less prone to recall bias, since patients may perceive DN and DR as more severe complications than dyslipidemia and hypertension. Furthermore, physicians may not always give a diagnosis to patients, despite elevated blood pressure and/or blood lipids.

Despite these differences, our findings on glycemic control as an important predictor for dyslipidemia [26], DR, DN [27], and hypertension [28] are in line with previous studies. Similarly, PA [29], BMI [30], and diabetes duration [5, 8] had been reported to associate with the risk of complications. With regard to the family and socioeconomic background, we found that not living with both biological parents increased the risk slightly. This is consistent with other studies that identified an association between family structure and glycemic control [12]. In addition, associations between SES [31] have been documented previously. Despite evidence for the use of CSII to improve glycemic control, we did not observe a protective association regarding diabetic complications, whilst others did (e.g., [32]). This could be explained, for instance, by the fact that we did not know for how long participants used CSII and if CSII was introduced before or after the onset of the complication(s). We found no protective association for patients taking part in a DMP. A systematic review of type 1 diabetes and type 2 diabetes DMPs concludes that DMPs can improve glycemic control modestly [33]. However, to our knowledge, there is no previous study which investigated the association between DMP and DHC in connection with complications in type 1 diabetes in Germany. With respect to DMPs on type 2 diabetes mellitus, some evaluation studies are in favor of DMP regarding survival [34, 35], whilst one study concludes that there are no differences between DMP participants and nonparticipants with regard to complications [36].

For the German general population, it is well known that SES is associated with many health outcomes leading to reduced life expectancy and increased mortality for groups with low SES (e.g., [37]). Less is known about health inequalities in the context of complications among type 1 diabetes patients. In the present study, we identified an inverse relationship between SES and complications. The differences we found might be caused, on the one hand, by diabetes-specific differences (e.g., glycemic control) and, on the other hand, by differences that also apply to the general population. For instance, it is known that BMI is inversely associated with SES among German children and adolescents [38], and we found BMI to be strongly associated with the risk of complications. One study found an inverse association between household income and the frequency of intentionally omitted insulin injections among adults but also reported high education to increase the risk of complications [39]. Future studies should investigate which pathways lead to social inequalities in the risk of complications in order to identify vulnerable groups and develop targeted interventions.

The central role of glycemic control to prevent complications is well acknowledged in clinical guidelines [40]. We used HbA1c as an indicator for long-term blood glucose levels and found a strong association with the risk of complications in an early-onset type 1 diabetes cohort. The exponential relationship between HbA1c and the risk of complications suggests that a one-unit increase in higher HbA1c ranges increases the risk more strongly than that in lower ranges. Thus, there might be a disproportionate potential for risk reduction in patients with high HbA1c values. In order to reduce complications in early-onset type

1 diabetes, it is essential to investigate underlying causes of elevated blood glucose.

Our results imply that participation in DMP and the use of the DHC were not associated with a reduced risk of complications. These programs are, amongst other aims, designed to prevent complications; wherefore, one would expect a reduced risk. Our negative results might call for improvement of these programs with special focus on the early-onset type 1 diabetes population. However, before drawing final conclusions on the effectiveness of DMP and use of a DHC, further evaluation is needed since we did not account for variables that potentially influence participation and also related to the risk of complications.

4.1. Strengths and Weaknesses. Our results are based on self-reports which may have led to measurement error and misclassification. Especially for nephropathy and retinopathy, direct standardized measurement would have been preferable to distinguish, for example, retinopathy with and without visual impairment. However, the fact that our dose-response curve for DN (Figure 1) corresponds to the survival curve for DN from Raile et al. [8] might suggest reasonably valid self-reported data on complications. Furthermore, by asking explicitly for physician-diagnosed retinopathy and nephropathy, we tried to improve the validity of the self-reported data, since most T1D patients in Germany are treated in diabetes centers under the same guidelines. In addition, under the assumption that the degree of the outcome misclassification does not depend on the values of the respective exposures, the shape of the dose-response curves can still be considered valid. With regard to HbA1c, a previous analysis using the data of survey$_{2009/10}$ showed good accordance of self-reported HbA1c with clinical documentation [16]. Nevertheless, we were not able to account for different laboratory methods of HbA1c, which recently have been suggested to influence HbA1c results [41].

Unfortunately, we had no information on puberty status, which has been suggested to be an important factor for the risk of complications [42, 43]. Our study might be prone to selection bias, since additional analyses showed that nonresponders were younger and had a longer diabetes duration. Furthermore, nonresponders who sent back a short questionnaire reported poorer overall health compared to participants with full questionnaires (results not shown). A major limitation is the fact that we collected exposure information retrospectively; wherefore, we could not establish a time order between exposure and outcome. Thus, all results should be considered exploratory. With regard to the statistical analyses, we defined a composite outcome variable to increase the number of events and thus the statistical power. However, it is probable that the relationships between exposure and the respective outcomes differ, which cannot be assessed with our results.

One strength of our study is the fact that it is built on a nationwide sample, drawn from a register with 97% coverage of all early-onset type 1 diabetes cases in Germany [15]. Furthermore, we were able to include sociodemographic and health care-related variables, which are often not available in clinical registries. In addition, we determined a time trend of the risk of complications between 2009/10 and 2015/16. With regard to the analyses, we used natural cubic spline regressions, which has the advantage of maintaining the continuous nature of exposure variables instead of artificially categorizing it in case of a nonlinear relationship. Therefore, we kept information, allowing us to characterize dose-response relationships. The use of log-binomial regression has the advantage of directly estimating RRs instead of odds ratios as is the case in logistic regression. Thus, we could avoid overestimating of RRs when the rare disease assumption does not hold.

4.2. Conclusion. Altogether, we provide evidence that in early-onset type 1 diabetes, there exists a considerable risk of complications and their predictors already in young ages. However, we observed a decreasing risk over time probably representing improved care among younger birth cohorts. Differences in the risk of complications with regard to SES, lifestyle-related variables, and glycemic control call for further improvement of care and development/implementation of prevention programs. Future research on early-onset type 1 diabetes should focus on prevention of diabetic complications in young ages and clarification of pathways of the associations found in this study.

Conflicts of Interest

All authors declare no conflicts of interest relevant to this article.

Acknowledgments

This work was supported by the Competence Network for Diabetes Mellitus [support codes 01GI0802, 01GI1109A, 01GI0859, and 01GI1106] and the German Center for Diabetes Research (DZD) both funded by the Federal Ministry of Education and Research (BMBF). The German Diabetes Centre is institutionally funded by the Ministry of Science and Research of the State of North Rhine-Westphalia and the German Federal Ministry of Health. Sponsors were not involved in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the article for publication.

References

[1] M. A. Atkinson, G. S. Eisenbarth, and A. W. Michels, "Type 1 diabetes," *The Lancet*, vol. 383, no. 9911, pp. 69–82, 2014.

[2] P. Hovind, L. Tarnow, K. Rossing et al., "Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes," *Diabetes Care*, vol. 26, no. 4, pp. 1258–1264, 2003.

[3] G. Pambianco, T. Costacou, D. Ellis, D. J. Becker, R. Klein, and T. J. Orchard, "The 30-year natural history of type 1 diabetes complications—the Pittsburgh Epidemiology of Diabetes Complications Study experience," *Diabetes*, vol. 55, no. 5, pp. 1463–1469, 2006.

[4] M. J. Fowler, "Microvascular and macrovascular complications of diabetes," *Clinical Diabetes*, vol. 29, no. 3, pp. 116–122, 2011.

[5] H. P. Hammes, W. Kerner, S. Hofer et al., "Diabetic retinopathy in type 1 diabetes—a contemporary analysis of 8,784 patients," *Diabetologia*, vol. 54, no. 8, pp. 1977–1984, 2011.

[6] American Diabetes Association, "Nephropathy in diabetes," *Diabetes Care*, vol. 27, pp. S79–S83, 2007.

[7] World Health Organization, *Global Report on Diabetes*, WHO Press, Geneva, Switzerland, 2016.

[8] K. Raile, A. Galler, S. Hofer et al., "Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes: effect of diabetes duration, A1C, hypertension, dyslipidemia, diabetes onset, and sex," *Diabetes Care*, vol. 30, no. 10, pp. 2523–2528, 2007.

[9] M. Lind, A.-M. Svensson, M. Kosiborod et al., "Glycemic control and excess mortality in type 1 diabetes," *The New England Journal of Medicine*, vol. 371, no. 21, pp. 1972–1982, 2014.

[10] K. O. Schwab, J. Doerfer, W. Hecker et al., "Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type 1 diabetes: cross-sectional data from the German diabetes documentation and quality management system (DPV)," *Diabetes Care*, vol. 29, no. 2, pp. 218–225, 2006.

[11] Diabetes Control Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus," *The New England Journal of Medicine*, vol. 329, no. 14, pp. 977–986, 1993.

[12] S. E. Watson, E. A. Kuhl, M. B. Foster et al., "The impact of insurance coverage and the family on pediatric diabetes management," *Pediatric Diabetes*, vol. 18, no. 4, pp. 315–319, 2017.

[13] T. Lawes, V. Franklin, and G. Farmer, "HbA1c tracking and bio-psychosocial determinants of glycaemic control in children and adolescents with type 1 diabetes: retrospective cohort study and multilevel analysis," *Pediatric Diabetes*, vol. 15, no. 5, pp. 372–383, 2014.

[14] C. C. Patterson, G. G. Dahlquist, E. Gyürüs, A. Green, G. Soltész, and EURODIAB Study Group, "Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study," *The Lancet*, vol. 373, no. 9680, pp. 2027–2033, 2009.

[15] A. Bendas, U. Rothe, W. Kiess et al., "Trends in incidence rates during 1999-2008 and prevalence in 2008 of childhood type 1 diabetes mellitus in Germany—model-based national estimates," *PLoS One*, vol. 10, no. 7, article e0132716, 2015.

[16] A. Stahl, K. Straßburger, K. Lange et al., "Health-related quality of life among German youths with early-onset and long-duration type 1 diabetes," *Diabetes Care*, vol. 35, no. 8, pp. 1736–1742, 2012.

[17] B.-M. Kurth, P. Kamtsiuris, H. Hölling et al., "The challenge of comprehensively mapping children's health in a nation-wide health survey: design of the German KiGGS-Study," *BMC Public Health*, vol. 8, no. 1, p. 196, 2008.

[18] K. Kromeyer-Hauschild, A. Moss, and M. Wabitsch, "Referenzwerte für den Body-Mass-Index für Kinder, Jugendliche und Erwachsene in Deutschland. Anpassung der AGA-BMI-Referenz im Altersbereich von 15 bis 18 Jahren. Adipositas – Ursachen, Folgeerkrankungen," *Thérapie*, vol. 9, pp. 123–127, 2015.

[19] T. J. Cole, "The LMS method for constructing normalized growth standards," *European Journal of Clinical Nutrition*, vol. 44, no. 1, pp. 45–60, 1990.

[20] R. Busse, "Disease management programs in Germany's statutory health insurance system," *Health Affairs*, vol. 23, no. 3, pp. 56–67, 2004.

[21] Bundesärztekammer (BÄK), *Kassenärztliche Bundesvereinigung (KBV), Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften (AWMF). Nationale VersorgungsLeitlinie Diabetes – Strukturierte Schulungsprogramme – Kurzfassung, 1. Auflage. Version 3*, Ärztliches Zentrum für Qualität in der Medizin, Berlin, Germany, 2013.

[22] D. Spiegelman and E. Hertzmark, "Easy SAS calculations for risk or prevalence ratios and differences," *American Journal of Epidemiology*, vol. 162, no. 3, pp. 199-200, 2005.

[23] S. Durrleman and R. Simon, "Flexible regression models with cubic splines," *Statistics in Medicine*, vol. 8, no. 5, pp. 551–561, 1989.

[24] Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group, D. M. Nathan, B. Zinman et al., "Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and pittsburgh epidemiology of diabetes complications experience (1983-2005)," *Archives of Internal Medicine*, vol. 169, no. 14, pp. 1307–1316, 2009.

[25] U. Samuelsson, J. Anderzén, S. Gudbjörnsdottir, I. Steineck, K. Åkesson, and L. Hanberger, "Teenage girls with type 1 diabetes have poorer metabolic control than boys and face more complications in early adulthood," *Journal of Diabetes and its Complications*, vol. 30, no. 5, pp. 917–922, 2016.

[26] A. S. Shah, D. M. Maahs, J. M. Stafford et al., "Predictors of dyslipidemia over time in youth with type 1 diabetes: for the SEARCH for Diabetes in Youth study," *Diabetes Care*, vol. 40, no. 4, pp. 607–613, 2017.

[27] T. R. Rohrer, J. Wolf, S. Liptay et al., "Microvascular complications in childhood-onset type 1 diabetes and celiac disease: a multicenter longitudinal analysis of 56,514 patients from the German-Austrian DPV Database," *Diabetes Care*, vol. 38, no. 5, pp. 801–807, 2015.

[28] B. L. Rodriguez, D. Dabelea, A. D. Liese et al., "Prevalence and correlates of elevated blood pressure in youth with diabetes mellitus: the SEARCH for Diabetes in Youth study," *The Journal of Pediatrics*, vol. 157, no. 2, pp. 245–251.e1, 2010.

[29] B. Bohn, A. Herbst, M. Pfeifer et al., "Impact of physical activity on glycemic control and prevalence of cardiovascular risk factors in adults with type 1 diabetes: a cross-sectional multicenter study of 18,028 patients," *Diabetes Care*, vol. 38, no. 8, pp. 1536–1543, 2015.

[30] M. Loredana Marcovecchio, R. Neil Dalton, A. Toby Prevost et al., "Prevalence of abnormal lipid profiles and the relationship with the development of microalbuminuria in adolescents with type 1 diabetes," *Diabetes Care*, vol. 32, no. 4, pp. 658–663, 2009.

[31] A. M. Secrest, T. Costacou, B. Gutelius, R. G. Miller, T. J. Songer, and T. J. Orchard, "Associations between socioeconomic status and major complications in type 1 diabetes: the Pittsburgh Epidemiology of Diabetes Complication (EDC) study," *Annals of Epidemiology*, vol. 21, no. 5, pp. 374–381, 2011.

[32] S. R. Johnson, M. N. Cooper, T. W. Jones, and E. A. Davis, "Long-term outcome of insulin pump therapy in children with type 1 diabetes assessed in a large population-based case–control study," *Diabetologia*, vol. 56, no. 11, pp. 2392–2400, 2013.

[33] K. Knight, E. Badamgarav, J. M. Henning et al., "A systematic review of diabetes disease management programs," *The American Journal of Managed Care*, vol. 11, no. 4, pp. 242–250, 2005.

[34] M. Laxy, R. Stark, C. Meisinger et al., "The effectiveness of German disease management programs (DMPs) in patients with type 2 diabetes mellitus and coronary heart disease: results from an observational longitudinal study," *Diabetology & Metabolic Syndrome*, vol. 7, no. 1, p. 77, 2015.

[35] A. Miksch, G. Laux, D. Ose et al., "Is there a survival benefit within a German primary care-based disease management program?," *The American Journal of Managed Care*, vol. 16, no. 1, pp. 49–54, 2010.

[36] R. Linder, S. Ahrens, D. Köppel, T. Heilmann, and F. Verheyen, "The benefit and efficiency of the disease management program for type 2 diabetes," *Deutsches Ärzteblatt International*, vol. 108, no. 10, pp. 155–162, 2011.

[37] T. Lampert and L. E. Kroll, "Social differences in mortality and life expectancy," *GBE kompakt*, vol. 5, 2014.

[38] C. Kleiser, A. S. Rosario, G. B. M. Mensink, R. Prinz-Langenohl, and B.-M. Kurth, "Potential determinants of obesity among children and adolescents in Germany: results from the cross-sectional KiGGS study," *BMC Public Health*, vol. 9, no. 1, p. 46, 2009.

[39] M. Peyrot, R. R. Rubin, D. F. Kruger, and L. B. Travis, "Correlates of insulin injection omission," *Diabetes Care*, vol. 33, no. 2, pp. 240–245, 2010.

[40] American Diabetes Association, "Standards of medical care in diabetes—2016: summary of revisions," *Diabetes Care*, vol. 39, Supplement 1, pp. S4–S5, 2016.

[41] J. Roth, N. Müller, T. Lehmann et al., "Comparison of HbA1c measurements using 3 methods in 75 patients referred to one outpatient department," *Experimental and Clinical Endocrinology & Diabetes*, vol. 126, no. 01, pp. 23–26, 2018.

[42] M. Svensson, L. Nyström, S. Schön, G. Dahlquist, and on behalf of the Swedish Childhood Diabetes Study and the Swedish Registry for Active Treatment of Uraemia, "Age at onset of childhood-onset type 1 diabetes and the development of end-stage renal disease—a nationwide population-based study," *Diabetes Care*, vol. 29, no. 3, pp. 538–542, 2006.

[43] Y. H. Cho, M. E. Craig, and K. C. Donaghue, "Puberty as an accelerator for diabetes complications," *Pediatric Diabetes*, vol. 15, no. 1, pp. 18–26, 2014.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 1875870, 13 pages
https://doi.org/10.1155/2018/1875870

Review Article

Oxidative Stress, Apoptosis, and Mitochondrial Function in Diabetic Nephropathy

Sonia Sifuentes-Franco,[1] **Diego Enrique Padilla-Tejeda**,[2] **Sandra Carrillo-Ibarra**,[1] **and Alejandra Guillermina Miranda-Díaz**[1]

[1]*Institute of Experimental and Clinical Therapeutics, Department of Physiology, University Health Sciences Centre, University of Guadalajara, Guadalajara, JAL, Mexico*
[2]*Programa de Químico Farmacéutico Biotecnologo, Escuela de Ciencias de la Salud, Campus Zapopan, Universidad del Valle de México, Guadalajara, JAL, Mexico*

Correspondence should be addressed to Alejandra Guillermina Miranda-Díaz; kindalex1@outlook.com

Received 12 December 2017; Accepted 7 February 2018; Published 1 April 2018

Academic Editor: Ilias Migdalis

Diabetic nephropathy (DN) is the second most frequent and prevalent complication of diabetes mellitus (DM). The increase in the production of oxidative stress (OS) is induced by the persistent hyperglycemic state capable of producing oxidative damage to the macromolecules (lipids, carbohydrates, proteins, and nucleic acids). OS favors the production of oxidative damage to the histones of the double-chain DNA and affects expression of the DNA repairer enzyme which leads to cell death from apoptosis. The chronic hyperglycemic state unchains an increase in advanced glycation end-products (AGE) that interact through the cellular receptors to favor activation of the transcription factor NF-κB and the protein kinase C (PKC) system, leading to the appearance of inflammation, growth, and augmentation of synthesis of the extracellular matrix (ECM) in DN. The reactive oxygen species (ROS) play an important role in the pathogenesis of diabetic complications because the production of ROS increases during the persistent hyperglycemia. The primary source of the excessive production of ROS is the mitochondria with the capacity to exceed production of endogenous antioxidants. Due to the fact that the mechanisms involved in the development of DN have not been fully clarified, there are different approaches to specific therapeutic targets or adjuvant management alternatives in the control of glycemia in DN.

1. Introduction

The global prevalence of diabetes mellitus (DM) in adults has increased considerably in recent decades. In the 7th edition of the diabetes mellitus (DM) Atlas published in 2015, it was reported that there are 415 million adults with DM in the world and it is estimated that there will be 693 million people living with DM by the year 2045 [1]. The lack of adequate control in consistently high levels of glucose leads to the appearance of serious micro- and macrovascular complications. The characteristic macrovascular complications include cardiovascular disease (CVD) with the propensity to suffer heart attacks and cerebrovascular accidents. Among the microvascular complications, neuropathy, retinopathy, and diabetic nephropathy (DN) stand out [2].

DN is characterized by the appearance of persistent clinical albuminuria (albumin excretion rate (AER) > 300 mg/24 h) and a reduction in the glomerular filtration rate (GFR) > 5 years in the absence of urinary tract infections, other kidney diseases, or cardiac insufficiency [3]. Proteinuria occurs in ~15–40% of type 1 DM patients and between 5 and 20% of individuals with type 2 DM. DN has significant long-term effects on the morbidity and mortality of DM patients [4]. Because the mechanisms in the appearance of DN have not yet been fully described, we proposed the approach of looking at diverse themes including the epidemiology, the mechanism of appearance, and the role of OS, the oxidative DNA damage, mitochondrial function, and cell death due to apoptosis in DN. Also described are some management alternatives that should be considered adjunctive in the control of

glycemia and for the traditional DN risk factors. We emphasize that the best management for patients with DM to avoid and control the development of kidney damage is the adequate control of glycemia.

1.1. Epidemiology. Diabetes mellitus is the most common cause of chronic kidney disease (CKD) in the world, where approximately 1 out of every 4 adults with DM has CKD [5] and 1 of every 10–20% of patients with DM dies due to CKD [6]. According to a report from the United States Renal Data System (USRDS) in 2015, DM was identified as the primary cause of end-stage renal disease (ESRD) in >50% of patients treated in Singapore, Malaysia, Jalisco (Mexico), and Chile [7]. Diabetic nephropathy affects the African-American population about 3–5 times more than the Caucasian population. In type 1 DM, CKD is relatively rare in the first 5–10 years; however, the incidence rapidly increases in the following 10 years until reaching a maximum of about ~3% per year after suffering from DM for 15 years. After 15 years with type 1 DM, the percentage of CKD diminishes in 1% in ≥40 years with the illness. The patients suffering with type 1 DM for >35 years who have not yet developed DN have a low risk of presenting it [8].

In type 2 DM, DN develops predominantly in patients with systemic arterial hypertension and in the population with previous kidney disease. DN sometimes occurs in young individuals and can accompany diabetic retinopathy. In older persons with type 2 DM, retinopathy and proteinuria can be absent or minimal, although other kidney diseases should be investigated and excluded. Diabetic retinopathy and proteinuria could also be related to arterial hypertension [9].

1.2. The Development of Diabetic Nephropathy. DN is a condition of multiple stages that requires several years to arrive at being clinically evident. The development of microalbuminuria and the progression to manifested proteinuria are the common clinical characteristics of DN [10].

Microalbuminuria is the first indicator of DN, which can be detected at ~1 year from the onset of the type 1 DM. Microalbuminuria is already present now in the diagnosis of type 2 DM. At the onset of type 2 DM, structurally significant glomerular disease exists and the GFR is already beginning to be affected. In the case that no intervention was to occur, the microalbuminuria progresses to clinical albuminuria in ~10–15 years. Studies reported suggest that the natural history of DN is variable: in ~20%, microalbuminuria progresses to clinical albuminuria in 5–9 years, while in 50%, it remains in microalbuminuria. Microalbuminuria in ~30% of patients returns to normal ranges (<30 μg/min). Microalbuminuria is a strong predictor of death by CVD in elderly patients with type 2 DM. Microalbuminuria is associated with generalized endothelial dysfunction in type 1 and type 2 DM. As proteinuria increases, the loss of GFR is quicker [9].

The natural history of DN differs between type 1 and type 2 DM. The five classic stages described in type 1 DM may not occur in type 2 DM because type 2 is diagnosed after other related but silent disturbances like arterial hypertension, proteinuria, or renal insufficiency. In type 1 DM, the alterations in kidney structure and function are found present at the onset of the illness. (A) Stage 1 is characterized by early hyperfunction and hypertrophy (changes are partially reversible with adequate treatment). (B) Stage 2 develops silently over the course of many years and is characterized by morphological lesions without clinical signs of illness. In this stage, good control of the DM does exist and the excretion of albumin is normalized. In deficient control of the hyperglycemic state, the excretion of albumin increases at rest and is greater during exercise. An important number of patients continue in stage 2 throughout their lives. (C) Stage 3 is characterized by incipient DN, which is the precursor to frank DN. Its main sign is the elevated urinary excretion of albumin. (D) Stage 4 is considered manifested DN. This is the classical entity characterized by persistent proteinuria (>0.5 g/24 h). When the arterial hypertension is not adequately treated, the GFR diminishes with an average fall of ~1 mL/min/month. (E) Stage 5 is considered ESRD with uremia [11]. In 2014, the group Committee on Diabetic Nephropathy reformulated the classification of DN [12] (Table 1).

The development and progression of DN are influenced by diverse factors, among which the most important are hyperglycemia, arterial hypertension, obesity, and an unhealthy lifestyle [13]. Actually, sufficient evidence exists that indicates that oxidative stress (OS) is a factor of great importance in the development of type 1 and type 2 DM, generated primarily by the hyperglycemic state. Hyperglycemia causes tissue and endothelial damage through five primary mechanisms: (1) increase in the flow of glucose through the activation of the alternative metabolic pathways of glucose, the polyol pathway; (2) increase in the formation of intracellular advanced glycation end-products (AGE); increase in expression of the AGE receptor and activation of the ligands; (4) activation of isoforms of the protein kinase C (PKC); and (5) hyperactivity of the hexosamine pathway [14] (Figure 1).

The intracellular events induced in the presence of an environment of high concentrations of glucose favor the accentuated flow of polyols and hexosamine. The generation of AGE and reactive oxygen species (ROS), the activation of PKC, the activation of the transforming growth factor β-mitogen-activated Smad proteins (TGF-β-Smad-MAPK). Enzymes that degrade the extracellular matrix (ECM) have the ability to cause structural damage in the kidneys through the aberrant expression of cyclin-dependent kinases. When the AGEs are increased, they stimulate the glomerular cells to produce TGF-β1, which contributes to glomerular sclerosis and interstitial tubular damage through the abnormal production of ECM [15, 16]. The increase of ECM leads to kidney fibrosis primarily generated by the accumulation of mesangial cells, which favors the depositing of ECM, thickening of the glomerular and tubular membranes, dysfunction of the podocytes, and unchaining cell death by apoptosis. All of these events are caused by alterations in the redox system that leads to the appearance of albuminuria, proteinuria, glomerulosclerosis, and interstitial tubular fibrosis [17]. When the redox equilibrium is slightly altered, pathological processes are unchained that initiate producing

Table 1: Classification of diabetic nephropathy [12].

Stage	Albuminuria (mg/g Cr) or proteinuria (g/g Cr)	GFR (mL/min/1.73 m^2)
Stage 1 (prenephropathy)	Normoalbuminuria (<30)	≥30
Stage 2 (incipient nephropathy)	Microalbuminuria (30–299)	≥30
Stage 3 (overt nephropathy)	Macroalbuminuria (≥300) or persistent proteinuria (≥0.5)	≥30
Stage 4 (kidney failure)	Any albuminuria/proteinuria *status*	<30
Stage 5 (dialysis therapy)	Any *status* on continued dialysis therapy	

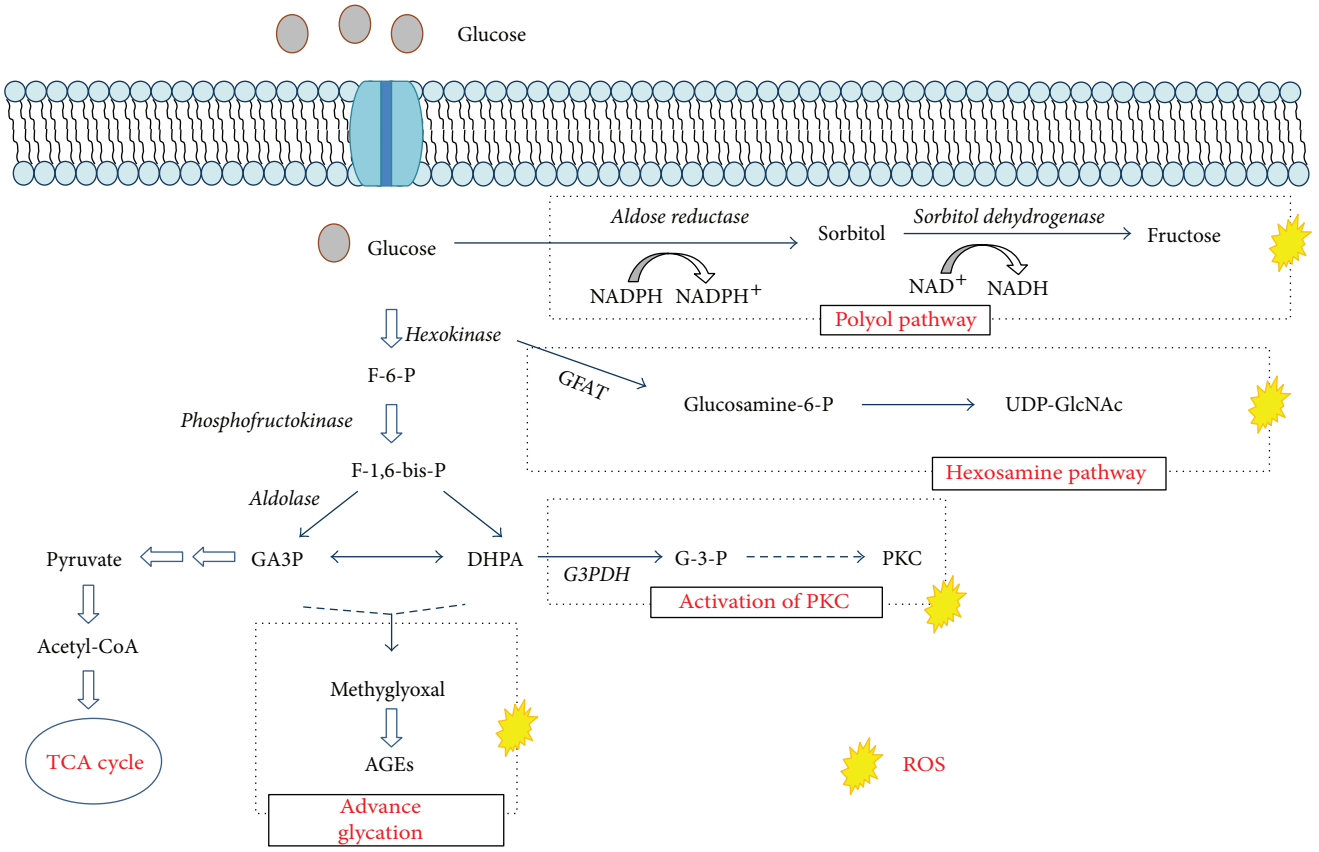


Figure 1: Mechanisms of cellular damage from the hyperglycemic state. Demonstration of the signaling pathways that are activated in the state of persistent hyperglycemia.

damage to the macromolecules and that favor cellular dysfunction by contributing to the pathogenesis of DN [18]. By being the cellular nucleus, one of the primary objectives of the ROS is to generate structural changes that lead to cell death by apoptosis [19].

1.3. Oxidative Stress and Diabetic Nephropathy. OS is considered a common and important factor that links hyperglycemia with the vascular complications through metabolic changes to the molecules of the target tissues and the alterations in renal hemodynamics. Therefore, the metabolic attacks and hemodynamics from the increase in ROS have adverse synergic effects in the target tissues. Many ROS possess unpaired electrons and are considered free radicals. The ROS are a family of molecules that include molecular oxygen and its derivatives, among which are the superoxide anion (O^{2-}), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^{-}$), hypochlorous acid (HClO), nitric oxide (NO), and lipid radicals. Excessive quantities of ROS, after overcoming various endogenous mechanisms of antioxidant defense, oxidize various biomolecules like DNA, proteins, carbohydrates, and lipids, producing OS [20]. The potential sources of ROS include the mitochondrial respiratory chain, the xanthine oxidase, the NADH/NADPH oxidases, the NO synthase, and some other hemoproteins. In having divergent sources of ROS production, it is conceivable that different mechanisms operate in the generation of ROS in a hyperglycemic environment [21], because hyperglycemia favors the production of ROS. The ROS play an important role in the pathogenesis of diabetic complications [22]. The

increase in the production of ROS can be so overwhelming, and the antioxidant defense systems are easily exhausted, favoring the increase in OS [23].

1.4. Mitochondrial Function and Diabetic Nephropathy. The ingested glucose generates energy for the production of adenosine triphosphate (ATP) through oxidative phosphorylation in the mitochondrial respiratory chain. After the glucose enters the cells, the majority is subjected to the process of glycolysis to form pyruvate and generate ATP, NADH, and FADH2. The generated NADH and FADH2 are transported to the mitochondria from the cytosol by the malate-aspartate enzyme or by the glycerol phosphate shuttle where they are used as electron donors during oxidative phosphorylation. The electrons from NADH or FADH2 are transferred to the molecular oxygen (O_2) in the mitochondrial respiratory chain complexes I–IV to generate ATP. During this process, the majority of O_2 in normal physiological states is reduced to water and <1% is converted into O^{2-}. However, when mitochondrial dysfunction exists, or in the state of persistent hyperglycemia, it produces an excessive leakage of electrons in complex I and in the interface between the coenzyme Q and complex III, since the mitochondrial respiratory chain is the primary source in the generation of O^{2-} in the cell. Therefore, the flow of O^{2-} outside the mitochondria is increased [24]. The protein complexes of the internal mitochondrial membrane are used to pump protons in the intermembranal space. This process is efficient, but a small percentage of the electrons can prematurely reduce the O_2 forming O^{2-} producing OS in the mitochondria [25]. The mitochondria are highly dynamic and constantly experiencing the processes of fission and fusion. The fission gives, as a result, the production of strips or short mitochondrial spheres, and fusion promotes the production of a long filament [26]. Mitochondrial fission 1 protein (FIS1) is a key component in the mechanics of mitochondrial fission in the cells of mammals. In a previous study, mitochondrial fragmentation and the increase in expression of FIS1 in recently isolated venous endothelial cells in patients with DM were reported. In silencing the expression of FIS1, the dynamin-1-like protein (drp1) through a small ARN of interference increased the production of the ROS induced by hyperglycemia, which suggests that the increase in mitochondrial fission can affect the endothelial function through the increase in ROS [27]. The mitochondrial ROS play an important role in the complications of DM including DN [28]. The target cells, including the glomerular mesangial cells, the capillary endothelial cells of the retina, and the neuronal cells, are incapable of adequately regulating the concentrations of intracellular glucose in the diabetic ambiance [11]. Therefore, these cells are subjected to the extraordinary OS mediated by ROS [11].

1.5. Oxidative DNA Damage and Apoptosis. The persistent OS in the state of hyperglycemia leads to modifications of the DNA capable of producing damage to the mitochondrial genetic material (mtDNA) and the nuclear DNA (nDNA) [29]. The ROS, on inducing damage to the DNA, cause breakage of the single or double strands, altering the bases (histones) [30]. Because the mtDNA is deficient in histones, it could be more susceptible to oxidative damage. It has been demonstrated that the mtDNA damage is more extensive and persistent than the nDNA damage in human cells subjected to OS [31]. The persistent oxidative damage on mtDNA favors the appearance of mutations in the mitochondrial genome [32] giving way to mitochondrial dysfunction that increases the production of ROS and forms a vicious cycle in the mitochondria by producing intense oxidative damage capable of unchaining cell death by necrosis or apoptosis [33, 34]. The 8-hydroxy-2-deoxyguanosine (8-OHdG) is a marker of oxidative DNA damage that increases in the presence of OS. The 8-OHdG marker is a potentially mutagenic compound that participates as an inductor of apoptosis through the activation of the initiator and executor caspases and increases the expression of p53 proteins [35–37]. Previous studies show that the urinary levels of the 8-OHdG marker are found elevated in patients with DN compared to healthy subjects, suggesting systemic oxidative DNA damage in DN. As well, it has been previously demonstrated that the progression of DN is positively associated with the levels of excretion of the 8-OHdG marker [38]. A previous study, after follow-up for five years of patients with DN, reported a significant progression of DN with increased 8-OHdG levels compared to patients with lower levels of excretion of this marker [39].

Apoptosis is the process of natural cell death, essential for the development and normal homeostasis of all multicellular organisms [40]. The state of hyperglycemia promotes apoptosis in various types of cells in DN, including the proximal tubule epithelial cells, without complete understanding of the mechanism [41]. Previously, it has been described that the apoptosis is caused by activation of a web of intracellular signaling pathways including the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway [42]. However, it is known that the hyperglycemic environment induces apoptosis and contributes to the gradual loss of renal function in DN [43]. Apoptosis has been found in tubular epithelial cells, endothelial, and interstitial cells in renal biopsies in patients with DN [44] Figure 2.

1.6. Diabetic Nephropathy Current Treatment Strategies. There is no current treatment available to prevent the development of DN. The primary therapeutic strategies are based on the strict control of the primary, modifiable risk factors among which are the arterial hypertension, glucose levels, and dyslipidemia, although the control of these entities does not always prevent the progression of DN [45].

1.6.1. Glycemia Control. The inadequate control of glycemia is the fundamental risk factor for the development and progression of DN. Consequently, it is imperative to avoid high levels of glucose [46]. Through previously published observational studies, the clear decrease in the incidence of DN in patients who were able to achieve better control of their glycemia is well known [47]. Strict glucose control reduces the risk of progression of severe albuminuria to the adequate GFT or the reduction of ESRD [48].

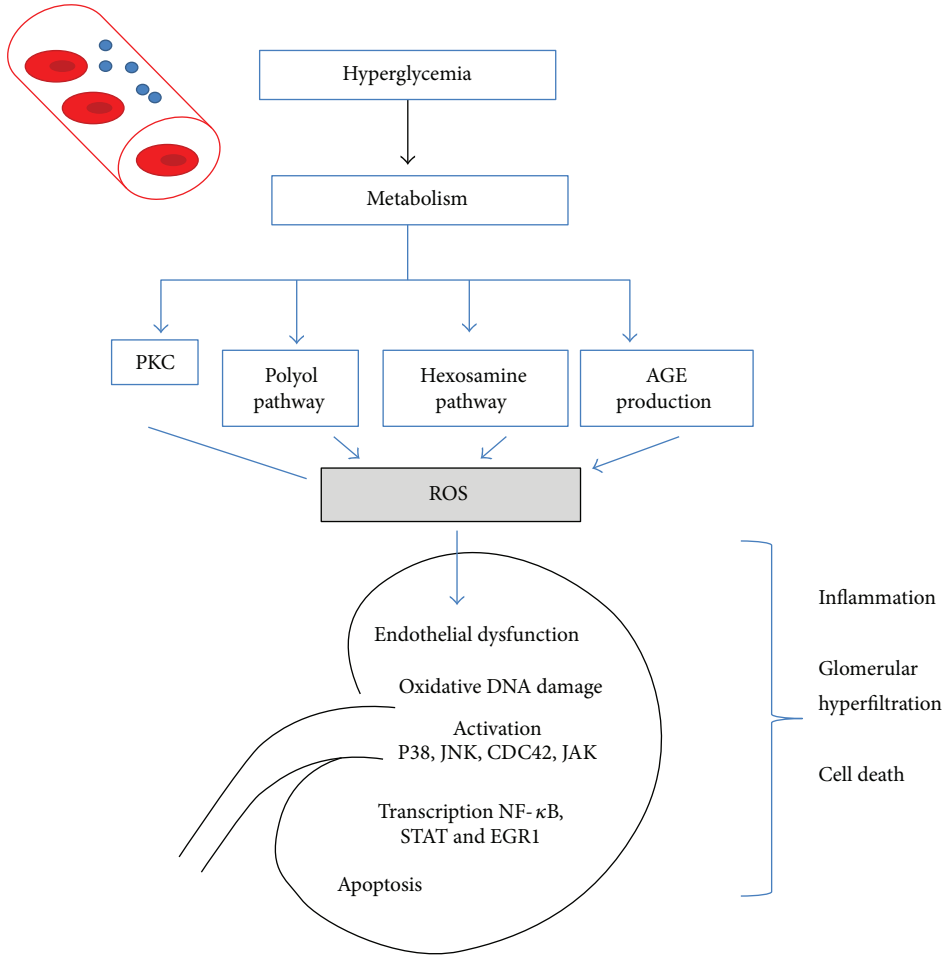


Figure 2: Hypothetical drawing of the apoptotic process in DN from hyperglycemia. Different signaling pathways that are activated in the state of hyperglycemia lead to OS and inflammation with capacity to carry out apoptosis in susceptible cells.

1.6.2. Renin-Angiotensin-Aldosterone System. The renin-angiotensin-aldosterone system (RAAS) is an important pathway implicated in the pathogenesis and progression of DN [49]. The therapeutic blockage of the RAAS is achieved with the angiotensin-converting enzyme inhibitors (ACEi) or with the use of angiotensin II (Ang II) receptor blockers. Both strategies are effective in reducing proteinuria and the slow progression of DN or nondiabetic nephropathy by the hemodynamic/antihypertensive action, as well as by its anti-inflammatory/antifibrotic action. Ang II activates the NF-κB and interacts with TGF-β. The anti-inflammatory action is produced through the inhibition of the NF-κB-dependent pathways [50]. The RAAS inhibitors, including the ACEi and the ANG II receptor blockers, are both widely utilized to control arterial hypertension in diabetic patients. These medications are considered superior to other categories of antihypertensive medications in the treatment of DN due to their capacity to reduce the intraglomerular pressure and prevent proteinuria by favoring the dilation of the efferent glomerular arteriole. The grade of proteinuria in glomerular disease is directly related to the intraglomerular pressure. Thus, the reduction induced by the excretion of proteins causes a decrease in the intraglomerular pressure by improving renal function. The decrease in the excretion of proteins has a predictive significance for better or worse renal prognosis [51].

1.6.3. Pentoxifylline. The pentoxifylline has important anti-inflammatory properties and offers beneficial effects on the microcirculatory blood flow due to its rheological properties. In patients with DM, it is associated with the reduction of urinary albumin excretion with possible beneficial effects on the GFR [52]. Pentoxifylline has also been demonstrated to reduce the urinary excretion of proteins in DM with normal renal function or with renal insufficiency [53]. The antiproteinuria effect has been related to reduction in concentrations of the tumor necrosis factor alpha (TNF-α), an important proinflammatory cytokine [54], inhibits the transcription of the TNF-α gene, and blocks the accumulation of mitochondrial ribonucleic acid (mtRNA), significantly reducing the levels of TNF-α and the urinary excretion of proteins without causing metabolic or hemodynamic changes [55]. The pentoxifylline has the capacity to modulate other cytokines and proinflammatory molecules (IFN, IL-10, and IL-6) on attenuating the processes of the inflammatory response (activation, adhesion, and phagocytosis) without causing metabolic or hemodynamic changes [56].

1.6.4. Albumin. The albumin is continuously exposed to OS [57]. OS is associated with renal dysfunction in patients with kidney failure, and the plasma albumin is the object of massive oxidation [58]. The albumin has antioxidant properties and is the master antioxidant protein of the plasma. The structural stress induced by nonenzymatic glycation or the presence of ROS deteriorates the antioxidant capacity of the albumin. The deterioration of the antioxidant capacity of the albumin is a factor strongly associated with the development of complications in DM [59]. Evidence of protein stress has been demonstrated through the detection of the carbonyl content and of the dityrosine in patients with DN [60]. A new component of the antioxidant capacity of albumin was described, and it has to do with the intrinsic component denominated response surplus (RS). This component represents the antioxidant response that is produced when the proteins suffer structural disorder due to some stress factor (temperature, short-wave ultraviolet (UV) light, and ROS). The change in antioxidant capacity of the proteins is narrowly related to its molecular structure. The changes in molecular structure are clearly related to the passive redox state of thiol groups and particularly with the active thiol group of the albumin redox (Cys-34). The antioxidant capacity of any biological system is very complex, and the albumin dependent on Cys-34 represents a passive component while the RS system represents an active component related to the changes in molecular structure [61]. The antioxidant capacity of albumin decreases with the reduction of the GFR and the advancement of the stages of DN because of the oxidation of the thiol groups, derived essentially from Cys-34. In the oxidative state, the free thiol groups react, which results in the formation of progressively more oxidized species. The reversible formation of sulfinic and sulfenic acid maintains the redox state of the plasma to the moderate, unprolonged exposure to OS [62]. With higher levels of ROS, the prolonged exposure to OS produces the formation of sulfonic acid as an irreversible or end-product of the oxidation of the Cys-34-SH [63].

1.6.5. Vitamins. Vitamin E suppresses albuminuria in patients with DM without cardiovascular disease preserving renal function [64]. Patients with oxidative stress due to low levels of genetically determined antioxidant haptoglobin, who received vitamin E, had significantly lower incidence of vascular events compared to healthy controls [65]. In a double-blind, placebo-controlled, crossover trial over 8 months, 36 subjects with type 1 DM and 9 subjects without diabetes were evaluated. The subjects ingested 1800 IU of vitamin E/day or placebo for 4 months and measured blood flow in the retina by fluorescein angiography and renal function by normalized creatinine clearance in urine samples. Treatment with vitamin E appears effective in normalizing hemodynamic abnormalities of the retina and improving renal function in patients with type 1 DM. The authors report no toxicity at the dose administered [66]. The administration of vitamin C alone or in combination with vitamin E has been recommended to decrease microalbuminuria. In a short study with small sample performed in type 1 DM patients with <10 years history of the illness who received a dose of 1800 IU/d of vitamin E, the authors demonstrated restoration of the renal function [65]. However, in the cardiac study (HOPE) with 4 years of follow-up where they evaluated the prevention in 3600 diabetic patients who received vitamin E supplements in a dose of 400 IU/day, they did not find a significant reduction in cardiovascular risk [67].

1.6.6. Alpha-Lipoic Acid. Lipoic acid is a derivative of the short-chain fatty acid octanoic acid. The α-lipoic acid is a coenzyme produced endogenously that undertakes an essential role in the reactions of the mitochondrial dehydrogenase. The production source of lipoic acid is the intestinal medium. The lipoic acid bound to the food proteins and can be released by an intestinal amidase. Some bacteria encode an amidase called lipoamidasa capable of releasing lipoic acid from the intact 2-oxo acid dehydrogenase complexes. In fact, a lipoamidasa enzyme activity must be responsible for being free and available lipoic acid in nature [68]. The α-lipoic acid or its reduced form (dihydrolipoic acid) extinguishes various ROS in the lipid phase and in the aqueous phase on chelating transition metals and preventing lipid peroxidation of the membrane and protein damage through interactions with vitamin C and glutathione. The α-lipoic acid participates in the recycling of vitamins C and E by increasing the cellular levels of glutathione and suppressing the nonenzymatic glycation [69]. Treatment with α-lipoic acid reduces the markers of OS in the plasma of patients with DM with deficient control of glycemia [70]. Experimental studies in rats reported that the lipoic acid synthetase (Lias) deficiency induces manifested DN with production of microalbuminuria, thickening of the basal glomerular membrane, proliferation of the mesangial matrix, and hypertension compared to the diabetic controls without Lias deficiency [71]. Wang et al. treated diabetic rats with α-lipoic acid and demonstrated that in serum and renal cortex, the content of malondialdehyde (MDA), the activity of the superoxide dismutase (SOD), and the mitochondrial swelling were significantly reduced and the mitochondrial membrane potential significantly increased compared to the group of diabetic rats without the α-lipoic acid [72].

1.6.7. Coenzyme Q10. The 2,3-dimethoxy-5-methyl-6-decaprenyl-benzoquinone is also called coenzyme Q10 or ubiquinone. The Q refers to the chemical quinone group and 10 to the number of chemical subunits of isoprene in the tail of the molecule. Coenzyme Q10 is present in mitochondria in the majority of the eukaryotic cells. Coenzyme Q10 is a vitamin-like substance; it is lipid soluble in nature and hydrophobic interior of the phospholipid bilayer of the cell membrane. Coenzyme Q10 exists in a wide range of dietary items including meat, fish, vegetable oils, and nuts and has a potent anti-inflammatory, antiulcer, antioxidant, and antidiabetic activity [73–76]. Coenzyme Q10 is an important component in the transport of electrons and participates in the cellular aerobic respiration generating energy in the ATP form. Ninety-five percent of the energy in the human body is generated by the mitochondria [77]. Coenzyme Q10 prevents alterations in function and mitochondrial morphology, glomerular hyperfiltration, and proteinuria in

diabetic rats, emphasizing the role of the mitochondria in the pathogenesis of DN and the benefits in preventing the increase in OS [78]. However, its effect on the prevention of glomerulosclerosis is controversial [79]. An experimental study in diabetic rats treated with coenzyme Q10, metformin, or coenzyme Q10 + metformin showed significant decrease in MDA levels ($p < 0.01$, $p < 0.05$, and $p < 0.001$), respectively, versus diabetic rats not treated. Coenzyme Q10 or coenzyme Q10 + metformin showed significant increase in the activity of antioxidant enzymes SOD and catalase ($p < 0.001$) [80]. On the other hand, segmental focal glomerulosclerosis and collapsed glomerulopathy are common causes of nephrotic syndrome. The PDSS2 gene is required for the synthesis of the decaprenyl tail of coenzyme Q10 in humans. The deficiency manifests itself in the lymphoblastoid cell lines [81].

1.6.8. Resveratrol. The resveratrol, 3,5,4′-trihydroxy-trans-stilbene, is a polyphenolic phytoalexin that is found in natural form in many plants such as grapes, berries, red wine, and legumes, presenting numerous health benefits [82]. The resveratrol is one of the most important natural stilbenes that has demonstrated having health promoter properties in possessing antioxidant, anti-inflammatory, cardioprotector, antidiabetic, anticancerous, chemoprotector, and neuroprotector effects [83]. Several studies once have informed on the potential health benefits with resveratrol in cardiac and kidney diseases [84]. The resveratrol has similar properties to insulin in DM. It has the capacity to protect the cells against OS in exhibiting concurrent anti- and proinflammatory effects. Resveratrol positively regulates the expression and activation of the AMP-activated protein kinase (AMPK), which can contribute to the beneficial effects in DN in the early stages [85].

1.6.9. Inhibitors of the HMG-CoA Reductase. Inhibitors of the 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase or "statins" are potent inhibitors of the biosynthesis of cholesterol. Statins are very useful for the treatment of patients with dyslipidemia, although the effect of the statins in patients with DN is controversial. The HMG-CoA reductase inhibitors could be key medications in the presence of low-grade inflammation and endothelial dysfunction in DN, by reducing the proinflammatory pathways [86]. Clinical studies performed to look for the beneficial effect of statins on kidney function in DN are still controversial. In a subanalysis, it was discovered that the management with 10 mg and 80 mg of atorvastatin increases the glomerular filtration rate [87], while in the test of preventive intervention in kidney disease and ESRD (PREVEND-IT), the treatment with 40 mg of pravastatin did not improve the GFR [88]. In a recent meta-analysis, the authors published that statins significantly reduce albuminuria, the rate of excretion of urinary albumin, that the efficacy of renal function is dependent on the length of history of DM, and that the effect is better in type 2 DM with nephropathy [89].

1.6.10. Inhibitors of COX-2 and Aspirin. The inhibitors of cyclooxygenase-2 (COX-2) are the primary anti-inflammatory agents. In a reported study, the authors suggested that the administration of aspirin could diminish albuminuria in patients with DN [90]. As well, it was reported that the combination of aspirin and AT1 receptor blockers decreased the progression of DN and inflammatory markers compared to the treatment with aspirin alone [91]. The COX-2 inhibitors could increase the renal hemodynamics by diminishing the expression of the profibrotic cytokines [92]. However, treatment with 200 mg/d of the COX-2 inhibitor for six weeks did not demonstrate a decrease in DN [93]. Therefore, the administration of COX-2 inhibitors for the treatment of DN continues to be controversial.

1.7. Diabetic Nephropathy Treatment Future Perspectives

1.7.1. Nuclear Factor-Like 2 NFE2. The nuclear factor-like 2 NFE2 (Nrf2) is considered the master regulator of the response of cellular detoxification and of the redox state. Nrf2 provides protective action for diverse damages caused by OS [94]. After cellular exposure to OS, Nrf2 free of Kelch-like ECH-associated protein (KEAP1) translocates to the nucleus and binds to elements sensitive to antioxidants in the genes that code for antioxidant enzymes like the NADPH quinone dehydrogenase (NQO1), glutathione S-transferase, heme oxygenase-1 (HO1), and γ-glutamil-cysteine-synthetase, increasing its expression to undertake a role of detoxification as an antioxidant and anti-inflammatory effects [95]. Therefore, the positive regulation of the expression and function of the Nrf2 by different methods could provide preventative effects on the oxidative damage induced by DM in DN. The first study in which the activator Nrf2 was used to prevent the damage induced by hyperglycemia was done by Yang et al. These authors used sulforaphane (SNF) [1-isothiocyanato-4-(methylsulfinyl)-butane] [96]. Sulforaphane is an isothiocyanate of natural origin produced by cruciferous vegetables such as broccoli. Sulforaphane has the ability to induce the nuclear translocation of Nrf2 with significant increase of the antioxidant genes downstream, (expression > 3–5 times of transketolase and glutathione reductase) [97]. The treatment with SNF significantly impeded the hyperglycemia from increasing the formation of ROS and the activation of the hexosamine and PKC pathways [98].

1.7.2. Adiponectin. The adiponectin is a hormone of 244 amino acids with a weight of 30 kDa produced by the adipocytes through the apM1 gene. The adiponectin structure is similar to the collagens VIII and X and the complement factor C1q. The hormone exists as a multimerer in the circulation of proteins of low, medium, and high molecular weights [99]. Two types of receptors have been reported for adiponectin (adipoR1 and adipoR2) in the skeletal muscle, liver, and endothelial cells. Its function includes the anti-atherogenesis, anti-inflammation, and sensitization to insulin [100]. The adiopoR1 receptor mediates the increase in protein kinase activated by the adenosine monophosphate 5′ (AMPK). adiopR2 is capable of activating the receptor for the peroxisome proliferator-activated receptor alpha (PPARα) [101]. It seems that the adiponectin of high

molecular weight improves the sensitivity to insulin more than the adiponectin of low molecular weight [102]. Kacso et al. studied patients with type 2 DM over one year and found that when the levels of adiponectin were low, they predicted progression of the kidney disease characterized by increase in the albumin/creatinine relationship in urine [103]. Adiponectin is found in breast milk, fish intake, Mediterranean diet, coffee consumption, and omega 3 [104, 105].

1.7.3. Inhibition of the NF-κB. Several lines of evidence support the fundamental role of the transcriptional factor NF-κB in the development of DN in the mesangial cells [106], in the glomerular endothelial cells [107], and in the podocytes [108]. Inhibition of the transcriptional factor NF-κB in the kidney using the peroxisome (proliferator-activated receptor-γ (PPAR-γ)) has the ability to improve DN in animal models [109]. Pioglitazone regulates the phosphorylation of p66 (Shc) by integrating many signaling pathways that affect mitochondrial function, by reducing protein kinase C-beta, and the PPAR-γ not only improve the metabolic alterations of DM and DN, they also protect against nondiabetic CKD in experimental models and could benefit aging-related renal injury by improving mitochondrial function [110]. Nevertheless, there is no existing clear evidence on the efficacy of inhibiting the NF-κB factor to slow the progression of DN [111]. The PPAR-γ and NF-κB could signify an interesting therapeutic target.

1.7.4. Rapamycin. The objective of the mammalian rapamycin (mTOR) is a serine/threonine-specific kinase that mediates cellular proliferation, survival, and size of the mass [112]. The rapamycin reduces the activity of mTOR that is augmented in the hyperglycemic state and mediates the renal changes in DN by favoring the mesangial proliferation [113]. The mTOR inhibitors are risk factors for DN because they cause hyperglycemia. Reports inform on their efficacy in the treatment of DN [114]. It should be considered that sirolimus inhibits the phosphorylation induced by the glucose of p70S6 kinase and its substrate, ribosomal protein S6 in mesangial cells. The inhibition of mTOR with sirolimus attenuates the morphogic and functional disturbances of the kidneys of diabetic patients, according to reports from a type 2 DM model in rats [115]. The rapamycin significantly reduces the accumulation of inflammatory cells, including monocytes and macrophages associated with the progression of DN, and it also reduces the liberation of proinflammatory cytokines and chemokines, including MCP-1, normal and secreted regulated T cells (RANTES), and IL-8 [116]. Thus, the administration of rapamycin is useful as an anti-inflammatory medication, which could suggest an attractive therapeutic treatment in the management of DN.

1.7.5. Inhibition of PKC Activation. The hyperglycemic state and resistance to insulin induce the activation of the PKC. Activation of the PKC alters the molecules of cellular signaling, including the proinflammatory cytokines like the NF-κB, IL-6, and TNF-α and plasminogen activator inhibitor-1 (PAI-1) in the vascular cells, including the endothelial and mesangial cells [117]. It has been shown that ruboxistaurin (RBX), a selective inhibitor of the PKCβ isoform, prevents DN in rodent models through the inhibition of the accumulation of the ECM and the TGF-β by improving insulin signaling [118]. The *nuls* diabetic rats with PKCβ demonstrated a decrease in albuminuria and mesangial proliferation [119]. In a phase II clinical trial, it was demonstrated that the treatment with RBX in DM significantly diminished albuminuria and maintained the estimated GFR (eGFR) stable. Hyperglycemia can activate the isoforms of PKCβ that potentiate the toxic effect of Ang II in glomerular endothelial cells and decrease receptor 1 of the glucagon-like peptide (GLP-1), which leads to resistance to treatment with GLP-1 [120].

1.7.6. Inhibitors of Sodium-Glucose Cotransporter-2 (SGLT-2). The role of the kidney in glucose homeostasis has led to the development of sodium-glucose cotransporter-2 inhibitors (SGLT-2). SGLT-2 is a new class of antiglycemic drugs with the ability to reduce blood glucose by inhibiting sodium-glucose transporter in the proximal tubule of the kidney by improving renal excretion of glucose. Several of these inhibitors have been commercialized for treatment of hyperglycemia in patients with type 2 DM [121]. The treatment with SGLT2 has shown improvements in glycosylated hemoglobin (HbA1c), reduction in body weight, and moderate decrease in blood pressure [122]. SGLT2i has the ability to reduce albuminuria thereby reducing renal risk in the DN [51]. In patients with type 2 DM and stage 3 CKD, 100 mg/day of canagliflozin reduces albuminuria ~22% [123]. A study in patients with DM and hypertension who received renin blockers angiotensin aldosterone and dapagliflozin 10 mg/day showed ~35% reduction in albuminuria compared to placebo. The reduction was independent of changes in HbA1c, systolic blood pressure, body weight, or eGFR [124]. The decrease in serum uric acid is another nephroprotective mechanism of SGLT2i. The high levels of uric acid correlate highly with the risk of kidney damage and microvascular disease in DM [125]. Recently, it was announced that the administration of empagliflozine is associated with a significant reduction of the progression of kidney disease, including the rate of decrease of the eGFR, the progression of albuminuria, and the onset of renal replacement therapy [57]. The usefulness of SGLT-2 is still not well defined.

1.7.7. Inhibitors of Dipeptidyl Peptidase 4 (DPP-4) and Glucagon-Like Peptide 1 (GLP-1). The inhibitors of dipeptidyl peptidase 4 (DPP-4) are oral hypoglycemic that are useful in treating patients with type 2 DM. DPP-4 cleaves polypeptides from the amine terminal position, with a proline/alanine in the penultimate position. Therefore, the net physiological effect is a complex interaction between the resulting substrate and product profile in an environment of particular illness (instead of a specific signaling pathway). The cut substrates can activate or inactivate without having relevant function. The DPP-4 inhibitors reduce the levels of glucose in the blood increasing the half-life of the endogenous incretins like GLP-1 and the glucose-dependent insulinotropic polypeptide. The capacity of DPP-4 to cleave an additional quantity of

substrate bound to the membrane that exercises nonenzymatic properties by the interaction or colocalization of other proteins/membrane receptors suggests that they could be a novel and stimulating therapeutic objective in DN [126].

2. Conclusion

Diabetic nephropathy is a microangiopathy that is prevalent in patients with type 1 and type 2 DM. The mechanism that unchains DN is still not entirely known. OS is a common and important factor that links hyperglycemia with the vascular complications through the metabolic changes to the molecules of the renal tissues and alterations in kidney hemodynamics. The persistent OS in the state of hyperglycemia favors oxidative DNA damage that is capable of producing damage to the genetic material mtDNA and to the nDNA upon favoring cell death by apoptosis. The kidney cells are predominantly susceptible to the hyperglycemic assault, resulting in greater flow of intracellular glucose and accelerating the oxidative phosphorylation of the mitochondria with the excessive leakage of O^{2-} and the significant decrease in the production of ATP. In patients with DN, it is convenient to stimulate the production of endogenous antioxidants or the administration of exogenous antioxidants that act as adjuvants to the management of the underlying pathology.

Conflicts of Interest

There are no conflicts of interest to report.

References

[1] International Diabetes Federation, "Atlas of diabetes," Seventh edition, 2015, December 2017, http://www.diabetesatlas.org.

[2] World Health Organization, "Diabetes programme," November 2017, http://www.who.int/diabetes/action_online/basics/en/index3.html.

[3] V. M. Muthuppalaniappan, M. Sheaff, and M. M. Yaqoob, "Diabetic nephropathy," *Medicine*, vol. 43, no. 9, pp. 520–525, 2015.

[4] V. Batuman, A. S. Soman, R. J. Schmidt, and S. S. Soman, "Diabetic nephropathy," Medscape. November 2017, https://emedicine.medscape.com/article/238946-overview.

[5] National Institute of Diabetes and Digestive and Kidney Diseases, "Diabetic kidney disease," November 2017, https://www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/diabetic-kidney-disease.

[6] Federación Mexicana de DiabetesNovember 2017, http://fmdiabetes.org/diabetes-México/.

[7] United States Renal Data System (USRDS)November 2017, https://www.usrds.org/.

[8] J. L. Gross, M. J. de Azevedo, S. P. Silveiro, L. H. Canani, M. L. Caramori, and T. Zelmanovitz, "Diabetic nephropathy: diagnosis, prevention, and treatment," *Diabetes Care*, vol. 28, no. 1, pp. 164–176, 2005.

[9] J. E. Grunwald, J. Alexander, G. S. Ying et al., "Retinopathy and chronic kidney disease in the Chronic Renal Insufficiency Cohort (CRIC) study," *Archives of Ophthalmology*, vol. 130, no. 9, pp. 1136–1144, 2012.

[10] A. Martínez-Castelao, J. Navarro-González, J. Górriz, and F. de Alvaro, "The concept and the epidemiology of diabetic nephropathy have changed in recent years," *Journal of Clinical Medicine*, vol. 4, no. 12, pp. 1207–1216, 2015.

[11] C. E. Mogensen, C. K. Christensen, and E. Vittinghus, "The stages in diabetic renal disease: with emphasis on the stage of incipient diabetic nephropathy," *Diabetes*, vol. 32, Supplement 2, pp. 64–78, 1983.

[12] M. Haneda, K. Utsunomiya, D. Koya et al., "A new classification of diabetic nephropathy 2014: a report from joint committee on diabetic nephropathy," *Clinical and Experimental Nephrology*, vol. 19, no. 1, pp. 1–5, 2015.

[13] P. Rossing, "Diabetic nephropathy: worldwide epidemic and effects of current treatment on natural history," *Current Diabetes Reports*, vol. 6, no. 6, pp. 479–483, 2006.

[14] P. J. Oates, "Polyol pathway and diabetic peripheral neuropathy," *International Review of Neurobiology*, vol. 50, pp. 325–392, 2002.

[15] D. Koya, M. R. Jirousek, Y. W. Lin, H. Ishii, K. Kuboki, and G. L. King, "Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats," *The Journal of Clinical Investigation*, vol. 100, no. 1, pp. 115–126, 1997.

[16] J. M. Forbes, V. Thallas, M. C. Thomas et al., "The breakdown of preexisting advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes," *The FASEB Journal*, vol. 17, no. 12, pp. 1762–1764, 2003.

[17] G. Manda, A. I. Checherita, M. V. Comanescu, and M. E. Hinescu, "Redox signaling in diabetic nephropathy: hypertrophy versus death choices in mesangial cells and podocytes," *Mediators of Inflammation*, vol. 2015, Article ID 604208, 13 pages, 2015.

[18] H. B. Lee, M. R. Yu, Y. Yang, Z. Jiang, and H. Ha, "Reactive oxygen species-regulated signaling pathways in diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 14, pp. S241–S245, 2003.

[19] F. Madeo, E. Fröhlich, M. Ligr et al., "Oxygen stress: a regulator of apoptosis in yeast," *The Journal of Cell Biology*, vol. 145, no. 4, pp. 757–767, 1999.

[20] J. M. Forbes, M. T. Coughlan, and M. E. Cooper, "Oxidative stress as a major culprit in kidney disease in diabetes," *Diabetes*, vol. 57, no. 6, pp. 1446–1454, 2008.

[21] M. R. Duchen, "Roles of mitochondria in health and disease," *Diabetes*, vol. 53, Supplement 1, pp. S96–S102, 2004.

[22] A. Ceriello, A. Morocutti, F. Mercuri et al., "Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy," *Diabetes*, vol. 49, no. 12, pp. 2170–2177, 2000.

[23] J. W. Baynes and S. R. Thorpe, "Role of oxidative stress in diabetic complications: a new perspective on an old paradigm," *Diabetes*, vol. 48, no. 1, pp. 1–9, 1999.

[24] F. Addabbo, M. Montagnani, and M. S. Goligorsky, "Mitochondria and reactive oxygen species," *Hypertension*, vol. 53, no. 6, pp. 885–892, 2009.

[25] D. Voet, J. G. Voet, and C. W. Pratt, "Fundamentals of biochemistry: life at the molecular level," *Chemistry & Biochemistry*, 2016.

[26] B. Westermann, "Mitochondrial fusion and fission in cell life and death," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 12, pp. 872–884, 2010.

[27] S. M. Shenouda, M. E. Widlansky, K. Chen et al., "Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus," *Circulation*, vol. 124, no. 4, pp. 444–453, 2011.

[28] I. Hwang, J. Lee, J. Y. Huh et al., "Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction," *Diabetes*, vol. 61, no. 3, pp. 728–738, 2012.

[29] P. Fernyhough, T. J. Huang, and A. Verkhratsky, "Mechanism of mitochondrial dysfunction in diabetic sensory neuropathy," *Journal of the Peripheral Nervous System*, vol. 8, no. 4, pp. 227–235, 2003.

[30] L. A. Esposito, S. Melov, A. Panov, B. A. Cottrell, and D. C. Wallace, "Mitochondrial disease in mouse results in increased oxidative stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 9, pp. 4820–4825, 1999.

[31] M. R. Lieber and Z. E. Karanjawala, "Ageing, repetitive genomes and DNA damage," *Nature Reviews Molecular Cell Biology*, vol. 5, no. 1, pp. 69–75, 2004.

[32] S. B. Hollensworth, C. C. Shen, J. E. Sim, D. R. Spitz, G. L. Wilson, and S. P. LeDoux, "Glial cell type-specific responses to menadione-induced oxidative stress," *Free Radical Biology & Medicine*, vol. 28, no. 8, pp. 1161–1174, 2000.

[33] S. Srinivasan, M. Stevens, and J. W. Wiley, "Diabetic peripheral neuropathy: evidence for apoptosis and associated mitochondrial dysfunction," *Diabetes*, vol. 49, no. 11, pp. 1932–1938, 2000.

[34] P. Ghafourifar, U. Schenk, S. D. Klein, and C. Richter, "Mitochondrial nitric-oxide synthase stimulation causes cytochrome*c* release from isolated mitochondria. Evidence for intramitochondrial peroxynitrite formation," *Journal of Biological Chemistry*, vol. 274, no. 44, pp. 31185–31188, 1999.

[35] A. P. Grollman and M. Moriya, "Mutagenesis by 8-oxoguanine: an enemy within," *Trends in Genetics*, vol. 9, no. 7, pp. 246–249, 1993.

[36] S. Loft, K. Vistisen, M. Ewertz, A. Tjønneland, K. Overvad, and H. E. Poulsen, "Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index," *Carcinogenesis*, vol. 13, no. 12, pp. 2241–2247, 1992.

[37] D. P. Lane, "p53, guardian of the genome," *Nature*, vol. 358, no. 6381, pp. 15-16, 1992.

[38] G. W. Xu, Q. H. Yao, Q. F. Weng, B. L. Su, X. Zhang, and J. H. Xiong, "Study of urinary 8-hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in diabetic nephropathy patients," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 36, no. 1, pp. 101–104, 2004.

[39] Y. Hinokio, S. Suzuki, M. Hirai, C. Suzuki, M. Suzuki, and T. Toyota, "Urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine as a predictor of the development of diabetic nephropathy," *Diabetologia*, vol. 45, no. 6, pp. 877–882, 2002.

[40] R. A. Schwartzman and J. A. Cidlowski, "Apoptosis: the biochemistry and molecular biology of programmed cell death," *Endocrine Reviews*, vol. 14, no. 2, pp. 133–151, 1993.

[41] D. A. Allen, S. Harwood, M. Varagunam, M. J. Raftery, and M. M. Yaqoob, "High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases," *The FASEB Journal*, vol. 17, no. 8, pp. 908–910, 2003.

[42] S. Simone, Y. Gorin, C. Velagapudi, H. E. Abboud, and S. L. Habib, "Mechanism of oxidative DNA damage in diabetes: tuberin inactivation and downregulation of DNA repair enzyme 8-oxo-7,8-dihydro-2′-deoxyguanosine-DNA glycosylase," *Diabetes*, vol. 57, no. 10, pp. 2626–2636, 2008.

[43] E. Adeghate, "Molecular and cellular basis of the aetiology and management of diabetic cardiomyopathy: a short review," *Molecular and Cellular Biochemistry*, vol. 261, no. 1, pp. 187–191, 2004.

[44] W. Droge, "Free radicals in the physiological control of cell function," *Physiological Reviews*, vol. 82, no. 1, pp. 47–95, 2002.

[45] T. H. Hostetter, "Prevention of end-stage renal disease due to type 2 diabetes," *The New England Journal of Medicine*, vol. 345, no. 12, pp. 910–912, 2001.

[46] I. H. de Boer, S. D. Sibley, B. Kestenbaum et al., "Central obesity, incident microalbuminuria, and change in creatinine clearance in the epidemiology of diabetes interventions and complications study," *Journal of the American Society of Nephrology*, vol. 18, no. 1, pp. 235–243, 2007.

[47] M. Bojestig, H. J. Arnqvist, G. Hermansson, B. E. Karlberg, and J. Ludvigsson, "Declining incidence of nephropathy in insulin-dependent diabetes mellitus," *The New England Journal of Medicine*, vol. 330, no. 1, pp. 15–18, 1994.

[48] I. H. de Boer and for the DCCT/EDIC Research Group, "Kidney disease and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study," *Diabetes Care*, vol. 37, no. 1, pp. 24–30, 2014.

[49] P. Ruggenenti, P. Cravedi, and G. Remuzzi, "The RAAS in the pathogenesis and treatment of diabetic nephropathy," *Nature Reviews Nephrology*, vol. 6, no. 6, pp. 319–330, 2010.

[50] F. T. Lee, Z. Cao, D. M. Long et al., "Interactions between angiotensin II and NF-κB-dependent pathways in modulating macrophage infiltration in experimental diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 15, no. 8, pp. 2139–2151, 2004.

[51] D. de Zeeuw, G. Remuzzi, H. H. Parving et al., "Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL," *Kidney International*, vol. 65, no. 6, pp. 2309–2320, 2004.

[52] J. F. Navarro and C. Mora, "Antiproteinuric effect of pentoxifylline in patients with diabetic nephropathy," *Diabetes Care*, vol. 22, no. 6, pp. 1006–1008, 1999.

[53] J. F. Navarro, C. Mora, M. Muros, M. Macıéa, and J. Garcıéa, "Effects of pentoxifylline administration on urinary *N*-acetyl-β-glucosaminidase excretion in type 2 diabetic patients: a short-term, prospective, randomized study," *American Journal of Kidney Diseases*, vol. 42, no. 2, pp. 264–270, 2003.

[54] J. F. Navarro, C. Mora, M. Muros, and J. García, "Additive antiproteinuric effect of pentoxifylline in patients with type 2 diabetes under angiotensin II receptor blockade: a short-term, randomized, controlled trial," *Journal of the American Society of Nephrology*, vol. 16, no. 7, pp. 2119–2126, 2005.

[55] G. M. Doherty, J. C. Jensen, H. R. Alexander, C. M. Buresh, and J. A. Norton, "Pentoxifylline suppression of tumor necrosis factor gene transcription," *Surgery*, vol. 110, no. 2, pp. 192–198, 1991.

[56] A. Cooper, A. Mikhail, M. W. Lethbridge, D. M. Kemeny, and I. C. Macdougall, "Pentoxifylline improves hemoglobin levels in patients with erythropoietin-resistant anemia in renal failure," *Journal of the American Society of Nephrology*, vol. 15, no. 7, pp. 1877–1882, 2004.

[57] F. Kouoh, B. Gressier, M. Luyckx et al., "Antioxidant properties of albumin: effect on oxidative metabolism of human neutrophil granulocytes," *Il Farmaco*, vol. 54, no. 10, pp. 695–699, 1999.

[58] R. Medina-Navarro, I. Corona-Candelas, S. Barajas-González, M. Díaz-Flores, and G. Durán-Reyes, "Albumin antioxidant response to stress in diabetic nephropathy progression," *PLoS One*, vol. 9, no. 9, article e106490, 2014.

[59] P. S. Lim, Y. M. Cheng, and S. M. Yang, "Impairments of the biological properties of serum albumin in patients on haemodialysis," *Nephrology*, vol. 12, no. 1, pp. 18–24, 2007.

[60] N. Yamada, A. Nakayama, K. Kubota, A. Kawakami, and E. Suzuki, "Structure and function changes of oxidized human serum albumin: physiological significance of the biomarker and importance of sampling conditions for accurate measurement," *Rinsho Byori*, vol. 56, no. 5, pp. 409–415, 2008.

[61] R. Medina-Navarro, G. Durán-Reyes, M. Díaz-Flores, and C. Vilar-Rojas, "Protein antioxidant response to the stress and the relationship between molecular structure and antioxidant function," *PLoS One*, vol. 5, no. 1, article e8971, 2010.

[62] M. Lamprecht, J. F. Greilberger, G. Schwaberger, P. Hofmann, and K. Oettl, "Single bouts of exercise affect albumin redox state and carbonyl groups on plasma protein of trained men in a workload-dependent manner," *Journal of Applied Physiology*, vol. 104, no. 6, pp. 1611–1617, 2008.

[63] L. Turell, H. Botti, S. Carballal et al., "Reactivity of sulfenic acid in human serum albumin," *Biochemistry*, vol. 47, no. 1, pp. 358–367, 2008.

[64] P. Gaede, H. E. Poulsen, H. H. Parving, and O. Pedersen, "Double-blind, randomised study of the effect of combined treatment with vitamin C and E on albuminuria in type 2 diabetic patients," *Diabetic Medicine*, vol. 18, no. 9, pp. 756–760, 2001.

[65] U. Milman, S. Blum, C. Shapira et al., "Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype: a prospective double-blinded clinical trial," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 2, pp. 341–347, 2008.

[66] S. E. Bursell, A. C. Clermont, L. P. Aiello et al., "High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes," *Diabetes Care*, vol. 22, no. 8, pp. 1245–1251, 1999.

[67] Heart Outcomes Prevention Evaluation Study Investigators, S. Yusuf, G. Dagenais, J. Pogue, J. Bosch, and P. Sleight, "Vitamin E supplementation and cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators," *The New England Journal of Medicine*, vol. 342, no. 3, pp. 154–160, 2000.

[68] J. E. Cronan, "Assembly of lipoic acid on its cognate enzymes: an extraordinary and essential biosynthetic pathway," *Microbiology and Molecular Biology Reviews*, vol. 80, no. 2, pp. 429–450, 2016.

[69] L. Packer, E. H. Witt, and H. J. Tritschler, "Alpha-lipoic acid as a biological antioxidant," *Free Radical Biology & Medicine*, vol. 19, no. 2, pp. 227–250, 1995.

[70] V. Borcea, J. Nourooz-Zadeh, S. P. Wolff et al., "α-Lipoic acid decreases oxidative stress even in diabetic patients with poor glycemic control and albuminuria," *Free Radical Biology & Medicine*, vol. 26, no. 11-12, pp. 1495–1500, 1999.

[71] X. Yi, L. Xu, S. Hiller et al., "Reduced expression of lipoic acid synthase accelerates diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 23, no. 1, pp. 103–111, 2012.

[72] L. Wang, C. G. Wu, C. Q. Fang et al., "The protective effect of α-lipoic acid on mitochondria in the kidney of diabetic rats," *International Journal of Clinical and Experimental Medicine*, vol. 6, no. 2, pp. 90–97, 2013.

[73] M. Kamei, T. Fujita, T. Kanbe et al., "The distribution and content of ubiquinone in foods," *International Journal for Vitamin and Nutrition Research*, vol. 56, no. 1, pp. 57–63, 1986.

[74] C. Schmelzer, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke, and F. Döring, "Functions of coenzyme Q_{10} in inflammation and gene expression," *BioFactors*, vol. 32, no. 1-4, pp. 179–183, 2008.

[75] Y. Kohli, Y. Suto, and T. Kodama, "Effect of hypoxia on acetic acid ulcer of the stomach in rats with or without coenzyme Q10," *The Japanese Journal of Experimental Medicine*, vol. 51, no. 2, pp. 105–108, 1981.

[76] G. Lenaz, R. Fato, G. Formiggini, and M. L. Genova, "The role of coenzyme Q in mitochondrial electron transport," *Mitochondrion*, vol. 7, pp. S8–S33, 2007.

[77] L. Ernster and G. Dallner, "Biochemical, physiological and medical aspects of ubiquinone function," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1271, no. 1, pp. 195–204, 1995.

[78] M. F. Persson, S. Franzén, S. B. Catrina et al., "Coenzyme Q10 prevents GDP-sensitive mitochondrial uncoupling, glomerular hyperfiltration and proteinuria in kidneys from *db*/*db* mice as a model of type 2 diabetes," *Diabetologia*, vol. 55, no. 5, pp. 1535–1543, 2012.

[79] K. C. Sourris, B. E. Harcourt, P. H. Tang et al., "Ubiquinone (coenzyme Q10) prevents renal mitochondrial dysfunction in an experimental model of type 2 diabetes," *Free Radical Biology & Medicine*, vol. 52, no. 3, pp. 716–723, 2012.

[80] R. A. Maheshwari, R. Balaraman, A. K. Sen, and A. K. Seth, "Effect of coenzyme Q10 alone and its combination with metformin on streptozotocin-nicotinamide-induced diabetic nephropathy in rats," *Indian Journal of Pharmacology*, vol. 46, no. 6, pp. 627–632, 2014.

[81] D. L. Gasser, C. A. Winkler, M. Peng et al., "Focal segmental glomerulosclerosis is associated with a *PDSS2* haplotype and, independently, with a decreased content of coenzyme Q_{10}," *American Journal of Physiology-Renal Physiology*, vol. 305, no. 8, pp. F1228–F1238, 2013.

[82] A. A. A. Bertelli and D. K. Das, "Grapes, wines, resveratrol, and heart health," *Journal of Cardiovascular Pharmacology*, vol. 54, no. 6, pp. 468–476, 2009.

[83] P. Brasnyó, G. A. Molnár, M. Mohás et al., "Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients," *The British Journal of Nutrition*, vol. 106, no. 3, pp. 383–389, 2011.

[84] T. P. Kondratyuk, E. J. Park, L. E. Marler et al., "Resveratrol derivatives as promising chemopreventive agents with

improved potency and selectivity," *Molecular Nutrition & Food Research*, vol. 55, no. 8, pp. 1249–1265, 2011.

[85] C. C. Chang, C. Y. Chang, Y. T. Wu, J. P. Huang, T. H. Yen, and L. M. Hung, "Resveratrol retards progression of diabetic nephropathy through modulations of oxidative stress, proinflammatory cytokines, and AMP-activated protein kinase," *Journal of Biomedical Science*, vol. 18, no. 1, p. 47, 2011.

[86] R. Ross, "Atherosclerosis — an inflammatory disease," *The New England Journal of Medicine*, vol. 340, no. 2, pp. 115–126, 1999.

[87] J. Shepherd, J. J. Kastelein, V. Bittner et al., "Intensive lipid lowering with atorvastatin in patients with coronary heart disease and chronic kidney disease: the TNT (Treating to New Targets) study," *Journal of the American College of Cardiology*, vol. 51, no. 15, pp. 1448–1454, 2008.

[88] F. P. Brouwers, F. W. Asselbergs, H. L. Hillege et al., "Long-term effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria: ten years of follow-up of Prevention of Renal and Vascular End-stage Disease Intervention Trial (PREVEND IT)," *American Heart Journal*, vol. 161, no. 6, pp. 1171–1178, 2011.

[89] X. Shen, Z. Zhang, X. Zhang et al., "Efficacy of statins in patients with diabetic nephropathy: a meta-analysis of randomized controlled trials," *Lipids in Health and Disease*, vol. 15, no. 1, p. 179, 2016.

[90] A. H. Hopper, H. Tindall, and J. A. Davies, "Administration of aspirin-dipyridamole reduces proteinuria in diabetic nephropathy," *Nephrology Dialysis Transplantation*, vol. 4, no. 2, pp. 140–143, 1989.

[91] S. R. Mulay, A. B. Gaikwad, and K. Tikoo, "Combination of aspirin with telmisartan suppresses the augmented TGFβ/smad signaling during the development of streptozotocin-induced type I diabetic nephropathy," *Chemico-Biological Interactions*, vol. 185, no. 2, pp. 137–142, 2010.

[92] H. F. Cheng, C. J. Wang, G. W. Moeckel, M. Z. Zhang, J. A. McKanna, and R. C. Harris, "Cyclooxygenase-2 inhibitor blocks expression of mediators of renal injury in a model of diabetes and hypertension," *Kidney International*, vol. 62, no. 3, pp. 929–939, 2002.

[93] M. Sinsakul, M. Sika, R. Rodby et al., "A randomized trial of a 6-week course of celecoxib on proteinuria in diabetic kidney disease," *American Journal of Kidney Diseases*, vol. 50, no. 6, pp. 946–951, 2007.

[94] M. Kobayashi and M. Yamamoto, "Nrf2–Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species," *Advances in Enzyme Regulation*, vol. 46, no. 1, pp. 113–140, 2006.

[95] J. B. de Haan, "Nrf2 activators as attractive therapeutics for diabetic nephropathy," *Diabetes*, vol. 60, no. 11, pp. 2683-2684, 2011.

[96] L. Yang, D. L. Palliyaguru, and T. W. Kensler, "Frugal chemoprevention: targeting Nrf2 with foods rich in sulforaphane," *Seminars in Oncology*, vol. 43, no. 1, pp. 146–153, 2016.

[97] M. L. Pall and S. Levine, "Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors," *Acta Physiologica Sinica*, vol. 67, no. 1, pp. 1–18, 2015.

[98] M. Xue, Q. Qian, A. Adaikalakoteswari, N. Rabbani, R. Babaei-Jadidi, and P. J. Thornalley, "Activation of NF-E2–related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease," *Diabetes*, vol. 57, no. 10, pp. 2809–2817, 2008.

[99] M. Adamczak and A. Wiecek, "The adipose tissue as an endocrine organ," *Seminars in Nephrology*, vol. 33, no. 1, pp. 2–13, 2013.

[100] T. Kadowaki and T. Yamauchi, "Adiponectin and adiponectin receptors," *Endocrine Reviews*, vol. 26, no. 3, pp. 439–451, 2005.

[101] T. Yamauchi and T. Kadowaki, "Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases," *International Journal of Obesity*, vol. 32, no. S7, pp. S13–S18, 2008.

[102] F. F. M. Fisher, M. E. Trujillo, W. Hanif et al., "Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males," *Diabetologia*, vol. 48, no. 6, pp. 1084–1087, 2005.

[103] I. Kacso, A. Lenghel, C. I. Bondor et al., "Low plasma adiponectin levels predict increased urinary albumin/creatinine ratio in type 2 diabetes patients," *International Urology and Nephrology*, vol. 44, no. 4, pp. 1151–1157, 2012.

[104] D. A. Fields, C. R. Schneider, and G. Pavela, "A narrative review of the associations between six bioactive components in breast milk and infant adiposity," *Obesity*, vol. 24, no. 6, pp. 1213–1221, 2016.

[105] E. Z. Fisman and A. Tenenbaum, "Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease?," *Cardiovascular Diabetology*, vol. 13, no. 1, p. 103, 2014.

[106] S. Menini, L. Amadio, G. Oddi et al., "Deletion of p66^{Shc} longevity gene protects against experimental diabetic glomerulopathy by preventing diabetes-induced oxidative stress," *Diabetes*, vol. 55, no. 6, pp. 1642–1650, 2006.

[107] A. Mima, J. Hiraoka-Yamomoto, Q. Li et al., "Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCβ activation in diabetes," *Diabetes*, vol. 61, no. 11, pp. 2967–2979, 2012.

[108] A. Mima, M. Kitada, P. Geraldes et al., "Glomerular VEGF resistance induced by PKCδ/SHP-1 activation and contribution to diabetic nephropathy," *The FASEB Journal*, vol. 26, no. 7, pp. 2963–2974, 2012.

[109] F. Kamal, N. Yanakieva-Georgieva, H. Piao, T. Morioka, and T. Oite, "Local delivery of angiotensin II receptor blockers into the kidney passively attenuates inflammatory reactions during the early phases of streptozotocin-induced diabetic nephropathy through inhibition of calpain activity," *Nephron*, vol. 115, no. 3, pp. e69–e79, 2010.

[110] H. C. Yang, S. Deleuze, Y. Zuo, S. A. Potthoff, L. J. Ma, and A. B. Fogo, "The PPARγ agonist pioglitazone ameliorates aging-related progressive renal injury," *Journal of the American Society of Nephrology*, vol. 20, no. 11, pp. 2380–2388, 2009.

[111] J. Wu, T. J. Guan, S. Zheng et al., "Inhibition of inflammation by pentosan polysulfate impedes the development and progression of severe diabetic nephropathy in aging C57B6 mice," *Laboratory Investigation*, vol. 91, no. 10, pp. 1459–1471, 2011.

[112] W. Lieberthal and J. S. Levine, "The role of the mammalian target of rapamycin (mTOR) in renal disease," *Journal of*

the American Society of Nephrology, vol. 20, no. 12, pp. 2493–2502, 2009.

[113] K. Nagai, T. Matsubara, A. Mima et al., "Gas6 induces Akt/mTOR-mediated mesangial hypertrophy in diabetic nephropathy," *Kidney International*, vol. 68, no. 2, pp. 552–561, 2005.

[114] C. Velagapudi, B. S. Bhandari, S. Abboud-Werner, S. Simone, H. E. Abboud, and S. L. Habib, "The tuberin/mTOR pathway promotes apoptosis of tubular epithelial cells in diabetes," *Journal of the American Society of Nephrology*, vol. 22, no. 2, pp. 262–273, 2011.

[115] H. Mori, K. Inoki, K. Masutani et al., "The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential," *Biochemical and Biophysical Research Communications*, vol. 384, no. 4, pp. 471–475, 2009.

[116] Y. Yang, J. Wang, L. Qin et al., "Rapamycin prevents early steps of the development of diabetic nephropathy in rats," *American Journal of Nephrology*, vol. 27, no. 5, pp. 495–502, 2007.

[117] K. K. Yerneni, W. Bai, B. V. Khan, R. M. Medford, and R. Natarajan, "Hyperglycemia-induced activation of nuclear transcription factor kappaB in vascular smooth muscle cells," *Diabetes*, vol. 48, no. 4, pp. 855–864, 1999.

[118] A. Mima, Y. Ohshiro, M. Kitada et al., "Glomerular-specific protein kinase C-β-induced insulin receptor substrate-1 dysfunction and insulin resistance in rat models of diabetes and obesity," *Kidney International*, vol. 79, no. 8, pp. 883–896, 2011.

[119] Y. Ohshiro, R. C. Ma, Y. Yasuda et al., "Reduction of diabetes-induced oxidative stress, fibrotic cytokine expression, and renal dysfunction in protein kinase Cβ–null mice," *Diabetes*, vol. 55, no. 11, pp. 3112–3120, 2006.

[120] R. E. Gilbert, S. A. Kim, K. R. Tuttle et al., "Effect of ruboxistaurin on urinary transforming growth factor-β in patients with diabetic nephropathy and type 2 diabetes," *Diabetes Care*, vol. 30, no. 4, pp. 995-996, 2007.

[121] R. D. Toto, "SGLT-2 inhibition: a potential new treatment for diabetic kidney disease?," *Nephron*, vol. 137, no. 1, pp. 64–67, 2017.

[122] L. Zanoli, A. Granata, P. Lentini et al., "Sodium-glucose linked transporter-2 inhibitors in chronic kidney disease," *Scientific World Journal*, vol. 2015, article 317507, 6 pages, 2015.

[123] J.-F. Yale, G. Bakris, B. Cariou et al., "Efficacy and safety of canagliflozin in subjects with type 2 diabetes and chronic kidney disease," *Diabetes, Obesity & Metabolism*, vol. 15, no. 5, pp. 463–473, 2013.

[124] H. J. L. Heerspink, E. Johnsson, I. Gause-Nilsson, V. A. Cain, and C. D. Sjöström, "Dapagliflozin reduces albuminuria in patients with diabetes and hypertension receiving renin-angiotensin blockers," *Diabetes, Obesity & Metabolism*, vol. 18, no. 6, pp. 590–597, 2016.

[125] M. Chonchol, M. G. Shlipak, R. Katz et al., "Relationship of uric acid with progression of kidney disease," *American Journal of Kidney Diseases*, vol. 50, no. 2, pp. 239–247, 2007.

[126] U. Panchapakesan and C. A. Pollock, "DPP-4 inhibitors—renoprotection in diabetic nephropathy?," *Diabetes*, vol. 63, no. 6, pp. 1829-1830, 2014.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 6539385, 6 pages
https://doi.org/10.1155/2018/6539385

Research Article

Elevated Serum Total Bilirubin Concentrations Are Negatively Associated with Diabetic Retinopathy among the Chinese Northeastern Population

Dan Zhang, Wei Zhang, Shi Jin, Wei Wang, Dan Guo, and Lu Wang

Department of Endocrinology, Fourth Hospital of China Medical University, Shenyang 110032, China

Correspondence should be addressed to Lu Wang; doctorwanglu@163.com

Received 25 December 2017; Revised 11 February 2018; Accepted 13 February 2018; Published 21 March 2018

Academic Editor: Ilias Migdalis

Objective. To evaluate the association between serum total bilirubin concentration (STBC) and diabetic retinopathy (DR) among the Chinese northeastern population. *Methods*. A cross-sectional study was conducted in Liaoning between January 2015 and May 2017. *Results*. A total of 742 subjects (419 men and 323 women) with type 2 diabetes mellitus (DM) who visited an ophthalmic clinic were included in this study. The mean age of the subjects was 59.55 ± 10.63 years, and 43.5% of the subjects were women. The mean of DM duration was 11.01 ± 7.35 years. STBC were negatively correlated with DM duration, urea nitrogen, serum creatinine, uric acid, and urine microalbumin. After adjusting for confounding factors, as a continuous variable, STBC was inversely associated with the risk of DR in total subjects (OR: 0.95, 95% CI: 0.93–0.99). When STBC was used as a tertiary variable, compared with the first tertile, the OR in the third tertile was 0.37 (95% CI: 0.22–0.64) in total subjects. *Conclusion*. Our results demonstrate that a significant negative association was found between STBC and DR. STBC might be an early clinical marker for predicting the occurrence of DR.

1. Introduction

As an important noncommunicable disease, diabetes mellitus (DM) has become one of the major public health problems in the world. DM does not only have an important influence on physical health and the quality of life but can also bring about multiple complications and negatively affect a person's mental health and well-being [1]. Currently, due to population ageing, urbanization, and lifestyle changes, the number of people with DM has increased sharply all over the world [2]. The International Diabetes Federation (IDF) reported that there were about 382 million diabetic patients in 2013, and almost 600 million people would develop DM by 2035 [3]. In America, there were 29.1 million diabetic patients in 2014, and it is predicted that about 50% of all Americans will develop prediabetes or diabetes by 2020 [4]. By 2035, Asia will be the center of the DM epidemic; the four Asian countries (China, India, Indonesia, and Japan) might have the largest diabetes populations in the world [5].

Bilirubin is one of the end products of heme catabolism and has been shown to have antioxidant and anti-inflammatory effects [6]. Due to the effects of heme oxygenase (HO), which contain two forms: HO-1 and HO-2, cyclic tetrapyrrole heme is divided into biliverdin, carbon monoxide (CO), and ferrous iron (Fe^{2+}). Bilirubin concentration increases as higher HO-1 expression increases [7]. It has been reported that the HO-1 system can act protectively in a variety of models of diseases, which included diabetic retinopathy (DR) [8]. Due to the potential role of oxidative stress in the pathogenesis of DR and the antioxidant and cytoprotective effects of bilirubin, the number of studies on the association between serum total bilirubin concentration (STBC) and the risk of DR was increasing, some studies found that elevated STBC might have a protective effect on DR [9, 10], and others did not find similar results [11]. In the meantime, some studies reported that serum bilirubin concentration was not only affected by age, gender, and BMI but also affected by altitude and ethnicity [12]. All these

factors could influence the biological effects of bilirubin on the human body. China has a vast territory; there are great differences in demographic characteristics and living and eating habits between the northern and southern population [13], but the number of high-quality studies on the association between STBC and risk of DR was too small. Therefore, we first performed a study on the northeastern Chinese population to analyze the association between STBC and risk of DR.

2. Materials and Methods

2.1. Participants and Study Design. All subjects with type 2 DM who visited the ophthalmic clinic in the Fourth Hospital of China Medical University between January 2015 and May 2017 were retrospectively included in the study. Type 2 DM patients were defined as self-reported doctor-diagnosed diabetes or taking antidiabetic medications or fasting glucose concentration higher than or equal to 7.0 mmol/L according to the World Health Organization (WHO) criteria [14]. DR was defined by an ophthalmologist following an eye examination. Fundus photographs taken for both eyes were analyzed and graded to confirm the presence and severity of DR. DR mainly included nonproliferative and proliferative types. Nonproliferative DR showed one or more of the following symptoms: microaneurysm, hemorrhage, exudates, or microvascular abnormalities; proliferative DR showed the generation of new vessels and fibrosis [15].

We excluded (1) patients who had type 1 DM (defined as presentation with diabetic ketoacidosis, acute hyperglycemia symptoms with heavy ketonuria, or the continuous requirement of insulin in the year succeeding diagnosis); (2) patients who had hepatobiliary, hematological system, cardiopulmonary dysfunction, cancer, and other severe chronic diseases; (3) patients who also had other fundus lesions; and patients (4) when the doctor could not see the fundus due to refractive medium opacity.

This study was approved by the ethical committee of the Fourth Hospital of China Medical University (EC-2015-KS-030). Due to the retrospective nature of this study, the harm to the participants is not more than the minimal risk; the ethical committee of the Fourth Hospital of China Medical University waived the requirement to obtain informed consent.

2.2. Collection of Demographic, Medical, and Laboratory Data. For this hospital-based cross-sectional study, basic demographic data from all subjects were collected from medical records, including sex, age, height, weight, duration of diabetes, systolic blood pressure (SBP), and diastolic blood pressure (DBP). The body mass index (BMI) was calculated as the ratio of weight in kilograms divided by the square of height in meters. Blood and urine samples taken on admission were used for the analysis during the study period. Laboratory data was measured from fasting blood samples using an autoanalyzer according to the manufacturer's instructions. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), urea nitrogen (BUN), serum creatinine (Cr), uric acid (UA), fasting plasma glucose (FPG), serum total bilirubin concentration (STBC), and urine microalbumin were measured on an ADVIA 2400 automatic biochemical analyzer (SIEMENS, Germany). Fasting insulin and C-peptide were measured on an ADVIA Centaur XP immunoassay analysis system (SIEMENS, Germany). Hemoglobin A1c (HbA1c) was measured on a TOSOH HLC-723G7 analyzer (Sysmex, Japan). Fibrinogen was measured on an ACL TOP700 automatic blood coagulation analyzer (Beckman, USA).

2.3. Statistical Analysis. Data were entered (double entry) using Microsoft Excel 2013. Statistical analysis was performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). Continuous variables were expressed as the means and standard deviations. Categorical variables were expressed by numbers and percentages. The normality test was used to check the distribution of continuous variables. *t*-test and one-way analysis of variance (ANOVA) were used for the comparison of continuous variables, and the chi-square test was used for the comparison of categorized variables. The correlation coefficient was determined using Pearson's coefficient to determine the association between STBC and various clinical parameters. Finally, multivariable logistic regression was performed to estimate the odds ratio (OR) and 95% confidence intervals (CIs) for DR according to STBC. Reported probabilities were two-sided; a difference was considered significant if the P value was less than 0.05.

3. Results

3.1. Clinical Characteristics of the Subjects. The clinical characteristics are shown in Table 1. The mean age of the subjects was 59.55 ± 10.63 years, and 56.5% of the participants were men. The mean of the DM duration was 11.01 ± 7.35 years. The subjects without DR had a shorter DM duration and lower SBP, DBP, TC, TG, LDL-C, BUN, Cr, UA, urine microalbumin, and fibrinogen. The STBC was significantly higher in the subjects without DR. There was no difference in age, BMI, FPG, HbA1c, HDL-C, Fasting insulin, and C-peptide.

3.2. Correlations between STBC and Various Clinical Parameters. In total subjects, STBC was negatively correlated with DM duration, SBP, TC, TG, LDL-C, BUN, Cr, UA, urine microalbumin, and fibrinogen, and there was no correlation with other related clinical factors. The detailed results on correlations between STBC and various parameters are shown in Table 2.

3.3. Comparison of Related Clinical Parameters in Each Tertile Group of STBC. The clinical characteristics of the subjects in each tertile group are shown in Table 3. In total subjects, DM duration, SBP, TC, TG, LDL-C, BUN, Cr, UA, fasting insulin, urine microalbumin, and fibrinogen in the third tertile were lower than those in the first tertile; SBP, TC, BUN, Cr, urine microalbumin, and fibrinogen in the second tertile were lower than those in the first tertile; and DM duration and UA in the third tertile

Table 1: Clinical characteristics of the subjects.

Characteristics	Total	Without DR	With DR	P
n	742	209	533	
Men (%)	56.5%	56.9%	56.3%	0.872
Age (Year)	59.55 ± 10.63	59.23 ± 10.90	59.67 ± 10.53	0.616
DM duration (year)	11.01 ± 7.35	7.03 ± 6.47	12.57 ± 7.09	<0.001
BMI (kg/m^2)	25.01 ± 2.85	24.90 ± 3.37	25.06 ± 2.61	0.506
SBP (mmHg)	140.49 ± 20.23	137.91 ± 21.15	141.49 ± 19.78	0.03
DBP (mmHg)	83.62 ± 11.32	82.31 ± 12.11	84.14 ± 10.97	0.048
FPG (mmol/L)	9.68 ± 3.68	9.75 ± 3.89	9.65 ± 3.60	0.743
HbA1c (%)	9.30 ± 1.52	9.31 ± 1.90	9.30 ± 1.34	0.972
TC (mmol/L)	4.86 ± 1.05	4.52 ± 1.05	5.00 ± 1.02	<0.001
TG (mmol/L)	2.04 ± 1.77	1.64 ± 1.23	2.20 ± 1.92	<0.001
HDL-C (mmol/L)	1.15 ± 0.32	1.16 ± 0.29	1.14 ± 0.33	0.568
LDL-C (mmol/L)	2.99 ± 0.84	2.81 ± 0.88	3.06 ± 0.81	<0.001
BUN (mmol/L)	6.69 ± 3.83	5.25 ± 1.47	7.26 ± 4.29	<0.001
Cr (μmol/L)	119.55 ± 127.32	87.28 ± 19.34	132.20 ± 147.86	<0.001
UA (μmol/L)	310.86 ± 88.27	280.88 ± 73.57	322.61 ± 90.80	<0.001
Fasting insulin (μU/mL)	13.35 ± 11.30	13.44 ± 11.66	11.32 ± 11.17	0.895
Urine microalbumin (mg/L)	70.88 ± 69.12	36.74 ± 54.42	84.27 ± 69.71	<0.001
C-peptide (ng/mL)	2.46 ± 1.20	2.50 ± 1.33	2.45 ± 1.14	0.647
Fibrinogen (g/L)	3.38 ± 1.22	3.15 ± 0.53	3.46 ± 1.39	0.002
STBC (μmol/L)	13.95 ± 7.08	17.04 ± 8.98	12.75 ± 5.75	<0.001

Table 2: Correlation of STBC and various clinical parameters in total subjects.

	Total	
Characteristics	r	P
Age (year)	0.018	0.620
DM duration (year)	−0.213	<0.001
BMI (kg/m^2)	−0.065	0.079
SBP (mmHg)	−0.079	0.030
DBP (mmHg)	−0.006	0.880
FPG (mmol/L)	0.037	0.319
HbA1c (%)	0.029	0.424
TC (mmol/L)	−0.113	0.002
TG (mmol/L)	−0.118	0.001
HDL-C (mmol/L)	0.045	0.221
LDL-C (mmol/L)	−0.066	0.073
BUN (mmol/L)	−0.230	<0.001
Cr (μmol/L)	−0.173	<0.001
UA (μmol/L)	−0.149	<0.001
Fasting insulin (μU/mL)	−0.077	0.035
Urine microalbumin (mg/L)	−0.176	<0.001
C-peptide (ng/mL)	−0.068	0.066
Fibrinogen (g/L)	−0.163	<0.001

was lower than those in the second tertile. By chi-square test, the percentage of DR in the third tertile was lower than that in the first tertile.

3.4. Association between STBC and the Risk of DR. In a multivariate analysis adjusted for sex, age, BMI, DM duration, SBP, DBP, FPG, HbA1c, TC, TG, HDL-C, LDL-C, BUN, Cr, UA, fasting insulin, urine microalbumin, C-peptide, and fibrinogen (Table 4; model 3), as continuous variables, STBC was inversely associated with the risk of DR (OR: 0.95, 95% CI: 0.93–0.99). When STBC was used as the tertiary variable, compared with the first tertile, the OR in the third tertile was 0.37 (95% CI: 0.22–0.64). The detailed results on the association between STBC and the risk of DR are shown in Table 4.

4. Discussion

In this study, we first analyzed the association between STBC and the risk of DR in the Chinese northeastern population. STBC was negatively correlated with DM duration, SBP, TC, TG, LDL-C, BUN, Cr, UA, urine microalbumin, and fibrinogen. Furthermore, we found that diabetic patients in the highest tertile of STBC have significantly decreased ORs for the risk of DR, even after adjusted for potential confounding factors in total subjects. We also found that STBC by one standard deviation was negatively associated with the risk of DR.

In recent years, DM has become a rapidly growing threat worldwide; it causes a huge burden for people affected by it and also has an important influence on physical health and life quality according to its multiple complications [1]. Thus, identifying high-risk diabetic individuals with a higher risk of complications is very important and may lead to improvements in preventing and decreasing the burden of this

Table 3: Comparison of clinical characteristics according to STBC tertiles in total subjects.

Characteristics	Q1 (<10.81)	Q2 (10.81–14.61)	Q3 (>14.61)
n	248	250	244
Age (year)	58.92 ± 11.02	60.02 ± 10.51	59.70 ± 10.35
DM duration (year)	12.12 ± 7.47	12.08 ± 7.32	8.79 ± 6.77[ab]
BMI (kg/m^2)	25.03 ± 2.88	25.16 ± 2.90	24.84 ± 2.75
SBP (mmHg)	144.19 ± 21.38	138.39 ± 19.02[a]	138.87 ± 19.77[a]
DBP (mmHg)	83.61 ± 11.10	82.71 ± 11.68	84.57 ± 11.14
FPG (mmol/L)	9.60 ± 4.19	9.58 ± 3.48	9.87 ± 3.32
HbA1c (%)	9.25 ± 1.43	9.38 ± 1.52	9.28 ± 1.62
TC (mmol/L)	5.02 ± 1.14	4.80 ± 0.97[a]	4.77 ± 1.02[a]
TG (mmol/L)	2.28 ± 1.95	1.98 ± 1.58	1.85 ± 1.74[a]
HDL-C (mmol/L)	1.12 ± 0.34	1.15 ± 0.32	1.17 ± 0.30
LDL-C (mmol/L)	3.07 ± 0.86	2.96 ± 0.81	2.93 ± 0.83
BUN (mmol/L)	8.14 ± 5.18	6.41 ± 3.30[a]	5.51 ± 1.64[ab]
Cr (μmol/L)	157.88 ± 188.64	109.82 ± 100.88[a]	90.55 ± 19.60[a]
UA (μmol/L)	327.97 ± 96.72	311.42 ± 89.86[a]	292.90 ± 73.17[ab]
Fasting insulin (μU/mL)	14.46 ± 11.94	13.56 ± 11.19	12.02 ± 10.62[a]
Urine microalbumin (mg/L)	87.60 ± 77.89	67.70 ± 64.14[a]	57.17 ± 60.86[a]
C-peptide (ng/mL)	2.62 ± 1.41	2.39 ± 1.06	2.38 ± 1.07
Fibrinogen (g/L)	3.68 ± 1.60	3.34 ± 1.03	3.10 ± 0.79
DR (%)	85.1	74.0	56.1

[a]$P < 0.05$ versus Q1 and [b]$P < 0.05$ versus Q2 (one-way ANOVA and post hoc test).

Table 4: Odds ratios (95% CI) for risk of DR according to STBC.

	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 3 OR (95% CI)	*P*
Continuous	0.91 (0.89, 0.94)	<0.001	0.93 (0.90, 0.96)	<0.001	0.95 (0.93, 0.99)	0.007
Tertiles						
Q1	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Q2	0.50 (0.32, 0.78)	0.002	0.46 (0.28, 0.73)	0.002	0.51 (0.30, 0.87)	0.014
Q3	0.23 (0.15, 0.35)	<0.001	0.26 (0.16, 0.41)	<0.001	0.37 (0.22, 0.64)	<0.001

Model 1: unadjusted model; Model 2: adjusted for sex, age, BMI, and duration of diabetes; Model 3: adjusted for the variables in model 2 and SBP, DBP, FPG, HbA1c, TC, TG, HDL-C, LDL-C, BUN, Cr, UA, Fasting insulin, urine microalbumin, C-peptide, and fibrinogen.

disease. Thus, in clinical practice, it is very important to find a simple and convenient indicator to predict the occurrence of DR. Due to its antioxidant and anti-inflammatory effects, the research field on bilirubin is one of the research hotspots in the world. The Heinz Nixdorf Recall study found that bilirubin showed a potential protective role in the atherosclerosis process [16]. Several studies have found that STBC was negatively associated with diabetic microvascular and macrovascular complications [17–19], and in 2017, a meta-analysis found that there was a negative association between STBC and diabetic complications [20]. A total of 674 patients with type 2 diabetes in Japan revealed that STBC was significantly lower in patients with retinopathy than in those without [10]. In 2014, Najam et al. found that diabetic patients in the higher STBC group were less likely (OR: 0.55; 95% CI: 0.33–0.91) to suffer from DR than patients in the lower STBC group in Shanghai [18]. Compared to domestic and international research results, our results were similar.

The biological mechanisms underlying the association between serum bilirubin levels and DR remain unclear, but there are several possible explanations. Oxidative stress is an important risk factor in the occurrence of DR [21]. As the end product of heme catabolism, bilirubin has a powerful antioxidant capacity. In the total antioxidant capacity of blood plasma, bilirubin was a major contributor; only 10 nM bilirubin scavenges a 10,000-fold higher concentration of hydrogen peroxide [22]. Bilirubin also plays an important role in physiological antioxidant by inhibiting the formation of reactive oxygen species through NADPH oxidase in the endothelial cell [23]. Fu et al. found that due to a lack in the enzyme uridine-diphosphate glucuronosyltransferase (UGT1A) and exhibiting an elevation of plasma

bilirubin concentration, the mice reduced streptozotocin-induced pancreatic β-cell dysfunction by weakening oxidative stress [24]. Similar results were found in the studies on genetic variants. Abbasi et al. performed Mendelian randomization in a prospective cohort of 3381 participants, used rs6742078 located in UGT1A 1, and found that elevated STBC is causally associated with the risk of DM [25]. Several studies also found that low-grade inflammation plays a critical role in the development of diabetic retinopathy [16, 26]. Bilirubin also has anti-inflammatory properties through interfering with the expression of cell adhesion molecules, complement activity, and T cell differentiation [22]. Furthermore, clinical studies have demonstrated a negative relationship between serum bilirubin and C-reactive protein levels, which is a robust marker of inflammatory status [27]. In subjects with Gilbert syndrome, Tapan et al. found that there was a negative association between serum bilirubin concentration and soluble forms of CD40 ligand and P-selectin [28]. The modulatory effects of bilirubin on T regulatory cell differentiation were recently reported [29], further underlining the protective role of bilirubin in the pathogenesis of chronic inflammatory as well as in autoimmune conditions.

Our study had several limitations. First, due to the hospital-based cross-sectional study, the subjects were mainly collected from the ophthalmic clinic; therefore, there may be selection bias in the collection [30], and we could not directly infer the causal relationship between serum bilirubin concentration and the risk of DR. Second, serum bilirubin concentration was measure only once and, therefore, could not reflect the fluctuation and mean level. Taking these things into consideration, the results of our study may not be applicable to the general population or patients with type 2 diabetes. So then, our study aimed at revealing and exploring the relationship between serum bilirubin concentration and the risk of DR. In the future, several more prospective studies are needed to confirm the present findings in the Chinese population.

5. Conclusions

Currently, the measuring method of STBC in most hospitals is performed routinely and is not expensive to patients. Thus, STBC can be used easily by clinicians as one of the risk factors for the development of DR. Our results indicated that STBC could be considered a biomarker to predict the risk of DR.

Data Availability

The datasets used to support this study are currently under embargo while the research findings are commercialized. Requests for data, 12 months after initial publication, will be considered by the corresponding author.

Ethical Approval

This study was approved by the ethical committee of the Fourth Hospital of China Medical University (EC-2015-KS-030).

Conflicts of Interest

The authors have no conflicts to disclose.

References

[1] W. Animaw and Y. Seyoum, "Increasing prevalence of diabetes mellitus in a developing country and its related factors," *PLoS One*, vol. 12, no. 11, article e0187670, 2017.

[2] L. Chen, D. J. Magliano, and P. Z. Zimmet, "The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives," *Nature Reviews Endocrinology*, vol. 8, no. 4, pp. 228–236, 2011.

[3] T. H. Grubesic, J. A. Miller, and A. T. Murray, "Geospatial and geodemographic insights for diabetes in the United States," *Applied Geography*, vol. 55, pp. 117–126, 2014.

[4] J. P. Boyle, T. J. Thompson, E. W. Gregg, L. E. Barker, and D. F. Williamson, "Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence," *Population Health Metrics*, vol. 8, no. 1, p. 29, 2010.

[5] D. R. Whiting, L. Guariguata, C. Weil, and J. Shaw, "IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030," *Diabetes Research and Clinical Practice*, vol. 94, no. 3, pp. 311–321, 2011.

[6] J. Zheng, Y. Wu, Z. Li et al., "Low serum Total bilirubin concentration was associated with increased high sensitive C reactive protein level in patients with impaired glucose tolerance and type 2 diabetes mellitus subjects," *Clinical Laboratory*, vol. 62, no. 5, pp. 901–907, 2016.

[7] Y. H. Chen, L. Y. Chau, J. W. Chen, and S. J. Lin, "Serum bilirubin and ferritin levels link heme oxygenase-1 gene promoter polymorphism and susceptibility to coronary artery disease in diabetic patients," *Diabetes Care*, vol. 31, no. 8, pp. 1615–1620, 2008.

[8] M. P. Soares and F. H. Bach, "Heme oxygenase-1: from biology to therapeutic potential," *Trends in Molecular Medicine*, vol. 15, no. 2, pp. 50–58, 2009.

[9] H. C. Cho, "The relationship among homocysteine, bilirubin, and diabetic retinopathy," *Diabetes & Metabolism Journal*, vol. 35, no. 6, pp. 595–601, 2011.

[10] R. Sekioka, M. Tanaka, T. Nishimura, and H. Itoh, "Serum total bilirubin concentration is negatively associated with increasing severity of retinopathy in patients with type 2 diabetes mellitus," *Journal of Diabetes and its Complications*, vol. 29, no. 2, pp. 218–221, 2015.

[11] E. J. Huang, W. W. Kuo, Y. J. Chen et al., "Homocysteine and other biochemical parameters in type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy," *Clinica Chimica Acta*, vol. 366, no. 1-2, pp. 293–298, 2006.

[12] J. T. Hughes, F. Barzi, W. E. Hoy et al., "Bilirubin concentration is positively associated with haemoglobin concentration and inversely associated with albumin to creatinine ratio among indigenous Australians: eGFR study," *Clinical Biochemistry*, vol. 50, no. 18, pp. 1040–1047, 2017.

[13] H. Y. Shi, J. R. Wang, J. Cao, Q. Y. Wang, and C. P. Liu, "Investigation on the difference of intolerance to food between southern and northern middle-aged Chinese and its association with eating habits," *Chinese Journal of Applied Physiology*, vol. 29, no. 3, pp. 283–286, 2013.

[14] K. G. M. M. Alberti and P. Z. Zimmet, "Definition, diagnosis and classification of diabetes mellitus and its complications.

Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation," *Diabetic Medicine*, vol. 15, no. 7, pp. 539–553, 1998.

[15] B. Zhu, X. Wu, K. Ning, F. Jiang, and L. Zhang, "The negative relationship between bilirubin level and diabetic retinopathy: a meta-analysis," *PLoS One*, vol. 11, no. 8, article e0161649, 2016.

[16] A. A. Mahabadi, N. Lehmann, S. Möhlenkamp et al., "Association of bilirubin with coronary artery calcification and cardiovascular events in the general population without known liver disease: the Heinz Nixdorf Recall study," *Clinical Research in Cardiology*, vol. 103, no. 8, pp. 647–653, 2014.

[17] S. Hamamoto, H. Kaneto, S. Kamei et al., "Low bilirubin levels are an independent risk factor for diabetic retinopathy and nephropathy in Japanese patients with type 2 diabetes," *Diabetes & Metabolism*, vol. 41, no. 5, pp. 429–431, 2015.

[18] S. S. Najam, J. Sun, J. Zhang et al., "Serum total bilirubin levels and prevalence of diabetic retinopathy in a Chinese population," *Journal of Diabetes*, vol. 6, no. 3, pp. 221–227, 2014.

[19] A. Dave, P. Kalra, B. H. Gowda, and M. Krishnaswamy, "Association of bilirubin and malondialdehyde levels with retinopathy in type 2 diabetes mellitus," *Indian Journal of Endocrinology and Metabolism*, vol. 19, no. 3, pp. 373–377, 2015.

[20] B. Zhu, X. Wu, Y. Bi, and Y. Yang, "Effect of bilirubin concentration on the risk of diabetic complications: a meta-analysis of epidemiologic studies," *Scientific Reports*, vol. 7, article 41681, 2017.

[21] S. Coccheri, "Approaches to prevention of cardiovascular complications and events in diabetes mellitus," *Drugs*, vol. 67, no. 7, pp. 997–1026, 2007.

[22] L. Vítek, "The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases," *Frontiers in Pharmacology*, vol. 3, p. 55, 2012.

[23] E. H. M. Temme, J. Zhang, E. G. Schouten, and H. Kesteloot, "Serum bilirubin and 10-year mortality risk in a Belgian population," *Cancer Causes and Control*, vol. 12, no. 10, pp. 887–894, 2001.

[24] Y. Y. Fu, K. J. Kang, J. M. Ahn et al., "Hyperbilirubinemia reduces the streptozotocin-induced pancreatic damage through attenuating the oxidative stress in the Gunn rat," *The Tohoku Journal of Experimental Medicine*, vol. 222, no. 4, pp. 265–273, 2010.

[25] A. Abbasi, P. E. Deetman, E. Corpeleijn et al., "Bilirubin as a potential causal factor in type 2 diabetes risk: a Mendelian randomization study," *Diabetes*, vol. 64, no. 4, pp. 1459–1469, 2015.

[26] L. B. Dudnik, O. A. Azyzova, N. P. Solovyova, A. P. Savchenkova, and M. A. Pokrovskaya, "Primary biliary cirrhosis and coronary atherosclerosis: protective antioxidant effect of bilirubin," *Bulletin of Experimental Biology and Medicine*, vol. 145, no. 1, pp. 18–22, 2008.

[27] H. J. Hwang, S. W. Lee, and S. H. Kim, "Relationship between bilirubin and C-reactive protein," *Clinical Chemistry and Laboratory Medicine*, vol. 49, no. 11, pp. 1823–1828, 2011.

[28] S. Tapan, T. Dogru, I. Tasci, C. N. Ercin, T. Ozgurtas, and M. K. Erbil, "Soluble CD40 ligand and soluble P-selectin levels in Gilbert's syndrome: a link to protection against atherosclerosis?," *Clinical Biochemistry*, vol. 42, no. 9, pp. 791–795, 2009.

[29] F. Rocuts, X. Zhang, J. Yan et al., "Bilirubin promotes de novo generation of T regulatory cells," *Cell Transplantation*, vol. 19, no. 4, pp. 443–451, 2010.

[30] K. Keyes and S. Galea, "What matters most: quantifying an epidemiology of consequence," *Annals of Epidemiology*, vol. 25, no. 5, pp. 305–311, 2015.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 6763586, 6 pages
https://doi.org/10.1155/2018/6763586

Review Article

Cataract as Early Ocular Complication in Children and Adolescents with Type 1 Diabetes Mellitus

Marko Šimunović,[1] **Martina Paradžik**,[2] **Roko Škrabić**,[3] **Ivana Unić**,[1] **Kajo Bućan**,[2] **and Veselin Škrabić**[1]

[1]*Department of Pediatrics, University Hospital Centre Split, Spinčićeva 1, 21000 Split, Croatia*
[2]*Department of Ophthalmology, University Hospital Centre Split, Spinčićeva 1, 21000 Split, Croatia*
[3]*School of Medicine, University of Split, Šoltanska 2, Split, Croatia*

Correspondence should be addressed to Veselin Škrabić; vskrabic@kbsplit.hr

Received 28 September 2017; Accepted 28 January 2018; Published 20 March 2018

Academic Editor: Ilias Migdalis

Cataract is a rare manifestation of ocular complication at an early phase of T1DM in the pediatric population. The pathophysiological mechanism of early diabetic cataract has not been fully understood; however, there are many theories about the possible etiology including osmotic damage, polyol pathway, and oxidative stress. The prevalence of early diabetic cataract in the population varies between 0.7 and 3.4% of children and adolescents with T1DM. The occurrence of diabetic cataract in most pediatric patients is the first sign of T1DM or occurs within 6 months of diagnosis of T1DM. Today, there are many experimental therapies for the treatment of diabetic cataract, but cataract surgery continues to be a gold standard in the treatment of diabetic cataract. Since the cataract is the leading cause of visual impairment in patients with T1DM, diabetic cataract requires an initial screening as well as continuous surveillance as a measure of prevention and this should be included in the guidelines of pediatric diabetes societies.

1. Introduction

Approximately half a million children in the world today have type 1 diabetes mellitus (T1DM) with an estimation of 80,000 new cases every year [1]. T1DM and its complications are one of the biggest public health issues today and a leading cause of morbidity and mortality later in life [2]. Occurrence and cause of ocular complications comprising retinopathy, macular edema, papillopathy, cataract, glaucoma, strabismus, and refractive changes are well established in adults with T1DM [2, 3]. Unfortunately, there is only limited information about prevalence and pathophysiology of T1DM ocular complications in population of children and adolescents [3].

Even though cataract is one of the principal causes of visual deficiency in adult population with T1DM, it is a rare ocular complication at an early phase of T1DM in the pediatric population [4, 5]. In addition to a series of short-case reports with interesting clinical observations, to date, there are only several clinical studies on the characteristics and prevalence of early diabetic cataract in the pediatric population [3, 5–19]. The surgical solution continues to be a gold standard in the treatment of diabetic cataract, but the real challenge remains elucidating new therapeutic principles that would influence the pathophysiological mechanism of early diabetic cataract in the pediatric population [4, 20].

Our group was interested in finding clear recommendations for screening for early diabetic cataract in the pediatric population but was not able to find guidelines. This review article aims at summarizing all published findings about diabetic cataract as one of the first ocular complications of newly diagnosed T1DM in childhood and at emphasizing the importance of early screening. Our secondary objective is to clarify possible etiology and to highlight other conservative

experimental therapeutic options for early cataract prevention and treatment in population of children and adolescents with T1DM.

2. Pathophysiology of Early Diabetic Cataract in Population of Children and Adolescents

The pathophysiological mechanism of early diabetic cataract has not been fully understood; however, there are many theories about the possible etiology of diabetic cataract early in childhood [14, 16]. Long-lasting hyperglycemia with consequential ketoacidosis and dehydration certainly plays an important role in the development of early diabetic cataract [5, 18]. Even though the majority of newly diagnosed pediatric patients with T1DM have aforementioned symptoms, only a small number of patients develop early diabetic cataract [16]. This observation certainly underlines the importance of other factors that influence the occurrence of early diabetic cataract including genetics, local factors, nutritional habits, and gender as well as growth and developmental changes in childhood [5, 15].

The activation of the polyol pathway under the influence of hyperglycemia and other cofactors is the most widely accepted hypothesis relating to the development of early diabetic cataract [4, 21]. A crucial enzyme in the cascade of polyol pathway is aldose reductase, which catalyzes reduction of glucose into sorbitol using NADPH prior to sorbitol reduction to fructose by sorbitol dehydrogenase with NAD^+ as a cofactor [21, 22]. In experimental conditions, using various animal models, it has been demonstrated that increased sorbitol levels cause hyperosmolar conditions resulting in fluid retention due to an osmotic gradient disorder [4, 21, 22]. Osmotic damage is considered to be a very important factor in the development of early cataracts in childhood, leading to a change in the lens structure, gradual fibrosis, and, eventually, the formation of cataract [4, 5, 23]. However, it is important to note that earlier studies demonstrate that levels of sorbitol in human diabetic lens are not as high as in the animal models, so the exact role of osmotic damage during the formation of cataract in patients with T1DM still needs to be clarified [15, 24, 25].

Furthermore, several other metabolic pathways such as oxidative stress, activation of mitogen-activated protein kinase and cyclooxygenase-2, accumulation of cytosolic calcium, activation of NF-κB, and activation of protein-1, which all relate to polyol pathway but signal through distinct mechanisms, were found important for the development of diabetic cataract [26–28]. The occurrence of oxidative stress plays a major role in gradual diabetic cataract development [26]. Reactive oxygen species (ROS) in diabetic patients are generated during oxidative stress in the process of advanced glycation end-product (AGEs) formation, but also as a byproduct of the polyol pathway due to accumulation of NADH and consequent NADH oxidase activity [21, 29]. The imbalance in antioxidant capacity results in increased availability of free radicals, which can also be associated with the possible formation of diabetic cataract [30].

In addition to this, a recent case report describes a patient with early diabetic cataract and an onset of monogenic-type diabetes caused by a mutation within the insulin gene (INS), which until now, has not been associated with diabetic cataract [31]. It is known that INS mutations are more frequent in patients with neonatal diabetes even though it is not entirely clear how this affects the formation of early diabetic cataract [31]. Altogether, these mechanisms have a certain impact on the occurrence of early diabetic cataract, but additional research is needed to unravel a clear pathophysiological pathway of early diabetic cataract in the pediatric population.

3. Prevalence and Other Clinical Study Features of Early Diabetic Cataract in Population of Children and Adolescents

The prevalence of early diabetic cataract in the population of children and adolescents depending on the authors varies between 0.7 and 3.4% [3, 5, 9, 10, 15, 32]. The highest prevalence has been described in the study from 1985, but these results might be explained by the substandard regulation of T1DM at the time and fewer therapeutic approaches [32]. Majority of the new studies reported the prevalence of approximately 1%, but a recent study in USA by Geloneck et al. reported a significantly higher prevalence of 3.3% [3, 9, 15]. This may be due to a slightly longer duration of T1DM before initial cataract diagnosis, but future studies including larger patient cohorts and multinational cooperation will fully clarify the precise prevalence in population [3]. However, the occurrence of cataract in most patients is the first sign of T1DM or it occurs within 6 months of diagnosis of T1DM, which indicates the importance of early screening. Wilson et al. note that T1DM should be considered in cases with acquired cataract of unknown etiology following cataract extraction, particularly in the younger patients [5].

In addition to this, majority of studies assessing prevalence of cataract in T1DM were performed in developed countries, so it is not entirely possible to exclude the influence of various environmental factors and socioeconomic status of the patients on the prevalence of early ocular T1DM complications including cataract. Summary of clinical and individual characteristics of patients that have been published in articles and case reports is shown in Table 1. The youngest patients described in the literature with early diabetic cataracts were 5 years old, but most of the patients were adolescents [5, 12, 15]. Iafusco et al. reported equal gender distribution in T1DM pediatric population with early diabetic cataract, while most of other authors had significantly more female patients [5, 9, 15]. The same group of authors describes the appearance of ketoacidosis as a sign of decompensated T1DM in all patients, which is in accordance with majority of older publications [15]. Interestingly, more recent findings suggest a smaller occurrence of ketoacidosis [17, 18]. Furthermore, there is a significant variability regarding the level of hemoglobin A1c (HbA_{1c}) at the diagnosis of diabetes itself, but also at the onset of early diabetic cataracts [3, 8, 9]. Iafusco et al. pointed out in their study that for each percentage point from 12.8 to 14.1% of HbA_{1c} level,

Table 1: Characteristics of studies and patients with early diabetic cataract in population of children and adolescents.

Authors	Country	Age at cataract diagnosis (yrs)	T1DM duration at diagnosis (yrs)	HbA_{1c} at T1DM diagnosis (%)	Mean HbA_{1c} (%)	Morphology of cataract	Gender female/male	Surgical treatment	Number of patients
Phillip et al. [6]	USA	14	0	17	/	PSC	1/0	0	1
Alouf and Pascual [7]	USA	9	0	22.2	/	S	1/0	0	1
Datta et al. [8]	UK	11 to 14	0 to 1	15.1 to 21.2	/	PSC, S, C, D	3/2	4	5
Montgomery and Batch [9]	Australia	9 to 16	0 to 13	7.2 to 15.2	6.5 to >14	PSC, C	8/1	8	9
Falck and Laatikainen [10]	Finland	9.1 to 17.5	0 to 3.9	/	/	PSC, S, DI	5/1	6	6
Awan et al. [11]	UK	18	0	10.5	/	C, D	0/1	1	1
Wilson et al. [5]	Multiple	5 to 16.5	/	/	/	Multiple	11/3	12	14
Costagliola et al. [12]	Italy	5.3 to 13.2	0 to 0.1	12.8 to 14.5	/	PSC, S, D, DI	0/3	3	3
Patel et al. [13]	USA	10	0	17.9	13.4	PSC, C, DI	0/1	1	1
Skrabic et al. [14]	Croatia	16.8	0.2	15.5	11.7	PSC	1/0	1	1
Iafusco et al. [15]	Italy	5.5 to 15	0 to 0.2	12.8 to >14	8 to 11.6	PSC, S, D, DI	3/3	5	6
Uspal and Schapiro [16]	USA	13	0	>14	/	D	1/0	1	1
Jin et al. [17]	China	9 to 11	0	30 to 31	14.7 to 20.4	PSC, S, C, D	2/0	0	2
Goturu et al. [18]	UK	13.3	0.3	16.6	11.6	PSC	1/0	1	1
Geloneck et al. [3]	USA	7.5 to 18	0 to 15	7.7 to >14	7.3 to >14	PSC, C, D	/	5	12
García García and García Robles [19]	Spain	12 to 13	0.5 to 10	14 to 14.5	8.7 to 10	S, C, DI	1/1	1	2

Yrs: years; T1DM: type 1 diabetes mellitus; HbA_{1c}: hemoglobin A1c; PSC: posterior subcapsular; S: snowflake; C: cortical; D: dense; DI: diffuse.

appearance of early diabetic cataract increases 3.6 times [15]. Altogether, these findings highlight the importance of good control of glycemia and HbA_{1c} levels as one of the risk factors for development of early diabetic cataract.

Regarding morphology of early diabetic cataract, Wilson et al. reported multiple morphologies including posterior subcapsular, lamellar, cortical, snowflake, and milky white type of early diabetic cataract, while most of other authors discriminate fewer types with posterior subcapsular cataract described as the most common type of diabetic cataract in childhood [5, 9, 14, 17, 18].

4. Prevention and Treatment of Early Diabetic Cataract in Population of Children and Adolescents

Typical early symptoms of T1DM such as polyuria, polydipsia, polyphagia, and weight loss need to be recognized as soon as possible thus reducing the exposure of the lens to hyperglycemia and other consequences of severe metabolic conditions, which altogether can have a positive impact on the formation of early diabetic cataracts in the pediatric population [5, 33]. American Diabetes Association (ADA) and the International Society for Pediatric and Adolescent Diabetes (ISPAD) as two major associations of pediatric diabetologists provide comprehensive guidelines for the prevention, diagnosis, and treatment of T1DM [34, 35]. Interestingly, ADA did not include any recommendation about screening for early diabetic cataract, although it is endorsed that the first ophthalmological examination for assessment of retinopathy should be done when the patient reaches the age of 10 years, after puberty occurs, or when the duration of T1DM is longer than 3 to 5 years [35]. In addition to this, ISPAD guidelines recommend that an initial eye examination should be considered in order to detect early diabetic cataract or major refractive errors, but there are no clear further instructions about extension of screening for diabetic cataract in population of children and adolescents [34].

In the past two decades, phacoemulsification is the most common technique of cataract extraction in the developed world [36]. Types of surgery differentiate between younger and older children. Attributable to soft cataract in younger children, use of phacoemulsification is not mandatory, whereas older children and adolescents should proceed to phacoemulsification [20, 37]. Most of the patients operated from 1982 underwent either intracapsular cataract extraction (ICCE), extracapsular cataract extraction (ECCE), or phacoemulsification surgery. Geloneck et al. reported that only 5 out of 12 of their patients had visually significant cataract and underwent cataract surgery [3]. However, cataract surgery is not without complications, and it is especially necessary to take into account the risks of long-term T1DM and the effects on growth and development of anterior eye segment [38]. The most common complications after cataract surgery are posterior capsular opacification (PCO), secondary glaucoma, retinal detachment, amblyopia, and acute complications (incision leakage, increased intraocular pressure, edema, and uveitis) [38, 39]. There are differences in management of PCO between younger and older children, as PCO occurs more often in younger children. It is generally advised that primary posterior capsulorhexis (PPC) should be performed in children younger than 4 years of life, since the risk of developing PCO even if posterior capsule remains intact is 100%, due to more reactive inflammatory response in younger age [20, 39, 40]. Even after PPC is performed, there is a substantial risk of secondary visual axis opacification (VAO) due to migration of lens epithelial cells from anterior vitreous; thus, it is recommended to perform anterior vitrectomy (AV) together with PPC in infants and young children [20, 41]. There are no clear guidelines whether PPC should be combined with AV in older children, which could be of great importance in children with diabetic cataract. Whitman and Vanderveen suggest that older children with simple PCO can undertake laser capsulotomy and those with both PCO and VAO can proceed to surgery [42]. Randomized controlled study in 27 children aged between 4 and 14 years who underwent the intervention of cataract surgery with or without PPC and AV demonstrated better visual acuity and significantly less PCO in the group that undergone cataract surgery with PPC and AV [39, 43]. Elkin et al. revised the incidence of PCO in all age groups of pediatric cataract patients who underwent cataract extraction followed by IOL implantation without PPC and AV and found occurrence of PCO up to 90% [44]. Khaja et al. shortly reported that 233 eyes of children younger than 18 with cataract that underwent cataract extraction followed by implantation of either *AcrySof* 1-piece lens (SN60AT) or 3-piece lens (MA60AC) without PPC and AV and had statistically higher incidence of VAO compared to groups with the same lens implantation that received PPC and AV, implicating that prospective study with longer follow-up should be performed in order to illuminate impact of VAO [45]. Additionally, few authors reported that cataract surgery had influenced the onset and progression of retinopathy [4]. Falck and Laatikainen demonstrated that out of 11 eyes that were surgically treated in pediatric patients with early diabetic cataract, only 3 eyes did not develop retinopathy after 8 years of follow-up [10]. Another important problem is the choice of an appropriate intraocular lens, which would provide adequate control of the posterior segment of the eye and possible need for laser treatment or vitrectomy in patients with progression of retinopathy [2, 5]. Despite the careful preoperative measurement of the eye, calculation of power of the IOL, and prediction of refractive outcome, refractive error can occur in adult age due to emmetropization of the eye [46]. The majority of published articles about calculation of IOL power refer to surgical approach to congenital cataracts in infants [47]. According to the case report describing the youngest T1DM patient with early diabetic cataract at the age of 5, even though risk for development of amblyopia is reduced, eye growth is still not finished and probability of refractive error persists. Eye growth after 18 months of life, in juvenile age, enters a slow phase of 0.01 mm in diameter per month and myopic shift occurs; therefore, targeted IOL power should be undercorrecting (hyperopic) in order to ensure emmetropia or low myopia in adult age [41, 48–50]. Consequently, long-term follow-up of patients with diabetic

cataract is needed to understand possible influence of surgical treatment on the development of ocular complications.

A small number of studies have shown gradual regression and resolution of diabetic cataracts in the pediatric population. Jin et al. reported two cases of reversible cataract that gradually disappeared over several months with good glycemic control [17]. Phillip et al. suggested that duration of T1DM symptoms prior to therapy has a key role in the reversibility of diabetic cataract [6].

Today, there are many experimental therapies for the treatment of diabetic cataracts, but most are at the stage of laboratory-level studies; however, only several have been tested in clinical trials, but there is no information specific for the pediatric population. Previously, studies have been published describing various aldose reductase inhibitors that clearly prevent the onset of cataract on induced diabetic mouse model, but unfortunately most of them have numerous side effects [22]. Recently, research focus has been shifted to unrefined nutrients extracted from plants, teas, and fruits, which inhibits aldose reductase [22, 51]. Other possible preventive supplements are also described in the literature including nutritional antioxidants such as pyruvates and vitamins C and E, but further studies are needed to fully clarify their role [52]. A positive effect of hyperbaric conditions on lowering the glucose level and delay of cataract onset in diabetic mouse model has also been described and it is assumed that it is related to inhibition of aldose reductase and other mechanisms of oxidative stress [30].

5. Conclusion

Early diabetic cataract, although a rare complication of T1DM in the pediatric population, requires an initial screening as well as continuous surveillance as a measure of prevention since it is the leading causes of visual impairment in pediatric T1DM patients, and this should be included in the guidelines of major pediatric diabetes societies. The prevention of long-term hyperglycemia and rapid implementation of intensive insulin therapy certainly reduce the prevalence of early diabetic cataract in children and adolescents. Furthermore, additional studies are needed to thoroughly explain the etiological cause and therefore improve the prevention and treatment of diabetic cataract in population of children and adolescents.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

Marko Šimunović and Martina Paradžik contributed equally to this work.

References

[1] M. Craig, C. Jefferies, D. Dabelea et al., "Definition, epidemiology, and classification of diabetes in children and adolescents," *Pediatric Diabetes*, vol. 15, no. S20, pp. 4–17, 2014.

[2] N. Sayin, N. Kara, and G. Pekel, "Ocular complications of diabetes mellitus," *World Journal of Diabetes*, vol. 6, no. 1, pp. 92–108, 2015.

[3] M. M. Geloneck, B. J. Forbes, J. Shaffer, G. Ying, and G. Binenbaum, "Ocular complications in children with diabetes mellitus," *Ophthalmology*, vol. 122, no. 12, pp. 2457–2464, 2015.

[4] A. Pollreisz and U. Schmidt-Erfurth, "Diabetic cataract—pathogenesis, epidemiology and treatment," *Journal of Ophthalmology*, vol. 2010, pp. 1–8, 2010.

[5] M. E. Wilson, A. V. Levin, R. H. Trivedi et al., "Cataract associated with type-1 diabetes mellitus in the pediatric population," *Journal of American Association for Pediatric Ophthalmology and Strabismus*, vol. 11, no. 2, pp. 162–165, 2007.

[6] M. Phillip, D. Ludwick, K. Armour, and M. Preslan, "Transient subcapsular cataract formation in a child with diabetes," *Clinical Pediatrics*, vol. 32, no. 11, pp. 684-685, 1993.

[7] B. Alouf and A. G. Pascual, "Cataracts as the presenting feature of diabetes mellitus in a child," *Clinical Pediatrics*, vol. 35, no. 1, pp. 37–39, 1996.

[8] V. Datta, P. G. Swift, G. H. Woodruff, and R. F. Harris, "Metabolic cataracts in newly diagnosed diabetes," *Archives of Disease in Childhood*, vol. 76, no. 2, pp. 118–120, 1997.

[9] E. L. Montgomery and J. A. Batch, "Cataracts in insulin-dependent diabetes mellitus: sixteen years' experience in children and adolescents," *Journal of Paediatrics and Child Health*, vol. 34, no. 2, pp. 179–182, 1998.

[10] A. Falck and L. Laatikainen, "Diabetic cataract in children," *Acta Ophthalmologica Scandinavica*, vol. 76, no. 2, pp. 238–240, 1998.

[11] A. Awan, T. Saboor, and L. Buchanan, "Acute irreversible diabetic cataract in adolescence: a case report," *Eye*, vol. 20, no. 3, pp. 398–400, 2006.

[12] C. Costagliola, R. Dell'Omo, F. Prisco, D. Iafusco, F. Landolfo, and F. Parmeggiani, "Bilateral isolated acute cataracts in three newly diagnosed insulin dependent diabetes mellitus young patients," *Diabetes Research and Clinical Practice*, vol. 76, no. 2, pp. 313–315, 2007.

[13] C. M. Patel, L. Plummer-Smith, and F. Ugrasbul, "Bilateral metabolic cataracts in 10-yr-old boy with newly diagnosed type 1 diabetes mellitus," *Pediatric Diabetes*, vol. 10, no. 3, pp. 227–229, 2009.

[14] V. Skrabic, M. Ivanisevic, R. Stanic, I. Unic, K. Bucan, and D. Galetovic, "Acute bilateral cataract with phacomorphic glaucoma in a girl with newly diagnosed type 1 diabetes mellitus," *Journal of Pediatric Ophthalmology & Strabismus*, vol. 47, pp. e1–e3, 2010.

[15] D. Iafusco, F. Prisco, M. R. Romano, R. Dell'Omo, T. Libondi, and C. Costagliola, "Acute juvenile cataract in newly diagnosed type 1 diabetic patients: a description of six cases," *Pediatric Diabetes*, vol. 12, no. 7, pp. 642–648, 2011.

[16] N. Uspal and E. Schapiro, "Cataracts as the initial manifestation of type 1 diabetes mellitus," *Pediatric Emergency Care*, vol. 27, no. 2, pp. 132–134, 2011.

[17] Y. Y. Jin, K. Huang, C. C. Zou, L. Liang, X. M. Wang, and J. Jin, "Reversible cataract as the presenting sign of diabetes mellitus : report of two cases and literature review," *Iranian Journal of Pediatrics*, vol. 22, no. 1, pp. 125–128, 2012.

[18] A. Goturu, N. Jain, and I. Lewis, "Bilateral cataracts and insulin oedema in a child with type 1 diabetes mellitus," *BMJ Case Reports*, vol. 2013, article bcr2012008235, 2013.

[19] E. García García and E. García Robles, "Cataract: a forgotten early complication of diabetes in children and adolescents," *Edocrinology, Diabetes and Nutrion*, vol. 64, no. 1, pp. 58-59, 2017.

[20] A. Medsinge and K. K. Nischal, "Pediatric cataract: challenges and future directions," *Clinical Ophthalmology*, vol. 9, pp. 77–90, 2015.

[21] A. Snow, B. Shieh, K. C. Chang et al., "Aldose reductase expression as a risk factor for cataract," *Chemico-Biological Interactions*, vol. 234, pp. 247–253, 2015.

[22] C. Sampath, S. Sang, and M. Ahmedna, "In vitro and in vivo inhibition of aldose reductase and advanced glycation end products by phloretin, epigallocatechin 3-gallate and [6]-gingerol," *Biomedicine and Pharmacotherapy*, vol. 84, pp. 502–513, 2016.

[23] M. B. Datiles 3rd and P. F. Kador, "Type I diabetic cataract," *Archives of Ophthalmology*, vol. 117, no. 2, pp. 284-285, 1999.

[24] K. Sestanj, F. Bellini, S. Fung et al., "N-[[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-N-methylglycine (Tolrestat), a potent, orally active aldose reductase inhibitor," *Journal of Medicinal Chemistry*, vol. 27, no. 3, pp. 255-256, 1984.

[25] C. Costagliola, G. Iuliano, M. Menzione, A. Nesti, F. Simonelli, and E. Rinaldi, "Systemic human diseases as oxidative risk factors in cataractogenesis. I. Diabetes," *Ophthalmic Research*, vol. 20, no. 5, pp. 308–316, 1988.

[26] I. G. Obrosova, S. S. Chung, and P. F. Kador, "Diabetic cataracts: mechanisms and management," *Diabetes/Metabolism Research and Reviews*, vol. 26, no. 3, pp. 172–180, 2010.

[27] I. G. Obrosova, "Increased sorbitol pathway activity generates oxidative stress in tissue sites for diabetic complications," *Antioxidants & Redox Signaling*, vol. 7, no. 11-12, pp. 1543–1552, 2005.

[28] P. J. Oates, "Aldose reductase, still a compelling target for diabetic neuropathy," *Current Drug Targets*, vol. 9, no. 1, pp. 14–36, 2008.

[29] W. H. Tang, K. A. Martin, and J. Hwa, "Aldose reductase, oxidative stress, and diabetic mellitus," *Frontiers in Pharmacology*, vol. 3, p. 87, 2012.

[30] F. Nagatomo, R. R. Roy, H. Takahashi, V. R. Edgerton, and A. Ishihara, "Effect of exposure to hyperbaric oxygen on diabetes-induced cataracts in mice," *Journal of Diabetes*, vol. 3, no. 4, pp. 301–308, 2011.

[31] H. Wasserman, R. B. Hufnagel, V. Miraldi Utz et al., "Bilateral cataracts in a 6-yr-old with new onset diabetes: a novel presentation of a known INS gene mutation," *Pediatric Diabetes*, vol. 17, no. 7, pp. 535–539, 2016.

[32] B. E. Klein, R. Klein, and S. E. Moss, "Prevalence of cataracts in a population-based study of persons with diabetes mellitus," *Ophthalmology*, vol. 92, no. 9, pp. 1191–1196, 1985.

[33] J. Couper, M. Haller, A. Ziegler et al., "Phases of type 1 diabetes in children and adolescents," *Pediatric Diabetes*, vol. 15, no. S20, pp. 18–25, 2014.

[34] K. C. Donaghue, R. P. Wadwa, L. A. Dimeglio et al., "Microvascular and macrovascular complications in children and adolescents," *Pediatric Diabetes*, vol. 15, no. S20, pp. 257–269, 2014.

[35] American Diabetes Association, "11. Children and adolescents," *Diabetes Care*, vol. 38, Supplement 1, pp. S70–S76, 2014.

[36] S. R. de Silva, Y. Riaz, and J. R. Evans, "Phacoemulsification with posterior chamber intraocular lens versus extracapsular cataract extraction (ECCE) with posterior chamber intraocular lens for age-related cataract," *Cochrane Database of Systematic Reviews*, no. 1, article CD008812, 2014.

[37] S. L. Robbins, B. Breidenstein, and D. B. Granet, "Solutions in pediatric cataracts," *Current Opinion in Ophthalmology*, vol. 25, no. 1, pp. 12–18, 2014.

[38] D. Chen, X. Gong, H. Xie, X. N. Zhu, J. Li, and Y. E. Zhao, "The long-term anterior segment configuration after pediatric cataract surgery and the association with secondary glaucoma," *Scientific Reports*, vol. 7, article 43015, 2017.

[39] C. Gasper, R. H. Trivedi, and M. E. Wilson, "Complications of pediatric cataract surgery," *Developments in Ophthalmology*, vol. 57, pp. 69–84, 2016.

[40] M. C. Ventura, V. V. Sampaio, B. V. Ventura, L. O. Ventura, and W. Nosé, "Congenital cataract surgery with intraocular lens implantation in microphthalmic eyes: visual outcomes and complications," *Arquivos Brasileiros de Oftalmologia*, vol. 76, no. 4, pp. 240–243, 2013.

[41] S. K. Khokhar, G. Pillay, E. Agarwal, and M. Mahabir, "Innovations in pediatric cataract surgery," *Indian Journal of Ophthalmology*, vol. 65, no. 3, pp. 210–216, 2017.

[42] M. C. Whitman and D. K. Vanderveen, "Complications of pediatric cataract surgery," *Seminars in Ophthalmology*, vol. 29, no. 5-6, pp. 414–420, 2014.

[43] J. Kumar, V. K. Misuriya, and A. Mishra, "Comparative analysis of outcome of management of pediatric cataract with and without primary posterior capsulotomy and anterior vitrectomy," *Journal of Evolution of Medical and Dental Sciences*, vol. 3, no. 24, pp. 6802–6811, 2014.

[44] Z. P. Elkin, W. J. Piluek, and D. R. Fredrick, "Revisiting secondary capsulotomy for posterior capsule management in pediatric cataract surgery," *Journal of AAPOS*, vol. 20, no. 6, pp. 506–510, 2016.

[45] W. A. Khaja, M. Verma, B. L. Shoss, and K. G. Yen, "Visual axis opacification in children," *Ophthalmology*, vol. 118, no. 1, pp. 224-225, 2011.

[46] M. A. O'Hara, "Pediatric intraocular lens power calculations," *Current Opinion in Ophthalmology*, vol. 23, no. 5, pp. 388–393, 2012.

[47] V. Vasavada, S. K. Shah, V. A. Vasavada et al., "Comparison of IOL power calculation formulae for pediatric eyes," *Eye*, vol. 30, no. 9, pp. 1242–1250, 2016.

[48] D. A. Plager, H. Kipfer, D. T. Sprunger, N. Sondhi, and D. E. Neely, "Refractive change in pediatric pseudophakia: 6-year follow-up," *Journal of Cataract and Refractive Surgery*, vol. 28, no. 5, pp. 810–815, 2002.

[49] M. Al Shamrani and S. Al Turkmani, "Update of intraocular lens implantation in children," *Saudi Journal of Ophthalmology*, vol. 26, no. 3, pp. 271–275, 2012.

[50] M. E. Wilson and R. H. Trivedi, "Axial length measurement techniques in pediatric eyes with cataract," *Saudi Journal of Ophthalmology*, vol. 26, no. 1, pp. 13–17, 2012.

[51] K. C. Chang, L. Li, T. M. Sanborn et al., "Characterization of Emodin as a therapeutic agent for diabetic cataract," *Journal of Natural Products*, vol. 79, no. 5, pp. 1439–1444, 2016.

[52] K. R. Hegde, S. Kovtun, and S. D. Varma, "Prevention of cataract in diabetic mice by topical pyruvate," *Clinical Ophthalmology*, vol. 5, pp. 1141–1145, 2011.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 8641942, 9 pages
https://doi.org/10.1155/2018/8641942

Review Article

Susceptible and Prognostic Genetic Factors Associated with Diabetic Peripheral Neuropathy: A Comprehensive Literature Review

L. B. L. Prabodha, N. D. Sirisena, and V. H. W. Dissanayake

Human Genetics Unit, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka

Correspondence should be addressed to L. B. L. Prabodha; lahiruprabodha@gmail.com

Received 23 October 2017; Accepted 29 January 2018; Published 15 March 2018

Academic Editor: Ilias Migdalis

Type 2 diabetes mellitus (T2D) is a disorder of glucose metabolism. It is a complex process involving the regulation of insulin secretion, insulin sensitivity, gluconeogenesis, and glucose uptake at the cellular level. Diabetic peripheral neuropathy (DPN) is one of the debilitating complications that is present in approximately 50% of diabetic patients. It is the primary cause of diabetes-related hospital admissions and nontraumatic foot amputations. The pathogenesis of diabetic neuropathy is a complex process that involves hyperglycemia-induced oxidative stress and altered polyol metabolism that changes the nerve microvasculature, altered growth factor support, and deregulated lipid metabolism. Recent literature has reported that there are several heterogeneous groups of susceptible genetic loci which clearly contribute to the development of DPN. Several studies have reported that some patients with prediabetes develop neuropathic complications, whereas others demonstrated little evidence of neuropathy even after long-standing diabetes. There is emerging evidence that genetic factors may contribute to the development of DPN. This paper aims to provide an up-to-date review of the susceptible and prognostic genetic factors associated with DPN. An extensive survey of the scientific literature published in PubMed using the search terms "Diabetic peripheral neuropathy/genetics" and "genome-wide association study" was carried out, and the most recent and relevant literature were included in this review.

1. Introduction

Diabetes mellitus is nowadays one of the foremost noncommunicable diseases affecting more than 387 million people worldwide [1]. Type 2 diabetes mellitus (T2D) is a disorder of glucose metabolism. It is a complex process involving the regulation of insulin secretion, insulin sensitivity, gluconeogenesis, and glucose uptake at the cellular level. Dysregulation of one or more of these processes due to environmental or genetic factors can lead to altered glucose metabolism causing diabetes mellitus [2, 3]. More than 90% of cases of T2D show higher incidence of insulin resistance. This phenomenon is acquired due to sedentary lifestyle in combination with multifactorial genetic susceptibility. T2D is associated with increased morbidity and mortality due to its debilitating and progressive nature and associated complications. The condition usually leads to multiorgan failure due to macrovascular and microvascular involvement (Figure 1) [2–5].

Uncontrolled T2D can complicate pregnancy outcomes. Different kinds of birth defects are more commonly seen in babies born to women with diabetes [3]. Twin studies can estimate the multifactorial genetic involvement in T2D more precisely and have reported high degree of heritability of diabetes-related conditions such as disorders of first phase insulin response and basal and insulin-stimulated glucose uptake [6]. There are different methods for mapping the genetic susceptibility loci in the pathogenesis of T2D. Candidate gene studies and genome-wide studies are commonly used to identify the association of susceptible genetic loci of T2D. The latter includes both genome-wide linkage studies (GWL) and genome-wide association studies (GWAS) [6].

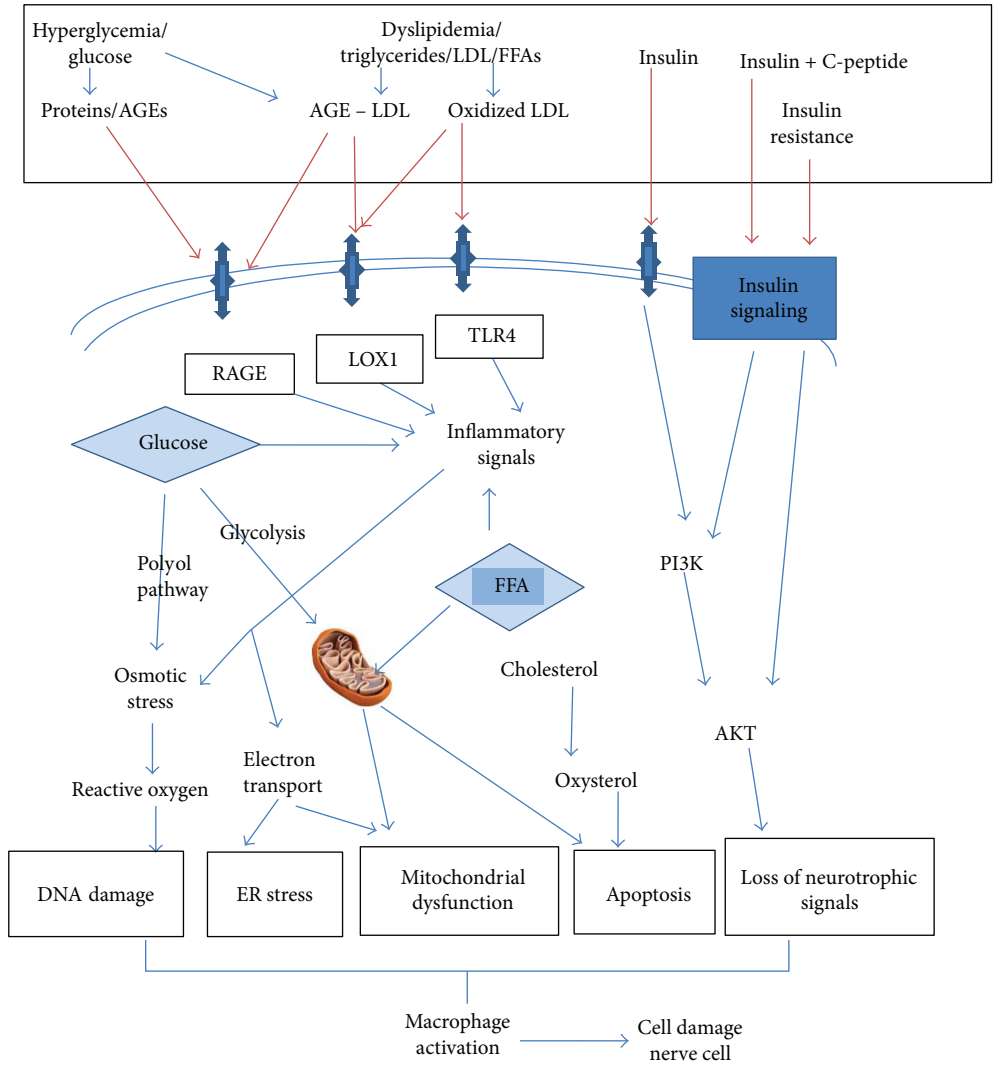


Figure 1: Mechanisms of diabetic neuropathy. Aetiological factors of diabetes initiate a cascade of events leading to DNA damage, endoplasmic reticulum stress, mitochondrial complex dysfunction, apoptosis, and loss of neurotrophic signaling. Ultimate activation of macrophages will cause cell damage in neurons, glial cells, and vascular endothelial cells, all of which can result in nerve dysfunction and neuropathy. AGE = advanced glycation end-products; LDL = low-density lipoprotein; FFA = free fatty acids; ER = endoplasmic reticulum; PI3K = phosphatidylinositol-3-kinase; LOX1 = oxidized LDL receptor 1; RAGE = receptor for advanced glycation end-products; TLR4 = Toll-like receptor 4.

With the advent of recent molecular genetic techniques and rapid screening methods, the method of investigation has shifted to the use of molecular genetic markers for understanding the genetic aetiology of T2D and its complications [6, 7]. The common susceptible genetic variants are known to have a prominent effect on the risk of T2D across the world in multiple ethnic groups [8, 9]. Some variants appear to exert more pronounced genetic effects in specific ethnic groups. Most loci associated with T2D map to regulatory or intronic regions of the genome [9].

Diabetic peripheral neuropathy (DPN) is one of the debilitating microvascular complications of diabetes that is present in approximately 50% of patients. It is the primary cause of diabetes-related hospital admissions and nontraumatic foot amputations [4, 5, 10]. The molecular mechanisms involved in the development of DPN is a complex process that includes activation of the polyol pathway, exaggerated oxidative stress, overactivity of protein kinase C and increased formation of advanced glycation end-products in the presence of hyperglycemia. In addition, there is increasing evidence that genetic factors could also contribute to the development of DPN [10, 11]. The consequences of diabetic neuropathy include neurogenic pain, numbness, lack of coordination of voluntary movements, and a susceptibility to foot ulceration that leads to infections and toe or foot amputations. The rate of toe or foot amputations is 15 times greater in diabetic patients compared with individuals without diabetes. To date, approximately 80 T2D susceptibility genetic loci have been reported in different ethnic groups worldwide [12–14]. Majority of studies on the prevalence

and associated aetiological factors of DPN have been conducted in Western countries. There is very limited data currently available for South Asian populations [15].

The objective of this paper is to provide an up-to-date review of the published scientific literature on the susceptible and prognostic genetic variants associated with DPN.

2. Methodology

This is a comprehensive review of the published literature on the susceptible and prognostic genetic variants associated with DPN. These variants were identified by an extensive survey of the scientific literature using the criteria described below. The most recent and relevant papers published in the last 15 years from January 2002 to July 2017 were searched in the PubMed database using the search terms "Diabetic peripheral neuropathy/genetics" and "genome-wide association study." Altogether, sixty studies describing single nucleotide variants (SNVs) in genes associated with the susceptibility and prognosis of DPN which were published as full text articles in English during the defined period of study were included in this review. Epigenetic modifications which regulate gene expression mainly at the tissue/cellular level were excluded as it was outside the scope of this review.

3. Diabetic Peripheral Neuropathy

According to the Toronto Consensus Panel on Diabetic Neuropathy, DPN is defined as a symmetrical, length-dependent sensorimotor polyneuropathy that develops on a background of longstanding hyperglycemia, associated derangements, and cardiovascular risk factors [16]. The mechanisms underlying the pathogenesis of DPN are different between type 1 and type 2 diabetes mellitus [17]. Recent literature has reported that there are different groups of susceptible genetic loci which are clearly involved in the development of DPN. Different studies reported that some patients with prediabetes develop neuropathic complications, whereas others reported little evidence of neuropathy even after long-standing diabetes. This observation confirms the involvement of genetic aetiological factors associated with the development of DPN [18]. The data from different studies suggest that T2D and its complications may have shared genetic risk factors [12, 18].

4. Genetic Aetiology and Pathogenesis of DPN

The pathogenesis of DPN is a complex process and is involved with hyperglycemia-induced oxidative stress and altered polyol metabolism that changes the nerve microvasculature, growth factor support, and lipid metabolism [4]. It is important to identify these factors alone or in combination to arrange effective DPN treatment, as better understanding of the mechanisms underlying the onset and progression of DPN is of prime importance in the process of management [19]. Different groups of cell types in diabetic complication-prone tissues are targets of damage due to uncontrolled hyperglycemia. Schwann cells are the prime target of hyperglycemia which results in cell damage leading to altered axon integrity and defective growth factor signaling [20, 21]. Defective inflammatory pathways including advanced glycation end-product/receptor (*AGE*/*RAGE*) signaling in axons and Schwann cells have been reported in experimental animals with diabetic neuropathy which contributed to nerve damage [22].

Lu et al. in China studied SNVs from previously identified ten genetic loci and analyzed the association of these loci with peripheral nerve function in patients with T2D. They found that rs5219 of *KCNJ11* gene polymorphism (*E23K, G>A*) was associated with peripheral nerve function. The results obtained from nerve conduction studies (NCS) showed that the allele "A" had a protective effect on peripheral nerve function. They also reported that SNVs rs7756992 of *CDKAL1* and rs7903146 of *TCF7L2* were associated with DPN in the Chinese T2D population [23].

Yigit et al. identified 230 unrelated patients with DPN at the outpatient clinics of the Physical Therapy and Rehabilitation Department of Gaziosmanpasa University, Tokat, in Turkey. They investigated the distributions of the genotype and allele frequencies of the *MTHFR* gene C677T variant among patients with DPN and a matched control group. A statistically significant difference of *MTHFR* gene C677T polymorphism between the patients with DPN and the control group was identified [24].

Decreased levels of peroxisome proliferator-activated receptor alpha (*PPARA*) in chromosome 22 and lipid metabolism-related gene apolipoprotein E (*APOE*) in chromosome 19 have been identified confirming the findings that altered lipid metabolism may play a role in the progression of DPN [25]. Monastiriotis et al. reviewed the literature to identify the association between *APOE* polymorphism and DPN and found that the $\varepsilon 4$ allele of the apolipoprotein E gene is significantly associated with the pathogenesis of DPN [25].

The alpha2B adrenergic receptor encoded by *ADRA2B* gene located on chromosome 2 is associated with an array of functions. A polymorphism (12Glu9) resulting in the insertion/deletion of three glutamic acid residues in the third intracellular loop has been described frequently in the literature [26]. In the nervous system, this polymorphism has been reported to be linked with autonomic nervous dysfunction. This is particularly increased with sympathetic nervous system activity, and Papanas et al. found a significant association in this indel allele distribution of alpha2B adrenoceptor gene among T2D patients with DPN in comparison with matched T2D patients without neuropathy [26].

5. Network of Genes Associated with Common Variants of DPN

Hur et al. examined two groups of DPN patients. A network of transcription factors jun (*JUN*), leptin (*LEP*), serpin peptidase inhibitor E type 1 (*SERPINE1*), apolipoprotein E (*APOE*), and peroxisome proliferator-activated receptor gamma (*PPARG*) was examined to identify their potential relationship (Figure 2). Further subsets of genes related to defense response, inflammatory response, regulation of lipid metabolic processes, and *PPAR* signaling pathways were then

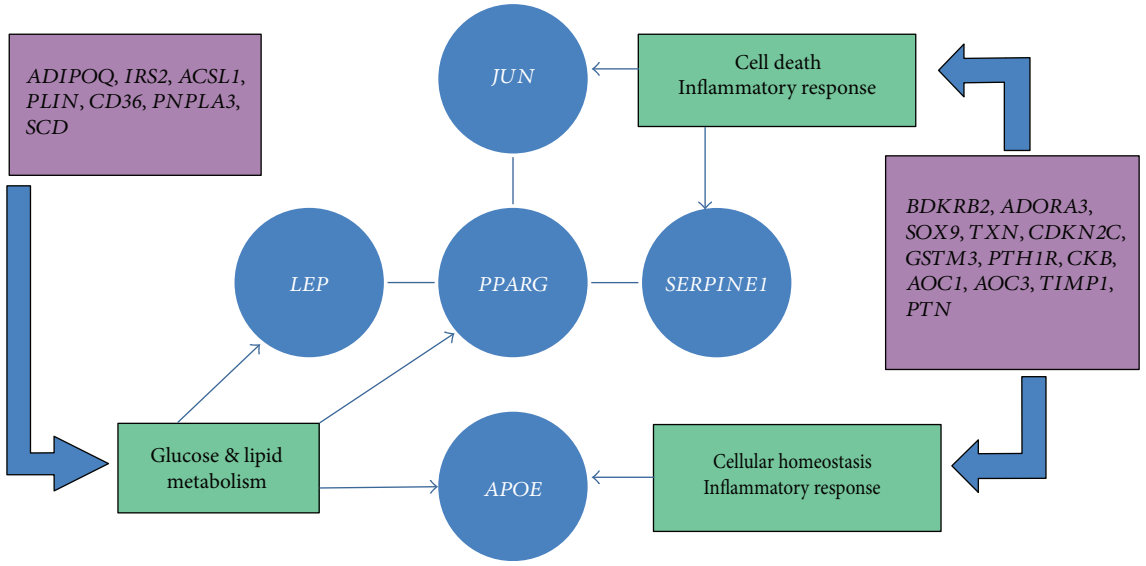


Figure 2: Five main genes (in blue circles) associated with diabetic peripheral neuropathy: *JUN*, *PPARG*, *LEP*, *SERPINE1*, and *APOE* and their relationship with defense response, inflammatory response, glucose, and lipid metabolism pathways (in green-coloured cages) are represented in the figure. Additional genes involved with DPN in relation to abovementioned metabolic pathways are indicated in purple-coloured cages. ADIPOQ = adiponectin, C1Q and collagen domain containing; IRS2 = insulin receptor substrate 2; ACSL1 = acyl-CoA synthetase long chain family member 1; PLIN = lipid storage droplet 2-like; CD36 = CD36 molecule; PNPLA3 = patatin-like phospholipase domain containing 3; SCD = stearoyl-CoA desaturase; BDKRB2 = bradykinin receptor B2; ADORA3 = adenosine A3 receptor; SOX9 = sex determining region Y-box 9; TXN = thioredoxin; CDKN2C = cyclin-dependent kinase inhibitor 2C; GSTM3 = glutathione S-transferase mu 3; PTH1R = parathyroid hormone 1 receptor; CKB = creatine kinase B; AOC1 = amine oxidase, copper containing 1; AOC3 = amine oxidase, copper containing 3; TIMP1 = TIMP metallopeptidase inhibitor 1; PTN = pleiotrophin.

analyzed to identify the association of gene expression and development of DPN (Figure 2) [27]. They demonstrated that increased glucose metabolism due to hyperglycemia resulted in increased oxidative stress, mitochondrial dysfunction, and cell death in both in vitro and in vivo models of diabetic neuropathy [27].

6. Variants Associated with Defense Response and Inflammatory Response in the Pathogenesis of DPN

Hur et al. reported that the molecules which are involved with the process of inflammation such as chemotactic agents and cytokines are involved with the development and progression of DPN as well as diabetic nephropathy [27, 28]. Kakoki et al. identified that the bradykinin receptor B2 (*BDKRB2*) is of particular interest in disease progression of DPN. *BDKRB2* gene was found to be involved in progressive glomerulosclerosis and also susceptibility to DPN [29].

Membrane-associated adenosine A3 receptor (*ADORA3*) is also involved in the pathogenesis of DPN [30]. Variants of *BDKRB2* and *ADORA3* were found to be involved in enhanced inflammation and dysregulated defense responses, thus contributing to more substantial nerve damage in patients with progressive DPN [27, 30]. Gene variants of *TXN*, *CDKN2C*, *GSTM3*, *PTH1R*, *CKB*, *AOC1*, *AOC3*, *TIMP1*, and *PTN* have been identified as other additional genes associated with defense response and inflammatory response in the pathogenesis of DPN (Figure 2) [27].

7. Variants Associated with Glucose Metabolic Processes and PPAR Signaling Pathway in the Pathogenesis of DPN

According to Hur et al., *PPARG*, which encodes a nuclear receptor for glitazone, plays a key role in regulating glucose and lipid metabolism [27, 31]. Agonists of *PPARG* are effective in treatment of DPN and nephropathy in experimental animal models [27, 32]. Another key gene is *APOE*, encoding an apolipoprotein, which regulates the normal catabolism of triglycerides and cholesterol. A polymorphism of this gene is linked to the progression of DPN [33]. Gene variants of *ADIPOQ*, *IRS2*, *ACSL1*, *PLIN*, *CD36*, *PNPLA3*, and *SCD* were identified as other additional gene variants associated with glucose metabolic processes and PPAR signaling pathway in the pathogenesis of DPN (Figure 2) [27].

8. Genetic Variants Involved in Different Phenotypes of DPN

According to Cheng et al., in an experiment involving both human and animal models, sensory neurodegeneration in the chronic stage of diabetes was found to be associated with early damage to the distal axons of both upper and lower limb neurons showing a pattern that accounts for the distribution of "glove-and-stocking" loss of sensation characteristically seen in DPN. These changes accompany widespread abnormalities involving electrophysiology and alterations in gene expression that indicate a degenerative phenotype.

However, existing knowledge on the development of DPN which includes oxidative and nitrergic stress, polyol accumulation, microangiopathy, inappropriate *AGE-RAGE* signaling, and/or mitochondrial dysfunction account for diverse mRNA changes that alter miRNA expression patterns resulting in diverse DPN phenotypes [34].

9. Genetic Variants Involved in Gender Dimorphism of DPN

Significant gender dimorphisms in the responsiveness of patients to antidiabetic drugs have been reported in the literature [35–37]. These observations highlighted the importance of understanding the gender-specific differences in manifestation of diabetes mellitus and its complications such as DPN.

O'Brien et al. reported the first instance of a female T2D mouse model presenting with a neuropathic phenotype including decreased intraepidermal nerve fiber density, impaired motor and sensory nerve conduction velocities, and thermal hypoalgesia [38]. A GWAS involving 961 diabetic neuropathic pain cases and 3260 diabetic controls in the Genetics of Diabetes Audit and Research Tayside by Meng et al. found that a cluster in the 1p35.1 region, the zinc finger and *SCAN* domain containing 20 (*ZSCAN20*) with a lowest p value of a variant at rs71647933 in females, and a cluster in the 8p23.1 region next to *HMGB1P46* with a lowest p value of a variant at rs6986153 in males were significantly associated with DPN. This GWAS on diabetic neuropathic pain provides evidence for the sex-specific involvement of 1p35.1 region (*ZSCAN20*) and 8p23.1 region (*HMGB1P46*) [39].

10. Other Gene Loci Involved in the Pathogenesis and Prognosis of DPN

A fibrinolysis-regulating gene, *SERPINE1*, which encodes for plasminogen activator inhibitor 1 (*PAI-1*) has been identified in association with higher incidences of diabetic complications such as diabetic neuropathy and nephropathy in knockout *PAI-1* mice [40, 41]. The cell cycle controlling *JUN* is also involved in the progression of DPN and is associated with inflammation and insulin resistance which is activated in multiple tissues including the peripheral sensory nerves of patients with types 1 and 2 diabetes [42].

Three other subsets of important gene variants were documented in literature associated with "cell projection and axonogenesis" involving nerve growth factor receptor (*NGFR*) and "cellular homeostasis and inflammatory response" involving thioredoxin, and "cytoskeletal protein binding" with stathmin 1 (*STMN1*) genes. *NGFR* exhibits protection against nerve cell and axonal damage, and the expression of nerve growth factor receptor protein in plasma correlates with DPN progression in diabetic rat models [43]. Thioredoxin, which regulates cellular oxidative stress with its antioxidant activity, also plays an important role in associated diabetes. Thioredoxin's antioxidant activity is significantly inhibited by hyperglycemic states in the blood. It complicates diabetes by playing an important role by deregulating vascular oxidative stress and inflammation in diabetic patients [44].

A study in North Catalonia, Spain, by Jurado et al. identified the protective effect of a single angiotensin-converting enzyme (*ACE*) gene polymorphism on the development of DPN in T2D patients. Despite *ACE* gene variants which are associated with diabetic renal disease and/or diabetic retinopathy, the heterozygous genotype stands as a protective factor against the development of DPN [45]. Heterozygous (D/I) *ACE* gene polymorphism reported a statistically significantly reduced risk of developing DPN whereas homozygous (D/D) *ACE* gene polymorphism reported an increased risk [45].

Mitochondrial transcription factor A (*TFAM*) is located in mitochondria, and its level regulates mitochondrial DNA (mtDNA) copy number. Chandrasekaran et al. showed that *TFAM* over expression prevented a decrease in mtDNA copy number in diabetic dorsal root ganglia (DRG) neurons, helped prevent DPN, and protected DRG neurons from oxidative stress in experimental mouse models [46].

Aldo-keto reductase family 1 member B (*AKR1B1*) in chromosome 7 encodes a member of the aldo-keto reductase superfamily, which consists of more than 40 known enzymes and proteins. This catalyzes the reduction of a number of aldehydes and is thereby implicated in the development of diabetic complications by catalyzing the reduction of glucose to sorbitol. Saraswathy et al. identified significant association of *AKR1B1* gene mutations in painful diabetic neuropathy [47].

There is increasing evidence that microRNAs (miRNAs) act as regulators of gene expression in multiple biological processes and associated complications [48]. Ciccacci et al. looked for an association between variants in miRNA genes and DPN. The results of this study identified a role for *MIR146a* and *MIR128a* SNVs in the susceptibility to DPN and were shown to have a significant association [49]. The rs2910164 (G>C) in *MIR146a* is associated with lower risk, and rs11888095 (C>T) in *MIR128a* is associated with higher risk of susceptibility to DPN [49].

Nitric oxide (NO) production and local release in the tissues significantly contributed to endothelial dysfunction. The process takes place by the modulation of the nitric oxide synthase (NOS) enzymes responsible for NO synthesis. Endothelium-derived NO plays a key role in the regulation of vascular tone and has vasoprotective effects by removing superoxide radicals and suppressing platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation. However, dysfunctional endothelial nitric-oxide synthase (eNOS) might play a critical role in the pathogenic pathway leading to diabetic vascular complications including DNP. Therefore, eNOS is considered as a candidate for the progression of DPN [50, 51].

In the early stages of DPN, abnormalities in the vasa nervorum and loss of nerve fibers can be seen in association with hyperglycemia. Damage to the nervous tissue results in increased intravascular endothelial growth factor (*VEGF*) plasma levels in diabetic animal models [52]. The ischemia and hypoxia in the nerves of patients with T2D due to microangiopathy of vasa nervorum have always been observed and

Table 1: Genetic variants associated with DPN.

Gene	Chromosomal location	Variants	Associated risk/remarks	Reference
Advanced glycation end receptor (*AGER*)	6p21.32	rs1800624	Higher risk/defective inflammatory pathways	[22]
Peroxisome proliferator-activated receptor alpha (*PPARA*)	3p25.2	rs1801282	Higher risk/defective inflammatory pathways	[27]
Bradykinin receptor B2 (*BDKRB2*)	14q32.2	rs1799722	Higher risk/defective inflammatory pathways/ African-Americans	[29]
Potassium voltage-gated channel subfamily J member 11 (*KCNJ11*)	11p15.1	E23K, G>A rs5219	Higher risk/Chinese population/altered signaling pathways	[23]
CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*)	6p22.3	rs7756992	Higher risk/Chinese population	[23]
Transcription factor 7-like 2 (*TCF7L2*)	10q25.2-q25.3	rs7903146	Higher risk/Chinese population	[23]
Methylenetetrahydro folate reductase (*MTHFR*)	1p36.22	C677T rs1801133	Higher risk/altered folate metabolism	[24]
Apolipoprotein E (*APOE*)	19q13.32	ε4 allele- rs429358 rs7412	Higher risk/altered lipid metabolism	[25]
Adrenoceptor alpha 2B (*ADRA2B*)	2q11.2	12Glu9 rs879255577	Higher risk/defects in regulation of neurotransmitter release from sympathetic nerves	[26]
microRNA 146a (*MIR146a*)	5q33.3	rs2910164 (G>C)	Lower risk	[49]
microRNA128a (*MIR128a*)	2q21.3	rs11888095 (C>T)	Higher risk	[49]
High mobility group box 1 pseudogene 46 (*HMGB1P46*)	8q23.1	rs6986153	Males/higher risk	[39]
Zinc finger and SCAN domain containing 20 (*ZSCAN20*)	1p35.1	rs71647933	Females/higher risk	[39]
Serpin family E member 1 (*SERPINE1*)	7q22.1	rs1799768	Progressive type of DPN	[40]
Nerve growth factor receptor (*NGFR*)	17q21.33	rs734194	Progressive type of DPN	[43]
Angiotensin-converting enzyme (*ACE*)	17q23.3	rs1799752 diallelic polymorphism: presence/absence of 287bp in intron 16 Heterozygous D/I Homozygous D/D	Japanese population Lower risk Higher risk	[45]
Aldo-keto reductase family 1 member B (*AKR1B1*)	7q33	rs5053 rs759853	Higher risk/altered glucose metabolism	[47]
Vascular endothelial growth factor (*VEGF*)	6p21.1	C936T/rs3025039 rs2010963 rs699947	Higher risk	[52]
Cytochrome b-245 alpha chain (*CYBA*)	16q24.2	C242T rs4673	Higher risk	[52]
Heat shock protein family A (Hsp70) member 5 (*HSPA5*)	9q33.3	Promoter region 57168556T>C rs391957	Higher risk	[54]
Adiponectin (*ADIPOQ*)	3q27.3	45T/G rs2241766 276G/T rs1501299	Higher risk Higher risk	[56, 57]

may be a key pathogenic mechanism of DPN [53]. An association study by Ghisleni et al. showed a clear association between diabetic polyneuropathy and the C936T polymorphism of the *VEGF* gene and the C242T polymorphism of the *p22phox* allele of *CYBA* gene [52].

Functional *GRP78* variants in heat shock protein family A (Hsp70) member 5 (*HSPA5*) genes are likely to have some influence on the gene expression, which results in the dysfunction of peripheral nerves and neuropathy. According to Jia et al., functional *GRP78* rs391957 variants, which are located in the promoter region, 57168556T>C, are known to cause abnormal promoter activities significantly associated with DPN [54].

Adiponectin gene (*ADPN*) serves as a protective factor in preventing diabetes progression by suppressing inflammatory responses and increasing insulin sensitivity [55]. SNVs of *ADPN* may influence T2DM, but *ADPN* variants SNV45 (45T/G, rs2241766) and SNV276 (276G/T, rs1501299) are the two most prominent variants influencing the disease progression, especially pathogenesis of DPN [56, 57]. A case-control study conducted by Ji et al. to evaluate the association between *ADPN* gene variants and pathogenesis of DPN in T2D patients indicated an increased risk of DPN in T2D patients, by downregulating *ADPN* expression which resulted in significantly reduced circulating *ADPN* plasma levels. Furthermore, they reported that the polymorphism frequencies of GG and GT haplotypes in the DPN group were significantly lower than those in the matched control group, while the frequency of the TG haplotype in the DPN group was markedly higher than that in the control group, showing a clear association between *ADPN* gene variants and the risk of DPN [58].

11. Genetic Variants of DPN in Different Ethnic Populations

Up to now, only few ethnic groups have described population-specific genetic variants associated with DPN. In a study conducted by Lu et al., 10 SNVs associated with pathogenesis of T2DM were studied. They reported that rs5219 on *KCNJ11* (E23K) gene is significantly associated with peripheral nerve function in a Chinese population with T2D [23]. Jia et al. studied the significance of functional *GRP78* gene variants in predicting the onset of type 2 DPN in the Chinese population. They suggested that the *GRP78* rs391957 promoter polymorphism is a potential risk factor for type 2 DPN in this population [54]. Prasad et al. studied forty-two patients with T2D from the Institute of Diabetology, Madras Medical College, and Rajiv Gandhi Government General Hospital in Chennai, Tamil Nadu, India. In this study, the extent of DNA damage in patients suffering from T2D, both with and without neuropathy, was analyzed. No genetic variants were evaluated in this study. The data demonstrated that the frequency of DNA damage was significantly higher in the T2D patients with DPN than in the controls [59]. Stoian et al. conducted a study in the University Center of Tırgu Mures, Romania. In their case-control study, which included a total of 182 participants, including 84 unrelated patients with T2D and an age-matched control group consisting of 98 unrelated individuals without T2D, they evaluated the influence of *GSTM1*, *GSTT1*, and *GSTP1* variants on T2D and DPN risk. Their data suggested that *GSTM1*, *GSTT1*, and *GSTP1* gene variants were not associated with individual susceptibility to developing DPN in patients with T2D in the Romanian population [60].

An association study of C936T polymorphism of the *VEGF* gene and the C242T polymorphism of the *p22phox* gene with T2D and DPN in a population of Caucasian ethnicity was studied by Ghisleni et al. According to their results, the C936T polymorphism of the *VEGF* gene and C242T polymorphism of the *p22phox* gene did not correlate with the risk of developing diabetes mellitus or neuropathic signs and symptoms. When considering the results of other studies, a substantial heterogeneity in the findings is observed, which demonstrates a complex link between the risk factors of DM and genetic predisposition to DPN [52]. Common susceptible and prognostic genetic factors associated with DPN in T2D are diverse in different pathophysiological pathways, and it is difficult to separate each genetic variant from the other as most of the variants are interrelated with each other (Table 1).

12. Conclusions

Although targeted gene sequencing is still a method of choice to identify rare functional mutations in monogenic disorders, exome sequencing becomes an attractive and cost-effective alternative when other disease-mapping strategies provide few or ambiguous results. This review has attempted to identify the common susceptibility and prognostic genetic factors associated with DPN in T2D. Knowledge about these factors is vital as DPN is one of the debilitating complications associated with T2D and identification of the common genetic variants would be valuable for the future development of gene panels targeted for the early detection and prognosis of DPN. Together with these gene panels, further gene expression studies will need to be conducted to modulate effective targeted therapies for DPN in these patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] International Diabetes Federation, *IDF Diabetes Atlas*, International Diabetes Federation, Brussels, Belgium, 6th edition, 2013.

[2] M. G. M. Wolfs, M. H. Hofker, C. Wijmenga, and T. W. van Haeften, "Type 2 diabetes mellitus: new genetic insights will lead to new therapeutics," *Current Genomics*, vol. 10, no. 2, pp. 110–118, 2009.

[3] S. Singh, "The genetics of type 2 diabetes mellitus: a review," *Journal of Scientific Research*, vol. 55, pp. 35–48, 2011, Banaras Hindu University, Varanasi.

[4] J. L. Edwards, A. M. Vincent, H. T. Cheng, and E. L. Feldman, "Diabetic neuropathy: mechanisms to management," *Pharmacology & Therapeutics*, vol. 120, no. 1, pp. 1–34, 2008.

[5] Centers for Disease Control and Prevention, *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014*, US Department of Health and Human Services, Atlanta, GA, USA, 2014.

[6] C. Guja, P. Gagniuc, and C. Ionescu-tîrgovişte, "Genetic factors involved in the pathogenesis of type 2 diabetes," *The Proceedings of the Romanian Academy, Series B*, vol. 1, pp. 44–61, 2012.

[7] U. J. Kommoju and B. M. Reddy, "Genetic etiology of type 2 diabetes mellitus: a review," *International Journal of Diabetes in Developing Countries*, vol. 31, no. 2, pp. 51–64, 2011.

[8] S. K. Kota, L. K. Meher, S. Jammula, S. K. Kota, and K. D. Modi, "Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities," *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, vol. 6, no. 1, pp. 54–58, 2012.

[9] N. Kato, "Insights into the genetic basis of type 2 diabetes," *Journal of Diabetes Investigation*, vol. 4, no. 3, pp. 233–244, 2013.

[10] A. M. Vincent, J. W. Russell, P. Low, and E. L. Feldman, "Oxidative stress in the pathogenesis of diabetic neuropathy," *Endocrine Reviews*, vol. 25, no. 4, pp. 612–628, 2004.

[11] S. Yagihashi, H. Mizukami, and K. Sugimoto, "Mechanism of diabetic neuropathy: where are we now and where to go?," *Journal of Diabetes Investigation*, vol. 2, no. 1, pp. 18–32, 2011.

[12] M. Imamura and S. Maeda, "Genetics of type 2 diabetes: the GWAS era and future perspectives [review]," *Endocrine Journal*, vol. 58, no. 9, pp. 723–739, 2011.

[13] E. Zeggini, L. J. Scott, R. Saxena et al., "Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes," *Nature Genetics*, vol. 40, no. 5, pp. 638–645, 2008.

[14] A. H. Ropper, K. C. Gorson, C. L. Gooch et al., "Vascular endothelial growth factor gene transfer for diabetic polyneuropathy: a randomized, double-blinded trial," *Annals of Neurology*, vol. 65, no. 4, pp. 386–393, 2009.

[15] D. Bansal, K. Gudala, H. Muthyala, H. P. Esam, R. Nayakallu, and A. Bhansali, "Prevalence and risk factors of development of peripheral diabetic neuropathy in type 2 diabetes mellitus in a tertiary care setting," *Journal of Diabetes Investigation*, vol. 5, no. 6, pp. 714–721, 2014.

[16] S. Tesfaye, A. J. M. Boulton, P. J. Dyck et al., "Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments," *Diabetes Care*, vol. 33, no. 10, pp. 2285–2293, 2010.

[17] B. C. Callaghan, H. T. Cheng, C. L. Stables, A. L. Smith, and E. L. Feldman, "Diabetic neuropathy: one disease or two?," *Current Opinion in Neurology*, vol. 25, no. 5, pp. 536–541, 2012.

[18] N. Papanas, A. I. Vinik, and D. Ziegler, "Neuropathy in prediabetes: does the clock start ticking early?," *Nature Reviews Endocrinology*, vol. 7, no. 11, pp. 682–690, 2011.

[19] C. Figueroa-Romero, M. Sadidi, and E. L. Feldman, "Mechanisms of disease: the oxidative stress theory of diabetic neuropathy," *Reviews in Endocrine and Metabolic Disorders*, vol. 9, no. 4, pp. 301–314, 2008.

[20] C. Yu, S. Rouen, and R. T. Dobrowsky, "Hyperglycemia and downregulation of caveolin-1 enhance neuregulin-induced demyelination," *Glia*, vol. 56, no. 8, pp. 877–887, 2008.

[21] J. F. McGuire, S. Rouen, E. Siegfreid, D. E. Wright, and R. T. Dobrowsky, "Caveolin-1 and altered neuregulin signaling contribute to the pathophysiological progression of diabetic peripheral neuropathy," *Diabetes*, vol. 58, no. 11, pp. 2677–2686, 2009.

[22] I. K. Lukic, P. M. Humpert, P. P. Nawroth, and A. Bierhaus, "The RAGE pathway: *activation and perpetuation in the pathogenesis of diabetic neuropathy*," *Annals of the New York Academy Sciences*, vol. 1126, no. 1, pp. 76–80, 2008.

[23] J. Lu, Y. Luo, J. Wang et al., "Association of type 2 diabetes susceptibility loci with peripheral nerve function in a Chinese population with diabetes," *Journal of Diabetes Investigation*, vol. 8, no. 1, pp. 115–120, 2017.

[24] S. Yigit, N. Karakus, and A. Inanir, "Association of *MTHFR* gene C677T mutation with diabetic peripheral neuropathy and diabetic retinopathy," *Molecular Vision*, vol. 19, pp. 1626–1630, 2013.

[25] C. Monastiriotis, N. Papanas, and G. Trypsianis, "The ε4 allele of the APOE gene is associated with more severe peripheral neuropathy in type 2 diabetic patients," *Angiology*, vol. 64, no. 6, pp. 451–455, 2013.

[26] N. Papanas, K. Papatheodorou, D. Papazoglou, S. Kotsiou, D. Christakidis, and E. Maltezos, "An insertion/deletion polymorphism in the alpha2B adrenoceptor gene is associated with peripheral neuropathy in patients with type 2 diabetes mellitus," *Experimental and Clinical Endocrinology & Diabetes*, vol. 115, no. 05, pp. 327–330, 2007.

[27] J. Hur, K. Sullivan, M. Pande et al., "The identification of gene expression profiles associated with progression of human diabetic neuropathy," *Brain*, vol. 134, no. 11, pp. 3222–3235, 2011.

[28] A. Rivero, C. Mora, M. Muros, J. Garcia, H. Herrera, and J. F. Navarro-Gonzalez, "Pathogenic perspectives for the role of inflammation in diabetic nephropathy," *Clinical Science*, vol. 116, no. 6, pp. 479–492, 2009.

[29] M. Kakoki, K. A. Sullivan, C. Backus et al., "Lack of both bradykinin B1 and B2 receptors enhances nephropathy, neuropathy, and bone mineral loss in Akita diabetic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 22, pp. 10190–10195, 2010.

[30] T. Pawelczyk, M. Grden, R. Rzepko, M. Sakowicz, and A. Szutowicz, "Region-specific alterations of adenosine receptors expression level in kidney of diabetic rat," *The American Journal of Pathology*, vol. 167, no. 2, pp. 315–325, 2005.

[31] S. Z. Duan, M. G. Usher, and R. M. Mortensen, "PPARs: the vasculature, inflammation and hypertension," *Current Opinion in Nephrology and Hypertension*, vol. 18, no. 2, pp. 128–133, 2009.

[32] S. Yamagishi, S. Ogasawara, H. Mizukami et al., "Correction of protein kinase C activity and macrophage migration in peripheral nerve by pioglitazone, peroxisome proliferator activated-γ-ligand, in insulin-deficient diabetic rats," *Journal of Neurochemistry*, vol. 104, no. 2, pp. 491–499, 2008.

[33] Y. Li, K. Tang, Z. Zhang et al., "Genetic diversity of the apolipoprotein E gene and diabetic nephropathy: a meta-analysis," *Molecular Biology Reports*, vol. 38, no. 5, pp. 3243–3252, 2011.

[34] C. Cheng, M. Kobayashi, J. A. Martinez et al., "Evidence for epigenetic regulation of gene expression and function in chronic experimental diabetic neuropathy," *Journal of Neuropathology & Experimental Neurology*, vol. 74, no. 8, pp. 804–817, 2015.

[35] L. A. Donnelly, A. S. Doney, A. T. Hattersley, A. D. Morris, and E. R. Pearson, "The effect of obesity on glycaemic response to metformin or sulphonylureas in type 2 diabetes," *Diabetic Medicine*, vol. 23, no. 2, pp. 128–133, 2006.

[36] Y. M. Kim, B. S. Cha, D. J. Kim et al., "Predictive clinical parameters for therapeutic efficacy of rosiglitazone in Korean type 2 diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 67, no. 1, pp. 43–52, 2005.

[37] M. Osterbrand, M. Fahlen, A. Oden, and B. A. Eliasson, "A method to predict the metabolic effects of changes in insulin treatment in subgroups of a large population based patient cohort," *European Journal of Epidemiology*, vol. 22, no. 3, pp. 151–157, 2007.

[38] P. D. O'Brien, J. Hur, N. J. Robell, J. M. Hayes, S. A. Sakowski, and E. L. Feldman, "Gender-specific differences in diabetic neuropathy in BTBR *ob*/*ob* mice," *Journal of Diabetes and its Complications*, vol. 30, no. 1, pp. 30–37, 2016.

[39] W. Meng, H. A. Deshmukh, L. A. Donnelly et al., "A genome-wide association study provides evidence of sex-specific involvement of Chr1p35.1 (*ZSCAN20-TLR12P*) and Chr8p23.1 (*HMGB1P46*) with diabetic neuropathic pain," *EBioMedicine*, vol. 4, no. 2no. 10, pp. 1386–1393, 2015.

[40] A. Festa, R. D'Agostino Jr., R. P. Tracy, and S. M. Haffner, "Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study," *Diabetes*, vol. 51, no. 4, pp. 1131–1137, 2002.

[41] S. B. Nicholas, E. Aguiniga, Y. Ren, J. Kim, J. Wong, and N. Govindarajan, "Plasminogen activator inhibitor-1 deficiency retards diabetic nephropathy," *Kidney International*, vol. 67, no. 4, pp. 1297–1307, 2005.

[42] R. Yang and J. M. Trevillyan, "c-Jun N-terminal kinase pathways in diabetes," *The International Journal of Biochemistry & Cell Biology*, vol. 40, no. 12, pp. 2702–2706, 2008.

[43] L. Chilton, A. Middlemas, N. Gardiner, and D. R. Tomlinson, "The p75 neurotrophin receptor appears in plasma in diabetic rats—characterisation of a potential early test for neuropathy," *Diabetologia*, vol. 47, no. 11, pp. 1924–1930, 2004.

[44] P. C. Schulze, J. Yoshioka, T. Takahashi, Z. He, G. L. King, and R. T. Lee, "Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein," *Journal of Biological Chemistry*, vol. 279, no. 29, pp. 30369–30374, 2004.

[45] J. Jurado, J. Ybarra, J. H. Romeo, M. Garcia, and E. Zabaleta-del-Olmo, "Angiotensin-converting enzyme gene single polymorphism as a genetic biomarker of diabetic peripheral neuropathy: longitudinal prospective study," *Journal of Diabetes and its Complications*, vol. 26, no. 2, pp. 77–82, 2012.

[46] K. Chandrasekaran, M. Anjaneyulu, T. Inoue et al., "Mitochondrial transcription factor A regulation of mitochondrial degeneration in experimental diabetic neuropathy," *American Journal of Physiology Endocrinology and Metabolism*, vol. 309, no. 2, pp. E132–E141, 2015.

[47] R. Saraswathy, S. Anand, S. K. Kunnumpurath, R. J. Kurian, A. D. Kaye, and N. Vadivelu, "Chromosomal aberrations and exon 1 mutation in the AKR1B1 gene in patients with diabetic neuropathy," *The Ochsner Journal*, vol. 14, no. 3, pp. 339–342, 2014.

[48] J. Lorenzen, R. Kumarswamy, S. Dangwal, and T. Thum, "MicroRNAs in diabetes and diabetes-associated complications," *RNA Biology*, vol. 9, no. 6, pp. 820–827, 2012.

[49] C. Ciccacci, R. Morganti, D. Di Fusco et al., "Common variants in MIR146a, MIR128a and MIR27a genes contribute to neuropathy susceptibility in type 2 diabetes," *Acta Diabetologica*, vol. 51, no. 4, pp. 663–671, 2014.

[50] S. S. Prabhakar, "Role of nitric oxide in diabetic nephropathy," *Seminars in Nephrology*, vol. 24, no. 4, pp. 333–344, 2004.

[51] T. Nakagawa, W. Sato, O. Glushakova et al., "Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 18, no. 2, pp. 539–550, 2007.

[52] M. M. Ghisleni, V. Biolchi, B. C. Jordon, C. Rempel, J. P. Genro, and A. Pozzobon, "Association study of C936T polymorphism of the VEGF gene and the C242T polymorphism of the p22phox gene with diabetes mellitus type 2 and distal diabetic polyneuropathy," *Molecular Medicine Reports*, vol. 12, no. 3, pp. 4626–4633, 2015.

[53] B. Wirostko, T. Y. Wong, and R. Simó, "Vascular endothelial growth factor and diabetic complications," *Progress in Retinal and Eye Research*, vol. 27, no. 6, pp. 608–621, 2008.

[54] Y. Jia, Y. Tong, and L. Min, "Significance of functional *GRP78* polymorphisms in predicting the onset of type 2 diabetic peripheral neuropathy in Chinese population," *Neurological Research*, vol. 37, no. 8, pp. 683–687, 2015.

[55] M. Abdelgadir, A. F. Karlsson, L. Berglund, and C. Berne, "Low serum adiponectin concentrations are associated with insulin sensitivity independent of obesity in Sudanese subjects with type 2 diabetes mellitus," *Diabetology & Metabolic Syndrome*, vol. 5, no. 1, p. 15, 2013.

[56] L. Y. Han, Q. H. Wu, M. L. Jiao et al., "Associations between single-nucleotide polymorphisms (+45T>G, +276G>T, −11377C>G, −11391G>A) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis," *Diabetologia*, vol. 54, no. 9, pp. 2303–2314, 2011.

[57] S. Sandy An, N. D. Palmer, A. J. Hanley et al., "Genetic analysis of adiponectin variation and its association with type 2 diabetes in African Americans," *Obesity*, vol. 21, no. 12, pp. E721–E729, 2013.

[58] Z. Y. Ji, H. F. Li, Y. Lei et al., "Association of adiponectin gene polymorphisms with an elevated risk of diabetic peripheral neuropathy in type 2 diabetes patients," *Journal of Diabetes and its Complications*, vol. 29, no. 7, pp. 887–892, 2015.

[59] M. Prasad, S. C. Bronson, T. Warrier et al., "Evaluation of DNA damage in type 2 diabetes mellitus patients with and without peripheral neuropathy: a study in south Indian population," *Journal of Natural Science, Biology and Medicine*, vol. 6, no. 1, pp. 80–84, 2015.

[60] A. Stoian, C. Bănescu, R. I. Bălaşa et al., "Influence of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms on type 2 diabetes mellitus and diabetic sensorimotor peripheral neuropathy risk," *Disease Markers*, vol. 2015, Article ID 638693, 10 pages, 2015.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 7631659, 9 pages
https://doi.org/10.1155/2018/7631659

Research Article

Incidence and Risk Factors of Diabetic Foot Ulcer: A Population-Based Diabetic Foot Cohort (ADFC Study)—Two-Year Follow-Up Study

Leila Yazdanpanah,[1] **Hajieh Shahbazian**,[1] **Iraj Nazari**,[2] **Hamid Reza Arti**,[3] **Fatemeh Ahmadi**,[4] **Seyed Ehsan Mohammadianinejad**,[5] **Bahman Cheraghian**,[6] **and Saeed Hesam**[7]

[1]*Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[2]*Department of Vascular Surgery, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[3]*Department of Orthopedic, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[4]*Infectious Disease Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[5]*Department of Neurology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[6]*Department of Epidemiology and Biostatistics, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[7]*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

Correspondence should be addressed to Leila Yazdanpanah; leila.yazdanpanah@gmail.com

Received 14 November 2017; Accepted 30 January 2018; Published 15 March 2018

Academic Editor: Nikolaos Papanas

Aim/Introduction. This study was carried out to assess the incidence and risk factors of diabetic foot ulcer (DFU). *Materials and Methods.* In this prospective cohort study in a university hospital, all the participants were examined and followed up for new DFU as final outcome for two years. To analyze the data, the variables were first evaluated with a univariate analysis. Then variables with P value < 0.2 were tested with a multivariate analysis, using backward-elimination multiple logistic regression. *Results.* Among 605 patients, 39 cases had DFU, so we followed up the remaining 566 patients without any present or history of DFU. A two-year cumulative incidence of diabetic foot ulcer was 5.62% (95% CI 3.89–8.02). After analysis, previous history of DFU or amputation [OR = 9.65, 95% CI (2.13–43.78), P value = 0.003], insulin usage [OR = 5.78, 95% CI (2.37–14.07), P value < 0.01], gender [OR = 3.23, 95% CI (1.33–7.83), P value = 0.01], distal neuropathy [OR = 3.37, 95% CI (1.40–8.09), P value = 0.007], and foot deformity [OR = 3.02, 95% CI (1.10–8.29), P value = 0.032] had a statistically significant relationship with DFU incidence. *Conclusion.* Our data showed that the average annual DFU incidence is about 2.8%. Independent risk factors of DFU development were previous history of DFU or amputation, insulin consumption, gender, distal neuropathy, and foot deformity. These findings provide support for a multifactorial etiology for DFU.

1. Introduction

Diabetes prevalence is increasing in developing and developed countries all over the world [1]. Diabetes complications are increasing too in this pandemic [2], making diabetes a major global health problem in different countries [3–5]. Among diabetes complications, managing diabetic foot remains as a major challenge for health care systems [6].

Diabetic foot is still the most frequent reason of hospitalization of patients with diabetes [7–10], and diabetes is the main cause of more than half of nontraumatic lower limb amputations [11–15]. In fact, every 30 seconds in the world, a lower limb is amputated due to diabetes [16], and it goes without saying that these amputations increase mortality rate [17].

About 15–25% of patients with diabetes may develop foot ulcer during their lifetime [15–20]. The annual risk of

developing diabetic foot ulcer in patients with diabetes is estimated to be about 2%, but this risk in patients with previous history of foot ulceration is expected to increase to 17–60% over the next three years [21]. The prevalence of diabetic foot ulcer is reported to be 1.3–12% in different studies [19–22].

Since the development of foot ulcers and amputations are preventable and this condition can greatly affect the quality of life of patients [23], prevention of this complication can relieve direct and indirect cost burdens on society. Based on the results of a study conducted in Iran, of the total costs of diabetes complications in this country, 10.7% were related to diabetic foot (107.1 billion USD) [3].

Large cohort studies on diabetic foot ulcer (DFU) incidence are rare [16–24]. In Iran, there has not been any cohort study conducted on this complication; furthermore, the socio-economic differences between different societies can affect the incidence rates. Therefore, this cohort was designed to identify diabetic foot incidence and risk factors to help health providers to reduce the burden of this complication.

2. Materials and Methods

This population-based prospective cohort study named ADFC (Ahvaz Diabetic Foot Cohort), was done in a tertiary care diabetes clinic in Golestan Hospital, a university hospital in Ahvaz (south-west of Iran) with a diabetic foot clinic that is the first diabetic foot clinic in the province. This clinic was the main center of this cohort study. All patients with diabetes (with or without foot problems) were recruited from July 2014 and followed up until July 2016.

Nonprobabilistic convenience sampling was used to select the patients. The inclusion criteria were as follows: (1) age ≥ 18 years; (2) diagnosed with diabetes mellitus, both types 1 and 2; (3) able to complete the consent form; and (4) able to walk. The exclusion criteria were (1) severe disabling disease or inability to walk, (2) severe mental illness preventing informed consent, and (3) current foot ulcer.

The research followed the tenets of the Declaration of Helsinki and was also approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. The procedure was described for all patients, and written informed consent was completed and signed by all of them at the first visit. A checklist including the following variables was completed for all participants: age, sex, blood pressure (BP), marital status, educational level, ethnicity, body mass index (BMI), waist circumference, job activity, smoking status, type of diabetes, diabetes duration, type of diabetes treatment (oral antidiabetes agents or insulin usage), diabetic retinopathy, diabetic nephropathy, having glucometer, history of DFU or amputation, present foot ulcer, preventive foot care, nail care, ill-fitting shoe, and patient training on feet.

Blood pressure was recorded as systolic and diastolic BP with a mercury sphygmomanometer. Marital status was categorized as single, married, dead partner, or divorced. Educational level was defined as illiterate, diploma or under diploma, and university degree. Ethnicities were defined as Arab, Fars, Lor, and other. BMI was measured in kg/m^2. Waist circumference was considered as midline of the lower ribs and upper outer edge of the right iliac crest. Job activity was categorized to low, moderate, high, and no job. Smoking status was described as present smoker, former smoker, and no history of smoking. Diabetic retinopathy was considered if the patients' medical record included pupil dilation followed by examination by fundoscope (nonproliferative or proliferative retinopathy, clinically significant macular edema). Diabetic nephropathy was defined based on the patients' medical record report of 24 hours' urine collection test (microalbuminuria or overt proteinuria) or azotemia, dialysis, or kidney transplantation. Preventive foot care was considered as washing the feet and doing daily feet self-exam, drying the feet after washing, moisturizing the feet, not walking barefoot, not putting the feet close to the heater, and wearing slippers and suitable socks at home. Nail care was defined as not cutting toenails very short, not cutting the corners of the toenails, and filing the toenails. Ill-fitting footwear was described as slippers, tight shoe, or shoes with pressure points on the feet. Suitable socks were considered as cotton socks having a soft elastic band. Patient training on feet was defined as self-training (reading books or brochures, visiting websites, or watching films) or participation in scheduled individual or group sessions.

All participants were then examined. The examination included the following: skin and nails, types of foot deformity, neurologic foot exams, and vascular foot exams. DFU was defined as a full thickness skin defect at least Wagner stage 1 [25]. Pressure sensation examination was performed by 10-gram monofilaments (Owen Mumford, UK). Nylon monofilaments were applied perpendicular on four sites (1st, 3rd, and 5th metatarsal heads and plantar surface of distal hallux) of each foot. Areas of ulcer, calluses, necrotic tissues, and scars were avoided during the test. Loss of ability to detect the monofilament at even one site of examination was considered as distal neuropathy [26]. For vascular examination, dorsalis pedis, tibialis posterior, popliteal, and femoral pulses were assessed. ABI (ankle-brachial index) was measured by a handheld Doppler device (Huntleigh Diabetic Foot Kit, UK) and calculated by the following formula: ABI = (maximum systolic pressure of dorsalis pedis artery or tibialis posterior)/(maximum systolic pressure of brachial artery) separately for each leg. ABI = 0.9–1.3 was considered as normal, ABI = 0.4–0.9 as vascular disease, and ABI < 0.4 as severe vascular disease [27].

The questionnaires were completed, and the clinical exams were performed by a trained general physician.

A blood sample was obtained from each person to measure their HbA1c. It was tested in the Diabetes Research Center laboratory using the NycoCard technique to assess the plasma glucose control. HbA1c of less than 7% was considered as good glycemic control [28], 7-8% as relatively good control, and more than 8% as poor glycemic control.

Then all the patients were followed up for new DFU as final outcome for two years.

The data were recorded on SPSS version 20. To describe the variables, mean ± SD was used for continuous data, and frequency and percentage were used for categorical data. To analyze the data, the variables were first evaluated with a univariate analysis with diabetic foot ulcer incidence as variable. The statistical methods in this part were independent

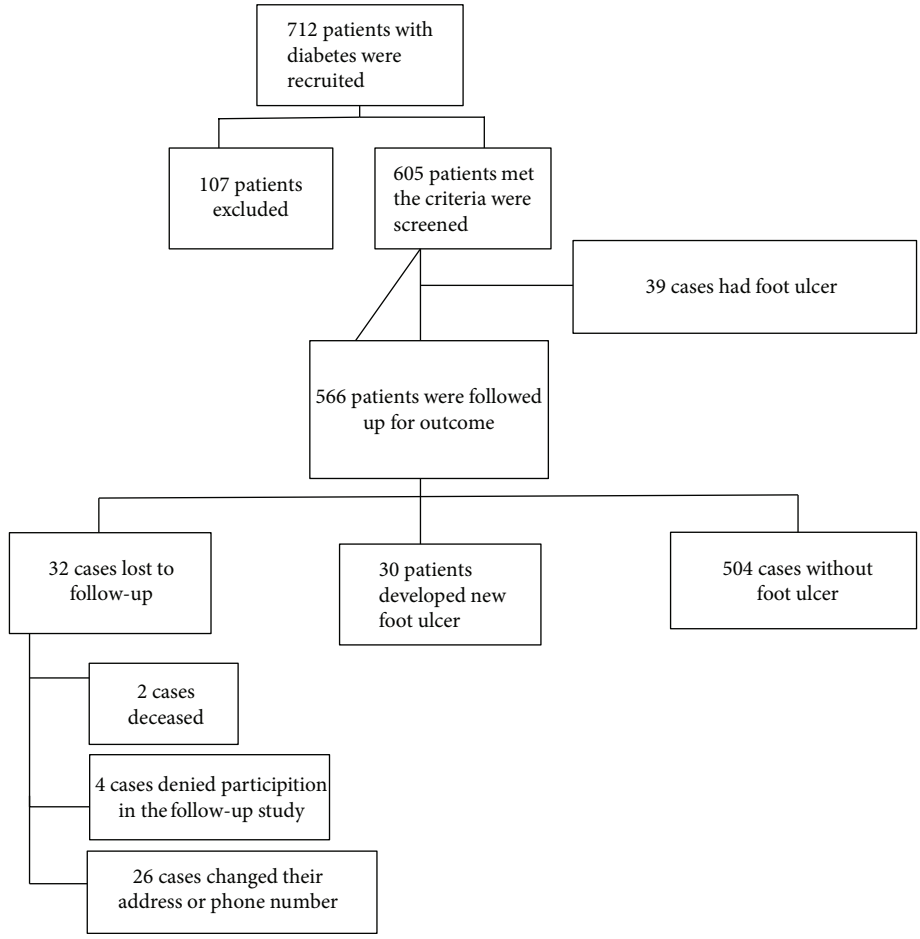


Figure 1: Diagram of the diabetic foot cohort participants.

t-test (Mann–Whitney test if the data were not normally distributed), chi-square, and Fisher's exact test. Variables with P value < 0.2 were tested with a multivariate analysis, using backward-elimination multiple logistic regression. Thus, the most statistically significant variables were identified as risk factors. In this study, P value ≤ 0.05 was considered as significant.

3. Result

Among all 712 patients with diabetes who were recruited in the study, 605 patients met the inclusion criteria. Thirty-nine cases (6.4%) had DFU, so we followed up 566 patients without any present or history of DFU for 24 months, of whom 32 were lost to follow-up (Figure 1). The mean duration of follow-up was 23.55 ± 2.39 months. The mean time at which the development of the ulcer occurred is 16.1 ± 6.6 months (minimum 4 months and maximum 24 months).

The mean (±SD) age of the participants was 53.52 ± 10.8 years. Of all cases, 307 (57.5%) were female and 521 (97.6%) had type 2 diabetes. The mean (±SD) duration of diabetes was 8.77 ± 6.87 years, and the mean HbA1c was 8.7 ± 1.7%. Seventy-nine cases (14.8%) had good glycemic control, 112 patients (21%) had HbA1c = 7-8%, and 343 cases (64.2%) had poor glycemic control.

The patients were followed up for diabetic foot ulceration as final outcome. The two-year cumulative incidence (risk) of diabetic foot ulcer was 5.62% (95% CI 3.89–8.02) (30 cases). The first-year incidence of foot ulceration was 1.50% (95% CI 0.70–3.05), and the second-year incidence was 4.18% (95% CI 2.70–6.36).

We excluded from data analysis patients who were lost to follow-up. Baseline characteristics of all participants and comparison between two groups (with and without diabetic foot ulcer) are shown in Table 1. In a univariate analysis, patients developing DFU were predominantly male, had longer duration of diabetes, had lower educational level, and were more likely to be smokers, compared with patients not developing DFU.

A univariate evaluation of risk factors of diabetic foot ulcer incidence and the comparison between patients developing and those not developing DFU are presented in Table 2. Patients developing DFU had more diabetic foot deformity, were less trained about their feet, and had more history of previous DFU or amputation, more decreased distal pulses, and more neuropathy, nephropathy, and retinopathy as opposed to patients not developing DFU.

In a multivariate logistic regression analysis, the variables with P value less than 0.2 were tested by backward elimination. Table 3 describes the risk factors that were in the model.

Table 1: Baseline characteristics of all participants and comparison between two groups (developing and not developing DFU).

Characteristics	All patients ($n = 534$)	Patients developing DFU ($n = 30$)	Patients not developing DFU ($n = 504$)	P value
*Age (years)**	53.52 ± 10.81	55.9 ± 13.47	53.38 ± 10.63	0.214
Gender				
Female	307 (57.5)	9 (30)	298 (59.1)	**0.002**
Male	227 (42.5)	21 (70)	206 (40.9)	
*Diabetes duration (year)**	8.77 ± 6.87	13.46 ± 8.48	8.49 ± 6.67	**0.001**
*BMI (kg/m^2)**	28.63 ± 4.42	28.33 ± 4.91	28.65 ± 4.40	0.700
*Waist circumference (cm)**	95.22 ± 11.02	95.33 ± 12.46	95.22 ± 10.94	0.710
*Blood pressure (mmHg)**				
Systolic BP	128.74 ± 11.87	129.67 ± 12.99	128.68 ± 11.81	0.879
Diastolic BP	81.11 ± 4.92	81.33 ± 5.71	81.10 ± 4.88	0.918
Ethnicity				0.974
Fars	158 (29.6)	8 (26.7)	150 (29.8)	
Arab	272 (50.9)	16 (53.3)	256 (50.8)	
Lor	86 (16.1)	5 (16.7)	81 (16.1)	
Other	18 (3.4)	1 (3.3)	17 (3.4)	
Education				**0.017**
Illiterate	129 (24.2)	11 (36.6)	118 (23.4)	
≤Diploma	366 (68.5)	14 (46.7)	352 (69.9)	
University degree	39 (7.3)	5 (16.7)	34 (6.7)	
Marital status				0.269
Single	12 (2.2)	0 (0)	12 (2.4)	
Married	498 (93.3)	27 (90)	471 (93.4)	
Divorced or dead partner	24 (4.5)	3 (10)	21 (4.2)	
Job activity				0.097
Low	446 (83.5)	21 (70)	425 (84.3)	
Moderate	41 (7.7)	5 (16.7)	36 (7.1)	
High	17 (3.2)	1 (3.3)	16 (3.2)	
No job	30 (5.6)	3 (10)	27 (5.4)	
Smoking status				**0.013**
Present	26 (4.9)	3 (10)	23 (4.6)	
Former	43 (8.1)	6 (20)	37 (7.3)	
No smoker	465 (87.1)	21 (70)	444 (88.1)	

DFU: diabetic foot ulcer; BP: blood pressure. *These variables are described as mean ± SD; other are presented as N (%).

Finally, history of previous DFU or amputation, insulin usage, gender, distal neuropathy, and foot deformity had a statistically significant relationship with DFU incidence. Patient training on feet did not have any significant correlation with DFU incidence, but it was borderline significant [OR = 6.66, 95% CI (0.75, 59.19), P value = 0.089].

By controlling other variables, we found that previous history of DFU or amputation increased the odds of DFU by 9.65 times. The odds of DFU in patients using insulin were 5.78 times greater than those in patients using oral agent or just having lifestyle modification. The odds of DFU were 3.23 times more in men than in women. DFU was 3.51 times more likely in patients who had neuropathy than in patients without. Patients with foot deformity were 3.02 times more likely to develop DFU than were patients without (Table 3).

The comparison between the two groups in terms of preventive foot care is demonstrated in Table 4. Only 4 cases (0.7%) had a complete care of their feet.

4. Discussion

In this study, we sought to determine the incidence and risk factors of DFU for the first time in the south-west of Iran. In a prospective cohort, the two-year cumulative incidence of DFU was 5.62% (95% CI 3.89–8.02). The mean duration of follow-up was 23.55 ± 2.39 months. Baseline characteristics and probable risk factors were compared between patients developing and not developing DFU. Based on the multivariate analysis, independent risk factors of DFU development in this study were history of previous DFU or amputation, insulin usage, gender, distal neuropathy, and foot deformity.

Table 2: Univariate evaluation of risk factors of diabetic foot ulcer incidence and the comparison between patients developing and not developing DFU.

Variable	All patients ($n = 534$)	Patients developing DFU ($n = 30$)	Patients not developing DFU ($n = 504$)	P value
Neuropathy				**<0.001**
Yes	172 (32.2)	21 (70)	151 (30)	
No	362 (67.8)	9 (30)	353 (70)	
Decreased distal pulses				0.99
Yes	6 (1.1)	0 (0)	6 (1.1)	
No	528 (98.9)	30 (100)	498 (98.9)	
ABI				0.28
Normal	528 (98.9)	29 (96.7)	499 (99)	
Abnormal	6 (1.1)	1 (3.3)	5 (1)	
Foot deformity				**0.001**
Yes	50 (9.4)	8 (26.7)	42 (8.3)	
No	484 (90.6)	22 (73.3)	462 (91.7)	
HbA1c*	8.73 ± 1.73	9.31 ± 1.79	8.69 ± 1.72	0.058
Retinopathy				
Yes	106 (19.9)	12 (40)	94 (18.7)	**0.004**
No	428 (80.1)	18 (60)	410 (81.3)	
Nephropathy				**0.004**
Yes	47 (8.8)	7 (23.3)	40 (7.9)	
No	487 (91.2)	23 (76.7)	464 (92.1)	
History of previous DFU or amputation				**<0.001**
Yes	11 (2.1)	6 (20)	5 (1)	
No	523 (97.9)	24 (80)	499 (99)	
Insulin consumption				**<0.001**
Yes	163 (30.5)	22 (73.7)	141 (28)	
No	371 (69.5)	8 (26.7)	363 (72)	
Patient training on feet				0.104
Yes	77 (14.4)	1 (3.3)	76 (15.1)	
No	457 (85.6)	29 (96.7)	428 (84.9)	
Ill-fitting footwear				0.433
Yes	338 (63.3)	21 (70)	317 (62.9)	
No	196 (36.7)	9 (30)	187 (37.1)	
Preventive foot care				0.999
Yes	4 (0.7)	0 (0)	4 (0.8)	
No	530 (99.3)	30 (100)	500 (99.2)	
Toenail care				0.246
Yes	32 (6)	0 (0)	31 (6.2)	
No	502 (94)	30 (100)	473 (93.8)	
Visit the physician less than 3 months ago				0.964
Yes	358 (67)	20 (66.7)	338 (67.1)	
No	176 (33)	10 (33.3)	166 (32.9)	
Having glucometer				0.840
Yes	383 (71.7)	22 (73.3)	361 (71.6)	
No	151 (28.3)	8 (26.7)	143 (28.4)	

* is presented in mean ± SD; other variables are presented as N (%).

Table 3: Independent risk factors of DFU using univariate and multivariate logistic regression analysis.

Risk factors (base)	Unadjusted OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
History of previous DFU or amputation (no)	24.95	7.11–87.57	<0.001	9.65	2.13–43.78	0.003
Insulin treatment (no)	7.08	3.08–16.27	<0.001	5.78	2.37–14.07	<0.01
Gender (female)	3.38	1.52–7.52	0.003	3.23	1.33–7.83	0.01
Distal neuropathy (no)	5.46	2.44–12.19	<0.001	3.37	1.40–8.09	0.007
Foot deformity (no)	4.00	1.68–9.54	0.002	3.02	1.10–8.29	0.032
Patient training on foot care (yes)	5.15	0.69–38.37	0.110	6.66	0.75–59.19	0.089

OR: odds ratio; CI: confidence interval.

Table 4: Comparison of preventive foot care in patients developing and not developing DFU.

Variable	All patients ($n = 534$)	Patients developing DFU ($n = 30$)	Patients not developing DFU ($n = 504$)	*P* value
Washing the feet daily				0.976
Yes	304 (56.9)*	17 (56.7)	287 (56.9)	
No	230 (43.1)	13 (43.3)	217 (43.1)	
Daily feet self-examination				0.266
Yes	120 (22.5)	4 (13.3)	116 (23)	
No	414 (77.5)	26 (86.7)	388 (77)	
Drying the feet after washing				0.757
Yes	54 (10.1)	2 (6.7)	52 (10.3)	
No	480 (89.9)	28 (93.3)	452 (89.7)	
Moisturizing the feet				0.617
Yes	108 (20.2)	5 (16.7)	103 (20.4)	
No	426 (79.8)	25 (83.3)	401 (79.6)	
Walking barefoot				0.999
Yes	14 (2.6)	0 (0)	14 (2.8)	
No	520 (97.4)	30 (100)	490 (97.2)	
Putting the feet close to the heater				0.561
Yes	60 (11.2)	2 (6.7)	58 (11.5)	
No	474 (88.8)	28 (93.3)	446 (88.5)	
Wearing slippers at home				0.222
Yes	39 (7.3)	0 (0)	39 (7.7)	
No	495 (92.7)	30 (100)	465 (92.3)	
Wearing suitable socks				0.999
Yes	48 (9)	2 (6.7)	46 (9.1)	
No	486 (91)	28 (93.3)	458 (90.9)	

*All are presented in number (%).

Prospective estimates of DFU incidence are rare [1-29]. Few studies, like ours have reported outcome in patients without any active or past foot ulcer [1]. The average annual DFU incidence in the present study was about 2.8%, which is comparable to that of other studies [22–30]. These studies are prospective (except Ramsey et al. [[22]]) with older patients and nearly the same duration of diabetes in comparison with our study. Leese et al. study showed 4.7% DFU incidence during 1.7 years' follow-up [26]. Abbott et al. reported 2.2% average annual incidence of DFU [24]. Crawford et al.'s study reported 1.93% new foot ulcer during an average 1-year follow-up [30], while Ramsey et al.'s study reported DFU incidence to be 5.8% over 3 years of observation [22]. A prospective study by Hurley et al., involving 18 months' follow-up of 563 patients, reported the same incidence too, but their patients had lower duration of diabetes [11]. Jiang et al.'s study, a cohort in China with 678 patients, reported DFU incidence to be 8.1% in a 1-year follow-up, which was higher than that in our study and that of western countries according to their own report [9].

According to the literature, the risk factors of foot ulceration vary from one study to another, but some of them are common. First, we used a univariate analysis to demonstrate the simple relationships between DFU and its potential risk

factors; however, many of these variables are substitutes for real factors. Consistent with other studies, previous history of DFU or amputation was the most associated risk factor of DFU in our study [1–32].

This is logical because patients with a history of ulceration may be predisposed to different micro- and macrovascular dysfunctions or peripheral neuropathy.

We found that patients treated with insulin were more likely to develop foot ulcer than were patients whose diabetes is managed with oral glycemic agent or lifestyle modification alone. This may be because of the fact that when patients acquiesce to start insulin, they may already have diabetes for a long time with greater associated complications. It could also be because of another confounding factor that we did not assess in our study, so insulin might play the role of that confounder. This might be due to the unequal number of patients in the two groups after the 2-year follow-up (those developing foot ulcer were 30 as opposed to 504 who did not develop foot ulcer). Nevertheless, this finding is compatible with few studies [9–33]. In a systematic review, 7 studies out of 16 reported an association between DFU and insulin treatment [1]. It seems that further studies are needed to elaborate on this variable by eliminating the possible confounding factors and providing more details.

The predominance of male patients developing DFU was another finding of this study, which was consistent with other studies [1–32]. This could be explained by the fact that men have more outside activity than have women, which may lead to more foot exposure to different risks and more plantar pressure on their feet. Some studies reported male association with DFU just in a univariate analysis, but it was not significant in a multivariate analysis [9–30].

Another risk factor we identified was distal neuropathy, which is a well-known risk factor for developing foot ulcers. Many studies recommend the use of 10 g monofilament, and this finding was similar to that of some other prospective studies [11–33] and cross-sectional studies [34].

Foot deformity was another risk factor of DFU in this study comparable with some studies [24–31]. In one study [33], it was significant just in a univariate analysis. Few patients in our study had foot deformity (9.4%) including hammer toe, hallux valgus, bunion, prominent metatarsal head, and just one Charcot joint.

Logically, we expected patient training on feet to have a relationship with DFU development, but in contrast to our previous study [10], it did not have any significant relationship with foot ulceration; however, it was borderline significant. This might be because of the severe unbalance between trained and untrained patients (Table 2).

Unrelated foot ulcer risk factors in a multivariate analysis were diabetes duration, educational level, marital status, job activity, smoking, glycemic control (HbA1c), retinopathy, nephropathy, and decreased peripheral pulses in this study. Some of these factors such as hyperglycemia, smoking, and decreased peripheral pulses have been described as risk factors in other studies [11, 14]. In this study, the small number of patients developing foot ulcer may have led to this result. Alternatively, smoking cessation in some patients or intensive blood glucose control when developing a complication may bring about these results.

Our study has a number of limitations. First, although this study was performed in a cohort design, our patients were from a university hospital, and this may affect the results by selection bias. However, our hospital was the referral center and the focal point of diabetes in the province, and this can be the strength of the study to have different patients with different conditions. Second, the small size of subgroups in analysis may lead to less precision in our estimates, and this was the reason why a wide range of confidence intervals for some estimates was obtained. Third, we did not consider some potential confounders in the occurrence of new foot ulceration such as health care provision level and patient behavioral factors like compliance with training on their foot care. Finally, differences in methods of neuropathy assessment may affect the results to be compared with those of other studies.

The strength of this study was its low lost to follow-up rate in comparison with that of other studies [11–30]. We have low missing data too. This study was the first population-based prospective cohort of diabetic foot in this area, and this paper is the first report of this study. Additionally, all patients with diabetes have to undergo an annual foot examination to identify risk factors of foot ulceration [28]. The results of this study could support this suggestion to reduce DFU incidence, but it is better to assess the cost-effectiveness of this annual screening in future studies. We recommend that future studies have a larger sample size and longer follow-up period.

In conclusion, our data reported the average annual DFU incidence to be about 2.8%. Independent risk factors of DFU development were history of previous DFU or amputation, insulin usage, gender, distal neuropathy, and foot deformity. This finding provides support for a multifactorial etiology of DFU.

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

This paper is based on a Ph.D. thesis, and financial support was provided by the Ahvaz Jundishapur University of Medical Sciences (Reg. no. D-9409).

References

[1] M. Monteiro-Soares, E. J. Boyko, J. Ribeiro, I. Ribeiro, and M. Dinis-Ribeiro, "Predictive factors for diabetic foot ulceration: a systematic review," *Diabetes/Metabolism Research and Reviews*, vol. 28, no. 7, pp. 574–600, 2012.

[2] S. Morbach, H. Furchert, U. Groblinghoff et al., "Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade," *Diabetes Care*, vol. 35, no. 10, pp. 2021–2027, 2012.

[3] M. Javanbakht, H. R. Baradaran, A. Mashayekhi et al., "Cost-of-illness analysis of type 2 diabetes mellitus in Iran," *PLoS One*, vol. 6, no. 10, article e26864, 2011.

[4] L. Yazdanpanah, H. B. Shahbazian, A. Moravej Aleali, A. Jahanshahi, S. Ghanbari, and S. M. Latifi, "Prevalence, awareness and risk factors of diabetes in Ahvaz (south west of Iran)," *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, vol. 10, no. 2, pp. S114–S118, 2016.

[5] M. A. Ahmed, G. L. Muntingh, and P. Rheeder, "Perspectives on peripheral neuropathy as a consequence of metformin-induced vitamin B12 deficiency in T2DM," *International Journal of Endocrinology*, vol. 2017, Article ID 2452853, 6 pages, 2017.

[6] K. A. Sriyani, S. Wasalathanthri, P. Hettiarachchi, and S. Prathapan, "Predictors of diabetic foot and leg ulcers in a developing country with a rapid increase in the prevalence of diabetes mellitus," *PLoS One*, vol. 8, no. 11, article e80856, 2013.

[7] A. Arsanjani Shirazi, M. Nasiri, and L. Yazdanpanah, "Dermatological and musculoskeletal assessment of diabetic foot: a narrative review," *Diabetes& Metabolic Syndrome: Clinical Research & Reviews*, vol. 10, no. 2, pp. S158–S164, 2016.

[8] H. Pham, D. G. Armstrong, C. Harvey, L. B. Harkless, J. M. Giurini, and A. Veves, "Screening techniques to identify people at high risk for diabetic foot ulceration: a prospective multicenter trial," *Diabetes Care*, vol. 23, no. 5, pp. 606–611, 2000.

[9] Y. Jiang, X. Wang, L. Xia et al., "A cohort study of diabetic patients and diabetic foot ulceration patients in China," *Wound Repair and Regeneration*, vol. 23, no. 2, pp. 222–230, 2015.

[10] H. Shahbazian, L. Yazdanpanah, and S. M. Latifi, "Risk assessment of patients with diabetes for foot ulcers according to risk classification consensus of International Working Group on Diabetic Foot (IWGDF)," *Pakistan Journal of Medical Sciences*, vol. 29, no. 3, pp. 730–734, 2013.

[11] L. Hurley, L. Kelly, A. P. Garrow et al., "A prospective study of risk factors for foot ulceration: the West of Ireland Diabetes Foot Study," *QJM: An International Journal of Medicine*, vol. 106, no. 12, pp. 1103–1110, 2013.

[12] L. A. Lavery, E. J. Peters, and D. G. Armstrong, "What are the most effective interventions in preventing diabetic foot ulcers?," *International Wound Journal*, vol. 5, no. 3, pp. 425–433, 2008.

[13] M. Riaz, Z. Miyan, S. I. Zaidi et al., "Characteristics of a large cohort of patients with diabetes having at-risk feet and outcomes in patients with foot ulceration referred to a tertiary care diabetes unit," *International Wound Journal*, vol. 13, no. 5, pp. 594–599, 2016.

[14] A. Wang, X. Sun, W. Wang, and K. Jiang, "A study of prognostic factors in Chinese patients with diabetic foot ulcers," *Diabetic Foot & Ankle*, vol. 5, no. 1, 2014.

[15] L. Yazdanpanah, M. Nasiri, and S. Adarvishi, "Literature review on the management of diabetic foot ulcer," *World Journal of Diabetes*, vol. 6, no. 1, pp. 37–53, 2015.

[16] M. A. Gershater, M. Londahl, P. Nyberg et al., "Complexity of factors related to outcome of neuropathic and neuroischaemic/ischaemic diabetic foot ulcers: a cohort study," *Diabetologia*, vol. 52, no. 3, pp. 398–407, 2009.

[17] K. Ikura, K. Hanai, S. Oka et al., "Brachial-ankle pulse wave velocity, but not ankle-brachial index, predicts all-cause mortality in patients with diabetes after lower extremity amputation," *Journal of Diabetes Investigation*, vol. 8, no. 2, pp. 250–253, 2017.

[18] D. Martins-Mendes, M. Monteiro-Soares, E. J. Boyko et al., "The independent contribution of diabetic foot ulcer on lower extremity amputation and mortality risk," *Journal of Diabetes and its Complications*, vol. 28, no. 5, pp. 632–638, 2014.

[19] R. R. Yotsu, N. M. Pham, M. Oe et al., "Comparison of characteristics and healing course of diabetic foot ulcers by etiological classification: neuropathic, ischemic, and neuro-ischemic type," *Journal of Diabetes and its Complications*, vol. 28, no. 4, pp. 528–535, 2014.

[20] L. N. McEwen, K. R. Ylitalo, W. H. Herman, and J. S. Wrobel, "Prevalence and risk factors for diabetes-related foot complications in Translating Research Into Action for Diabetes (TRIAD)," *Journal of Diabetes and its Complications*, vol. 27, no. 6, pp. 588–592, 2013.

[21] M. Dubsky, A. Jirkovska, R. Bem et al., "Risk factors for recurrence of diabetic foot ulcers: prospective follow-up analysis in the Eurodiale subgroup," *International Wound Journal*, vol. 10, no. 5, pp. 555–561, 2013.

[22] S. D. Ramsey, K. Newton, D. Blough et al., "Incidence, outcomes, and cost of foot ulcers in patients with diabetes," *Diabetes Care*, vol. 22, no. 3, pp. 382–387, 1999.

[23] K. Winkley, D. Stahl, T. Chalder, M. E. Edmonds, and K. Ismail, "Risk factors associated with adverse outcomes in a population-based prospective cohort study of people with their first diabetic foot ulcer," *Journal of Diabetes and its Complications*, vol. 21, no. 6, pp. 341–349, 2007.

[24] C. A. Abbott, A. L. Carrington, H. Ashe et al., "The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort," *Diabetic Medicine*, vol. 19, no. 5, pp. 377–384, 2002.

[25] R. G. Frykberg, T. Zgonis, D. G. Armstrong et al., "Diabetic foot disorders. A clinical practice guideline (2006 revision)," *The Journal of Foot & Ankle Surgery*, vol. 45, no. 5, pp. S1–S66, 2006.

[26] G. P. Leese, F. Reid, V. Green et al., "Stratification of foot ulcer risk in patients with diabetes: a population-based study," *International Journal of Clinical Practice*, vol. 60, no. 5, pp. 541–545, 2006.

[27] L. Potier, C. Abi Khalil, K. Mohammedi, and R. R. Use, "Utility of ankle brachial index in patients with diabetes," *European Journal of Vascular & Endovascular Surgery*, vol. 41, no. 1, pp. 110–116, 2011.

[28] "Standards of medical care in diabetes—2017: summary of revisions," *Diabetes Care*, vol. 40, Supplement 1, pp. S4–S5, 2017.

[29] L. Prompers, M. Huijberts, J. Apelqvist et al., "Optimal organization of health care in diabetic foot disease: introduction to the Eurodiale study," *The International Journal of Lower Extremity Wounds*, vol. 6, no. 1, pp. 11–17, 2007.

[30] F. Crawford, C. McCowan, B. D. Dimitrov et al., "The risk of foot ulceration in people with diabetes screened in community settings: findings from a cohort study," *QJM: An International Journal of Medicine*, vol. 104, no. 5, pp. 403–410, 2011.

[31] E. J. Boyko, J. H. Ahroni, V. Cohen, K. M. Nelson, and P. J. Heagerty, "Prediction of diabetic foot ulcer occurrence using commonly available clinical information: the Seattle Diabetic Foot Study," *Diabetes Care*, vol. 29, no. 6, pp. 1202–1207, 2006.

[32] S. B. Hadi and M. A. Reza, "Assessment of diabetic foot ulcer's predisposing factors and its outcomes in patients with diabetic foot syndrome hospitalized in Hazrat Rasoul-e-A kram Hospital in Tehran during 1996–2001," *Razi Journal of Medical Sciences*, vol. 11, no. 39, pp. 77–83, 2004.

[33] K. Al-Rubeaan, M. Al Derwish, S. Ouizi et al., "Diabetic foot complications and their risk factors from a large retrospective cohort study," *PLoS One*, vol. 10, no. 5, article e0124446, 2015.

[34] M. C. Parisi, A. Moura Neto, F. H. Menezes et al., "Baseline characteristics and risk factors for ulcer, amputation and severe neuropathy in diabetic foot at risk: the BRAZUPA study," *Diabetology & Metabolic Syndrome*, vol. 8, no. 1, p. 25, 2016.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 2401094, 7 pages
https://doi.org/10.1155/2018/2401094

Clinical Study

Direct Photocoagulation Guided by Merged Retinal Images for the Treatment of Focal Diabetic Macular Edema

Yoshihiro Takamura, Takehiro Matsumura, Shogo Arimura, Makoto Gozawa, Masakazu Morioka, YutakaYamada, and Masaru Inatani

Department of Ophthalmology, Faculty of Medical Sciences, University of Fukui, Eiheiji-cho, Yoshida-gun, Fukui-ken 910-1193, Japan

Correspondence should be addressed to Yoshihiro Takamura; takamurayoshihiro@gmail.com

Received 10 December 2017; Accepted 31 January 2018; Published 12 March 2018

Academic Editor: Ilias Migdalis

Purpose. To introduce a novel laser photocoagulation (PC) protocol named merged image-guided PC (MIG-PC), which included merging the images of the fundus, optical coherence tomography (OCT) map, and fluorescein angiography (FA). We compared the anatomical and functional results between MIG-PC and FA-guided PC (FG-PC) for the treatment of focal diabetic macular edema (DME). *Method*. We examined the treatment outcomes in 27 consecutive eyes treated with MIG-PC compared with 28 matched eyes treated with FG-PC. We identified the microaneurysms (MAs) located in the focal edema areas and ablated them using focal PC. Best-corrected visual acuity (BCVA) and retinal thickness (RT) measured using OCT were compared between the groups at baseline and 2, 4, 8, 12, and 24 weeks after treatment. *Results*. The foveal and perifoveal RT were reduced after treatment in both the groups, and the perifoveal RT in the MIG-PC group was significantly lower than that in the FG-PC group at 4 weeks and thereafter. BCVA in the MIG-PC group was significantly higher than that in the FG-PC group at 12 and 24 weeks. The numbers of laser spots ($p = 0.0001$), additional laser treatments ($p = 0.0121$), and intravitreal injection of ranibizumab ($p = 0.0012$) in the MIG-PC group were significantly lower than those in the FG-PC group (Mann–Whitney test). *Conclusion*. MIG-PC contributed to the improvement in BCVA and reduction in RT, number of laser shots required, and retreatment rates. Based on our data, MIG-PC can be recommended for the treatment of focal DME. This trial is registered with ID UMIN000030390.

1. Introduction

Diabetic macular edema (DME) is the most common cause of visual loss in patients with diabetes [1]. DME is generally differentiated into diffuse and focal DME, and microaneurysms (MAs) are associated with the pathogenesis of both these types. Typically, MAs are found in the area of thickened retina with a circinate ring of exudation [2]. The leakage of blood constituents from MAs into the retinal tissue results in the swelling of the retina. Focal laser photocoagulation (PC) for MAs is considered as the standard treatment [3, 4]. The potency of focal PC may be partially attributed to the closure of MAs. The Early Treatment Diabetic Retinopathy Study (ETDRS) showed that focal/grid PC reduced the risks of losing ≥3 lines of vision by approximately 50% for 3 years after treatment [5]. Based on the evaluation of prospective clinical trials, the mainstays in the treatment for DME have shifted to new modalities, such as the use of anti-vascular endothelial growth factor (VEGF) [6, 7]. However, several DME cases show a low response to anti-VEGF treatment, and repeated injections are required to maintain its therapeutic effects [8]. Direct PC aiming at MAs remains an important option in the treatment of extrafoveal DME.

In focal PC, the goal is to close MAs and stop the leakage, while avoiding retinal scarring. Focal PC is delivered with very low energy compared with panretinal photocoagulation (PRP); nevertheless, some enlargement of laser scarring may lead to the atrophy of the retinal pigment epithelium (RPE) and photoreceptor cells. If the laser scar is placed very close to the foveal center, visual acuity may be severely impaired.

Therefore, ophthalmologists should pay attention to performing focal PC accurately with minimum number of shots and avoid overzealous treatment.

In focal PC for treating MAs, accurate information regarding the location of MA is important. On fundus examination, MAs and small hemorrhages can be recognized as tiny round red dots. In fluorescein angiography (FA), MAs and hemorrhages appear as hyper- and hypofluorescent spots, respectively, and are thus distinguishable. MAs located in the thickened area may be associated with DME pathogenesis. Recently, mapping images using spectral domain-optical coherence tomography (SD-OCT) has provided information regarding the degree and distribution of macular thickness [9].

In this study, we overlapped the images of the ocular fundus, FA, and OCT map of the eyes with DME. Using this novel protocol, we could visualize the location of MAs on macular lesions and apply direct focal PC to these MAs; we compared the anatomical and visual outcomes with those for the eyes treated with FA-guided PC.

2. Method

This retrospective comparative study adhered to the tenets of the Declaration of Helsinki and was approved by the University of Fukui Institutional Review Board. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) of Japan (UMIN000030390). This study included 27 consecutive eyes with focal DME treated with FA-guided PC (FG-PC group) between November 2014 and December 2015 and 28 eyes with focal DME treated with merged images of fundus photographs and OCT maps (MIG-PC group) between January 2016 and May 2017 at the Fukui University Hospital. We enrolled patients (age, >20 years) diagnosed with type 2 diabetes mellitus with focal DME. In this study, focal DME was defined as the leakage from MAs on FA and focal swelling in retinal topography on OCT. Exclusion criteria included a history of prior vitrectomy in the study eye, history of anti-VEGF injections received within 3 months, history of PRP within 6 months, and the presence of medial opacity, such as severe cataract, corneal opacity, or vitreous hemorrhage. All patients underwent comprehensive ophthalmic examinations, including visual acuity testing, slit-lamp biomicroscopy, measurement of intraocular pressure (IOP) using the Goldmann applanation tonometer, and dilated fundoscopic examination. Color fundus photographs and FAs were captured using a Kowa VX-10i fundus camera (Kowa Ltd., Nagoya, Japan) and Spectralis Heidelberg Retinal Angiograph+OCT (Heidelberg Engineering, Heidelberg, Germany), respectively, at baseline before laser PC. Using Cirrus OCT (Carl Zeiss Meditec, Dublin, CA), we measured the retinal thickness (RT) using the fast macular thickness protocol at baseline and 2, 4, 8, 12, and 24 weeks. The analysis of RT reconstructed a false-color topographic image displayed with numerical averages of the thickness values for all nine map sectors defined by ETDRS (Figure 1(a)). The scanned areas were segmented into the center, inner, and outer rings, with diameters of 1, 3, and 6 mm,

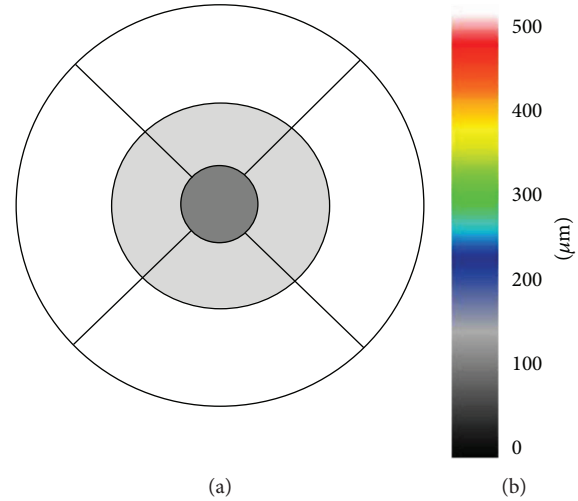
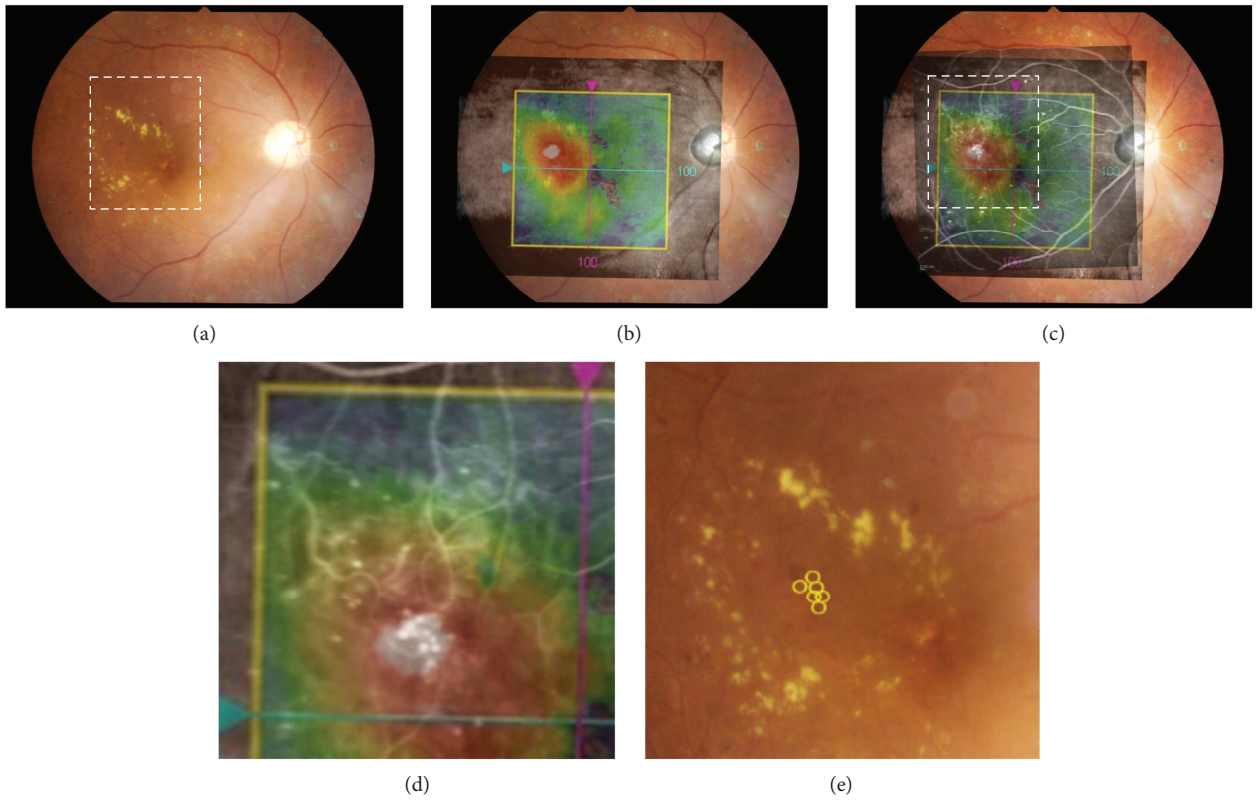


Figure 1: (a) Location of the three fields on the macular thickness map. Center: dark-gray field; inner ring: light-gray field; outer ring: white field. (b) A sample of the false-color topographic image of optical coherence tomography (OCT).

respectively. We calculated the average RT using four corresponding sections in the inner and outer rings and ratio of the edematous areas in the macular area. The number of pixels in highly swollen areas, indicated by the white area on OCT color maps, representing >500 μm, was calculated using Adobe software (Photoshop CS6 Extended, Adobe Systems Inc., San Jose, CA) and divided by the pixels in the macular area indicated by black lines in Figure 1(b).

2.1. Treatment Protocols. To determine the location of MAs in focal DME, images of the OCT map and FA were superimposed and aligned to fundus photographs according to retinal landmarks, such as retinal vessels and the optic disc, using Adobe Photoshop (CS2, Adobe Systems Inc.). To complete this process, images were enlarged and placed as needed. The layers of OCT and FA images became transparent when opacity was set to 70% and 50%, respectively, so that the underlying images of MAs in the ocular photographs showed through (Figure 2). We marked the MAs located in the focal edema expressed by red or white colors with a circle on the ocular photograph (Figure 2(e)). Finally, fundus photographs marked with MAs were displayed on the computer screen besides the patient and used as a guide while performing laser treatment. To perform direct PC, we referred to FA images alone to identify the location of MAs.

Laser PC was performed following pupillary dilation and instillation of topical anesthesia using H-R Centralis® (Volk Optical Inc., Mentor, OH, USA) by a single retina specialist (YT). We used a PASCAL photocoagulator (Optimedica Corporation, Santa Barbara, CA, USA) with a frequency-doubled neodymium-doped yttrium aluminum garnet solid-state laser with a wavelength of 577 nm. The laser parameters were as follows: (1) spot size of 60 μm, (2) pulse duration of 20 ms, (3) single spot, and (4) burn intensity of 100–150 mW, which was increased until a gray/white lesion was attained. Patients were eligible for additional PC if edematous areas indicated by white color in a false-color



(a) (b) (c) (d) (e)

Figure 2: Steps for marking the location of microaneurysms (MAs) in the merged image-guided photocoagulation (MIG-PC) method. (a) Ocular fundus photograph shows focal diabetic macular edema accompanied by a hard exudate ring. (b) OCT map is overlaid on the fundus photograph. (c) Image of fluorescein angiography (FA) is merged with the fundus photograph and OCT map. (d) High-magnification image corresponding to the rectangular area is indicated with white-dashed line in Figures 2(a) and 2(c). (e) MAs are marked on the ocular fundus as yellow circles.

topographic image were still present. Additionally, patients were eligible for the intravitreal injection of ranibizumab (IVR) (Lucentis, Genentech Inc., San Francisco, CA, USA), anti-VEGF drugs if the central RT (CRT) was >350 μm at 8, 12, and 24 weeks after the initial treatment.

2.2. Statistical Analysis. We performed statistical analyses using JMP (SAS institute Inc., Tokyo, Japan). We used Bartlett's test to examine equal variances across samples, followed by assessing the statistical significances between the groups using the Mann–Whitney test. Furthermore, statistical analyses were performed using the Wilcoxon signed-rank test (pre- and posttreatment data in the same group). Values were expressed as the mean ± standard deviation. Differences were considered statistically significant at $p < 0.05$.

3. Results

We analyzed 28 eyes in the MIG-PC group and 27 in the FG-PC group. There was no significant difference in the baseline characteristics, visual acuity, and CRT between the groups (Table 1). Figure 3 shows a sample case of the MIG-PC group. The merged images indicated that some MAs visualized by FA (Figure 3(a)) were located in the highly thickened areas (Figures 3(b) and 3(c)). These MAs are marked by yellow circles (Figures 3(d) and 3(e)). The ratio of the swollen area to the macular area was 28.1 ± 8.3% and 25.2 ± 6.9% in the MIG-PC and FG-PC groups, respectively (Table 2); the difference between the groups was insignificant. In the central area, a significant decrease in CRT was observed in both the groups; at 4 weeks and thereafter, CRT in the MIG-PC group was significantly lower than that in the FG-PC group at 4 and 8 weeks after laser treatment (Figure 4(a)).

Table 1: Baseline characteristics at the time of registration.

	MIG-PC group[a] ($n = 28$)	FG-PC group[b] ($n = 27$)	p value
Mean age (years)	65.3 ± 6.3	63.6 ± 7.2	0.47[a]
Mean hemoglobin A1c (%)	7.2 ± 0.3	7.5 ± 0.4	0.42[a]
Insulin therapy	15 (53.6%)	13 (48.1%)	0.58[a]
Left eye : right eye	12 : 14	14 : 13	0.64[b]
Gender (male/female)	16/12	15/12	0.57[b]
Mean duration of DM (years)	13.3 ± 2.3	12.6 ± 2.1	0.48[b]
Mean serum creatinine	2.11 ± 0.32	2.04 ± 0.22	0.38[a]

[a]Mann–Whitney test; [b]chi-square test; DM: diabetes mellitus; MIG-PC: merged image-guided photocoagulation; FG-PC: fluorescein angiography-guided photocoagulation.

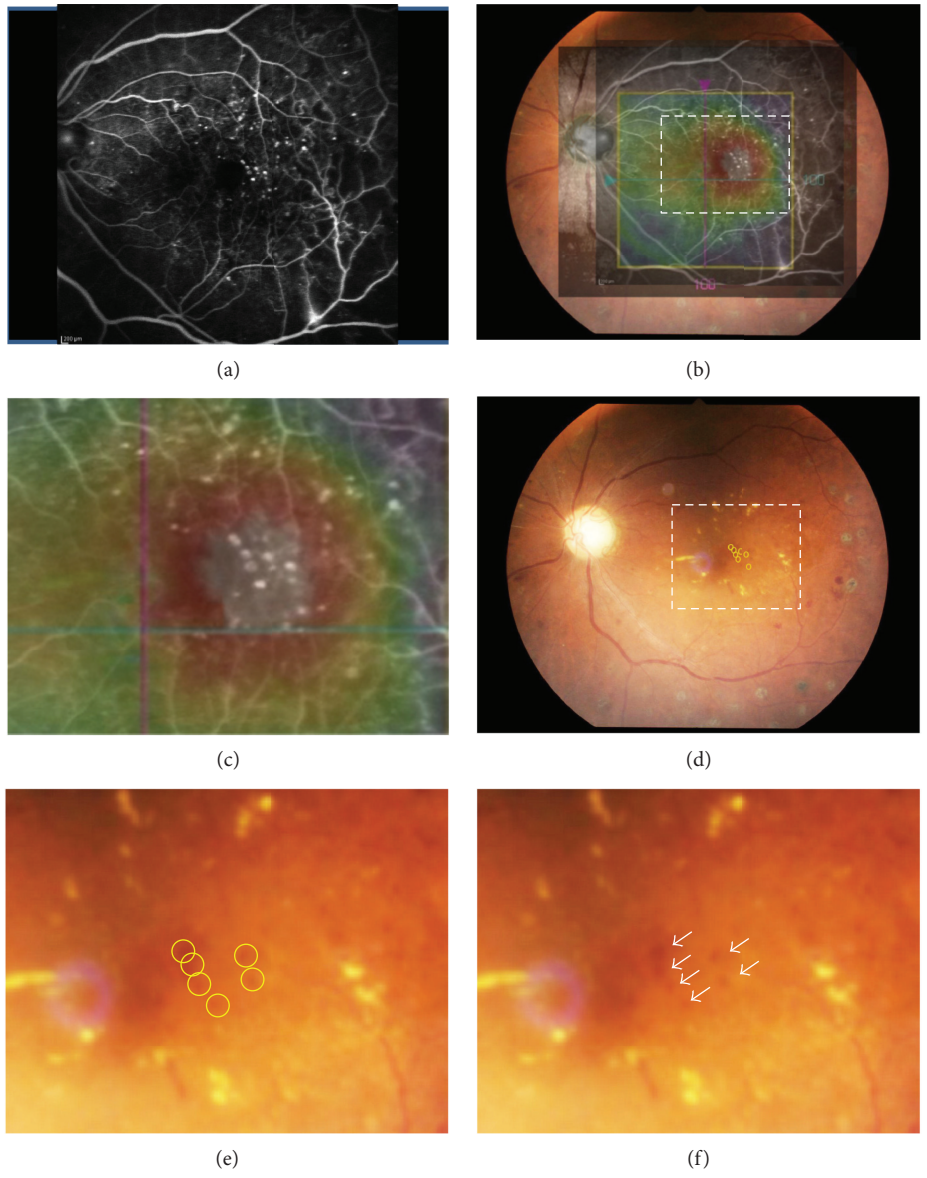


Figure 3: A sample of the MIG-PC method. (a) FA image shows the MAs as dots of hyperfluorescein. (b) The OCT map image is merged with the FA image and fundus photograph. (c) High-magnification image corresponding to the rectangle area is indicated with white-dashed line in Figures 3(b) and 3(d). MAs were noticed in the thickened retinal area. (d) MAs are marked as yellow circles on the ocular fundus photograph. (e) High-magnification image corresponding to the rectangle area is indicated with white-dashed line in Figure 3d.

In the inner ring, a significant decrease in RT was found at 2 weeks in the MIG-PC group, but only at 4 weeks in the FG-PC group (Figure 4(b)). RT in the MIG-PC group was significantly lower than that in the FG-PC group at 2, 4, 8, 12, and 24 weeks after surgery. In the outer ring, a significant decrease in RT was observed in both the groups at 2 weeks after the treatment; no significant difference was found between the groups (Figure 4(c)). Visual acuity was significantly improved in both the groups at 12 and 24 weeks from baseline. BCVA in the MIG-PC group was significantly higher than that in the FG-PC group at 12 ($p = 0.026$) and 24 ($p = 0.032$) weeks (Figure 5).

The data of other parameters in the treatment are shown in Table 2. The number of additional laser treatments was 0.87 ± 0.62 in the FG-PC group and 0.42 ± 0.58 in the MIG-

Table 2: Data relating treatment.

	MIG-PC group[a] ($n = 28$)	FG-PC group[b] ($n = 27$)	p value
Edematous areas (%)	28.1 ± 8.3	25.2 ± 6.9	0.24[a]
Injection of ranibizumab (n)	12.6 ± 2.1	26.5 ± 9.2	0.012[a]
Laser shots (n)	0.72 ± 0.58	1.87 ± 0.62	0.00012[a]
Additional laser (n)	1.25 ± 0.56	2.41 ± 0.49	0.00001[a]

[a]Mann–Whitney test; [b]chi-square test; MIG-PC: merged image-guided photocoagulation; FG-PC: fluorescein angiography-guided photocoagulation.

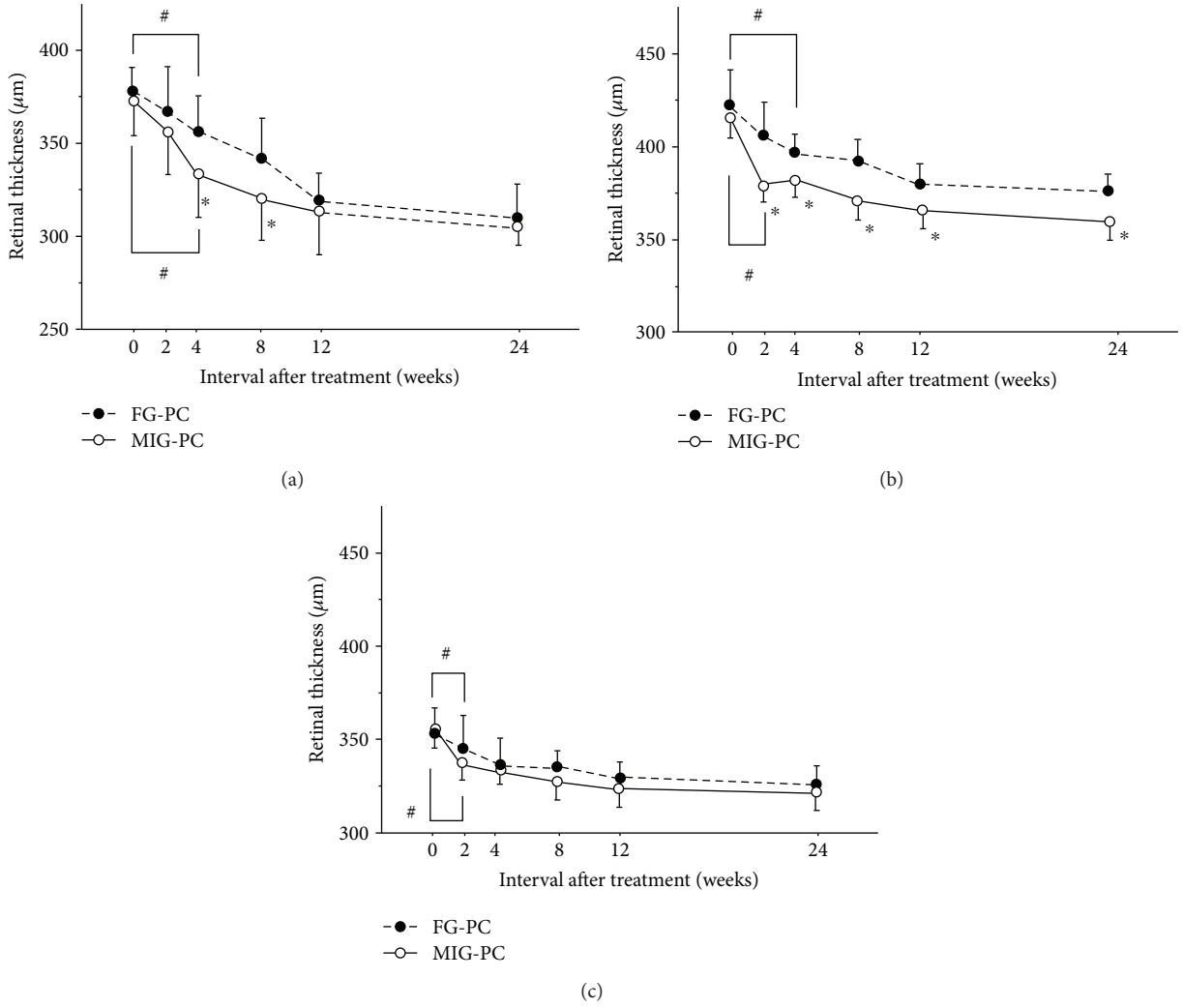


Figure 4: Changes in retinal thickness (RT) in the center (a), inner ring (b), and outer ring (c) after fluorescein angiography-guided focal photocoagulation (FG-PC) or merged image-guided PC (MIG-PC). Data represent the mean ± standard error (SE). $^{\#}p < 0.05$ (compared with RT at PC initiation) and $^{*}p < 0.05$ (MIG-PC group versus FG-PC group).

PC group; the difference was significant ($p = 0.0121$). Also, the number of IVR was greater in the FG-PC (0.91 ± 0.59) than in the MIG-PC group (0.35 ± 0.56; $p = 0.0012$). The difference between the average number of laser spots in the initial laser treatment (26.5 ± 9.2 in the FG-PC group and 16.4 ± 6.7 in the MIG-PC group) was significant ($p = 0.0001$).

4. Discussion

Targeting MAs using focal PC is a strong tool for improving macular swelling [3]. However, accurate focal laser treatment is technically difficult, and the failure of the procedure results in the presence of DME. In this study, we introduced a novel focal PC protocol guided with the merged images of FA, OCT map, and ocular fundus photography, named MIG-PC. Based on our data, focal MIG-PC resulted in the rapid reduction of perifoveal RT, indicating the superiority of MIG-PC compared with FG-PC. Based on FA findings, MAs could be defined as dots with hyperfluorescence, and their location could be determined in reference to the path of the main

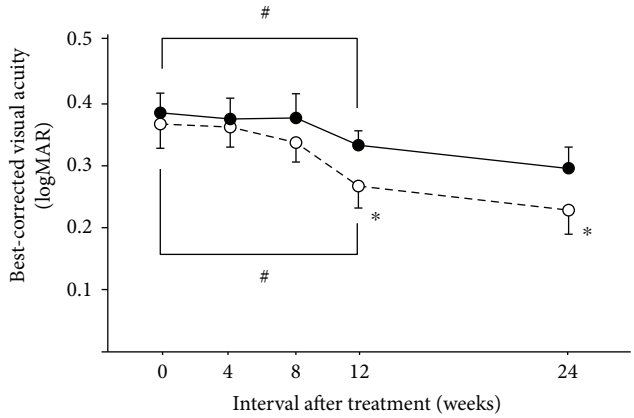


Figure 5: Changes in best-corrected visual acuity (BCVA) (logarithm of the minimum angle of resolution (logMAR)) after focal laser treatment in the MIG-PC and FG-PC groups. Data represent the mean ± standard error (SE). $^{\#}p < 0.05$ (compared with RT at PC initiation) and $^{*}p < 0.05$ (MIG-PC group versus FG-PC group).

vessels. However, the positioning of existing MAs between large numbers of branching vessels is challenging. Using MIG-PC, we can utilize not only the path of blood vessels but also the hard exudate and hemorrhages and dot hemorrhages in determining the location of the MAs. Accurate ablation of MA with the aid of the merged images may thus contribute to better treatment outcomes.

The leakage from MAs which are located within the highly thickened retinal areas is probably responsible for the formation of focal edema [10]. In MIG-PC, we targeted the MAs located in the thickened areas. On the other hand, as FA does not provide us information regarding the geographical relationship between MAs and focal macular areas, MAs not only within but also surrounding the focal edema may have been photocoagulated in the FG-PC group. Although the width of the edematous areas was similar, the number of PC shots in the MIG-PC group was significantly lower than that in the FG-PC group. A suitable direct PC can achieve the closure of MAs, leaving cells in retinal layers intact. However, excessive thermal burns sometimes result in the destruction of the photoreceptor cells and RPE, leading to permanent vision loss [11]. Based on our data, MIG-PC is beneficial in decreasing the number of laser spots and thus preventing the onset of laser-related complications.

In the MIG-PC group, we photocoagulated MAs located in the swollen areas. MAs surrounding the edematous areas were not ablated even if they showed focal leakage in FA. Nevertheless, focal DME was improved more rapidly in the MIG-PC group than in the FG-PC group. Thus, MAs surrounding the focal edema may not be responsible for retinal swelling. Previous reports have shown that some MAs with dye leakage by FA did not completely overlap with retinal thickening on OCT [12–15]. The combination of FA and OCT map is informative in determining the MAs that are responsible for retinal swelling and treating the focal DME.

In this study, the numbers of IVR (anti-VEGF drug) and direct PC shots in the MIG-PC group were significantly lower than those in the FG-PC group. These data indicated that MIG-PC may be superior than FG-PC in terms of both the retreatment rate and overall injection burden. Minimizing the frequency of IVR would contribute to reducing the risks of cerebral infarction, endophthalmitis, and retinal detachment. In the retreatment criteria of our study, additional IVR was allowed at 8 weeks and thereafter. The difference in CRT between the groups was insignificant at the same time points, possibly due to the rapid therapeutic effects of retreatment. MIG-PC not only enhanced the decrease in CRT but also improved the final outcome of BCVA. As a significant improvement in BCVA followed the reduction in CRT, it is likely that the improved visual outcome resulted from the earlier recovery from DME.

In conclusion, our study reported MIG-PC as a new approach to performing focal laser treatment for focal DME. MIG-PC has some benefits over the conventional methods, specifically the increased accuracy in photocoagulating MAs and a reduced need for retreatment. MIG-PC may accelerate and enhance the improvement of visual acuity and DME achieved using conventional focal laser treatment based on FA images alone.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] S. E. Moss, R. Klein, and B. E. Klein, "The 14-year incidence of visual loss in a diabetic population," *Ophthalmology*, vol. 105, no. 6, pp. 998–1003, 1998.

[2] T. Murakami, K. Nishijima, A. Sakamoto, M. Ota, T. Horii, and N. Yoshimura, "Foveal cystoid spaces are associated with enlarged foveal avascular zone and microaneurysms in diabetic macular edema," *Ophthalmology*, vol. 118, no. 2, pp. 359–367, 2011.

[3] S. N. Lee, J. Chhablani, C. K. Chan et al., "Characterization of microaneurysm closure after focal laser photocoagulation in diabetic macular edema," *American Journal of Ophthalmology*, vol. 155, no. 5, pp. 905–912.e2, 2013.

[4] "Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group," *Archives of Ophthalmology*, vol. 103, no. 12, pp. 1796–1806, 1985.

[5] D. S. Fong, S. F. Strauber, L. P. Aiello et al., "Comparison of the modified Early Treatment Diabetic Retinopathy Study and mild macular grid laser photocoagulation strategies for diabetic macular edema," *Archives of Ophthalmology*, vol. 125, no. 4, pp. 469–480, 2007.

[6] J. A. Wells, A. R. Glassman, A. R. Ayala et al., "Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema," *The New England Journal of Medicine*, vol. 372, no. 13, pp. 1193–1203, 2015.

[7] Q. D. Nguyen, S. M. Shah, A. A. Khwaja et al., "Two-year outcomes of the ranibizumab for edema of the mAcula in diabetes (READ-2) study," *Ophthalmology*, vol. 117, no. 11, pp. 2146–2151, 2010.

[8] M. Ashraf, A. Souka, and R. Adelman, "Predicting outcomes to anti-vascular endothelial growth factor (VEGF) therapy in diabetic macular oedema: a review of the literature," *The British Journal of Ophthalmology*, vol. 100, no. 12, pp. 1596–1604, 2016.

[9] J. Y. Shin, S. H. Byeon, and O. W. Kwon, "Optical coherence tomography-guided selective focal laser photocoagulation: a novel laser protocol for diabetic macular edema," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 253, no. 4, pp. 527–535, 2015.

[10] H. Wang, J. Chhablani, W. R. Freeman et al., "Characterization of diabetic microaneurysms by simultaneous fluorescein angiography and spectral-domain optical coherence tomography," *American Journal of Ophthalmology*, vol. 153, no. 5, pp. 861–867.e1, 2012.

[11] E. K. Deschler, J. K. Sun, and P. S. Silva, "Side-effects and complications of laser treatment in diabetic retinal disease," *Seminars in Ophthalmology*, vol. 29, no. 5-6, pp. 290–300, 2014.

[12] N. P. Blair, M. Shahidi, W. W. Lai, and R. Zelkha, "Correlation between microaneurysms and retinal thickness in diabetic macular edema," *Retina*, vol. 28, no. 8, pp. 1097–1103, 2008.

[13] M. Shahidi, Y. Ogura, N. P. Blair, M. M. Rusin, and R. Zeimer, "Retinal thickness analysis for quantitative assessment of diabetic macular edema," *Archives of Ophthalmology*, vol. 109, no. 8, pp. 1115–1119, 1991.

[14] C. L. Lobo, R. C. Bernardes, and J. G. Cunha-Vaz, "Alterations of the blood-retinal barrier and retinal thickness in preclinical retinopathy in subjects with type 2 diabetes," *Archives of Ophthalmology*, vol. 118, no. 10, pp. 1364–1369, 2000.

[15] Early Treatment Diabetic Retinopathy Study Research Group, "Focal photocoagulation treatment of diabetic macular edema. Relationship of treatment effect to fluorescein angiographic and other retinal characteristics at baseline: ETDRS report no. 19," *Archives of Ophthalmology*, vol. 113, no. 9, pp. 1144–1155, 1995.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 5607208, 7 pages
https://doi.org/10.1155/2018/5607208

Research Article

A Comparative Assessment of Cardiovascular Autonomic Reflex Testing and Cardiac ^{123}I-Metaiodobenzylguanidine Imaging in Patients with Type 1 Diabetes Mellitus without Complications or Cardiovascular Risk Factors

Triantafyllos Didangelos,[1] **Efstratios Moralidis**,[2] **Eleni Karlafti**,[1] **Konstantinos Tziomalos**,[1] **Charalambos Margaritidis**,[1] **Zisis Kontoninas**,[1] **Ioannis Stergiou**,[1] **Maria Boulbou**,[3] **Marianthi Papagianni**,[1] **Emmanouel Papanastasiou**,[4] **and Apostolos I. Hatzitolios**[1]

[1]*Diabetes Center, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki, Greece*
[2]*Laboratory of Nuclear Medicine, Medical School, Aristotle University of Thessaloniki, Papageorgiou Hospital, Thessaloniki, Greece*
[3]*Department of Internal Medicine, Medical School, University of Thessaly, Larissa, Greece*
[4]*Department of Medical Physics, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Correspondence should be addressed to Triantafyllos Didangelos; didang@med.auth.gr

Received 21 November 2017; Accepted 31 January 2018; Published 12 March 2018

Academic Editor: Ilias Migdalis

Aim. To compare the cardiovascular autonomic reflex tests (CARTs) with cardiac sympathetic innervation imaging with ^{123}I-metaiodobenzylguanidine (MIBG) in patients with type 1 diabetes mellitus (T1DM). *Patients and Methods*. Forty-nine patients (29 males, mean age 36 ± 10 years, mean T1DM duration 19 ± 6 years) without cardiovascular risk factors were prospectively enrolled. Participants were evaluated for autonomic dysfunction by assessing the mean circular resultant (MCR), Valsalva maneuver (Vals), postural index (PI), and orthostatic hypotension (OH). Within one month from the performance of these tests, patients underwent cardiac MIBG imaging and the ratio of the heart to upper mediastinum count density (H/M) at 4 hours postinjection was calculated (abnormal values, H/M < 1.80). *Results*. Twenty-nine patients (59%) had abnormal CARTs, and 37 (76%) patients had an H/M_4 < 1.80 ($p = 0.456$). MCR, PI, Vals, and OH were abnormal in 29 (59%), 8 (16%), 5 (10%), and 11 (22%) patients, respectively. When using H/M_4 < 1.80 as the reference standard, a cutoff point of ≥2 abnormal CARTs had a sensitivity of 100% but a specificity of only 33% for determining CAN. *Conclusions*. CARTs are not closely associated with ^{123}I-MIBG measurements, which can detect autonomic dysfunction more efficiently than the former. In comparison to semiquantitative cardiac MIBG assessment, the recommended threshold of ≥2 abnormal CARTs to define cardiovascular autonomic dysfunction is highly sensitive but of limited specificity and is independently determined by the duration of T1DM.

1. Introduction

Diabetic neuropathy is the most common cause of neuropathy worldwide and the most common chronic complication of diabetes mellitus (DM) [1, 2]. Autonomic neuropathy is an underestimated complication of DM and may remain asymptomatic in early stages, especially in young patients with type 1 DM (T1DM) [3]. In the setting of DM, cardiovascular autonomic neuropathy (CAN) is defined as the impairment of the autonomic control of the cardiovascular system after the exclusion of other causes [2, 4]. CAN is one of the most serious consequences of DM due to its life-threatening complications, including silent myocardial infarction, ventricular arrhythmias, intraoperative cardiovascular instability, and

sudden cardiac death, as well as because of its relation with other microvascular comorbidities [5, 6].

Over the years, much attention has been directed to the early diagnosis of CAN using standardized cardiovascular reflex tests (CARTs) [7]. These tests assess the parasympathetic system by evaluating beat-to-beat variations during deep breathing, moving from the supine to the standing position and during the Valsalva maneuver (Vals) [8]. On the other hand, orthostatic hypotension (OH) and the blood pressure response to Vals mostly evaluate the sympathetic system [8].

Nuclear imaging is also useful for the assessment of cardiac sympathetic innervation, by visualizing and measuring the uptake and storage of radiolabeled neurotransmitters into the presynaptic nerve terminals [9]. Several studies using ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) imaging have demonstrated the presence of abnormalities in sympathetic adrenergic innervation in diabetic patients [10–13].

It is noteworthy that most data on CAN are based on older studies, at a time when the treatment of diabetic patients might not have been optimal or the tests for identifying CAN might have been limited in number, performed in a nonstandardized manner, or influenced by comorbidities or medications [13, 14]. Moreover, the relation between CARTs and the more objective ^{123}I-MIBG scintigraphy, in terms of direct visualization and quantification of cardiac sympathetic innervation, is not well determined [10, 15, 16].

The aim of the present study was to assess the CAN by both CARTs and ^{123}I-MIBG scintigraphy in well-characterized T1DM patients, with disease duration > 5 years, without complications or cardiovascular risk factors, and who were only receiving insulin.

2. Patients and Methods

2.1. Patient Recruitment. Over an 18-month period, consecutive adult patients with T1DM who fulfilled the following criteria were enrolled in the study: (a) regular attendance of the outpatient diabetes clinic of our hospital to ensure systematic monitoring of glycemic control and diabetic complications, (b) diabetes duration > 5 years [17], (c) no symptoms associated with CAN (e.g., exercise intolerance, orthostatic intolerance, and syncope), (d) no clinically overt diabetic complications, other than CAN, or known cardiovascular risk factors and hence treated only with insulin, and (e) a normal adenosine myocardial perfusion scintigraphy. Ten patients had mild background retinopathy, and 6 patients had microalbuminuria. Thyroid stimulating hormone and thyroid hormones were measured in all patients because Hashimoto's thyroiditis is common in patients with T1DM. The levels of both thyroid stimulating hormone and thyroid hormones were within the normal range in all patients.

All patients had good glycemic control using insulin alone according to widely accepted standards of care [18]. Patients were well characterized and recruited for a broader study on diabetic autonomic dysfunction, which had been approved by the Ethics Committee of the Aristotle University of Thessaloniki. All patients provided informed consent.

2.2. Cardiovascular Autonomic Reflex Testing. Patients were submitted to a battery of CARTs early in the morning, as endorsed by international guidelines [7, 8, 19]. Subjects were instructed to abstain from caffeine or alcoholic beverages and smoking for a minimum of 8 h prior to testing and also from strenuous exercise for at least 24 h prior to testing. Patients with arrhythmias, fever, hypoglycemia, and emotional distress on the day before testing were excluded from testing. The following CARTs were performed:

(1) The heart rate variation during deep breathing at 6 cycles per minute for 5 minutes, over 120 consecutive beats, was assessed with the mean circular resultant (MCR), which was measured by a vector analysis technique, as described elsewhere [18]. Patients, while lying down, were ordered to perform a deep and slow inspiration up to the maximum total lung capacity for 5 seconds, which was followed by a forced expiration down to the residual volume for 5 seconds. The time to alternate the respiratory cycle was signalled directly to the patient by the attending physician.

(2) The postural index (PI) was calculated by measuring the heart rate response to the change of position from recumbent to standing over 180 seconds after getting up. This was calculated as the "30–15 ratio," defined as the longest RR interval of beats 20–40 divided by the shortest RR interval of beats 5–25, starting from the first beat during the process of standing up.

(3) For the assessment of heart rate variability during Vals, patients with the nose closed were asked to exhale into a mouthpiece connected to a manometer and to perform a continuous expiratory effort, which was equivalent to an intraoral pressure of 40 mmHg, for 15 seconds. The expiratory overstrain was then released, and patients were asked to breathe regularly until the end of the test. The ratio of the longest RR interval following the pressure release to the shortest RR interval during the maneuver was determined. The test was performed 3 times, and the mean value was used in the analyses.

(4) For the OH test, blood pressure was measured with the patients in recumbent position, every minute for three minutes, and then while standing, every minute for 5 minutes. A drop of ≥20 mmHg in systolic blood pressure or ≥10 mmHg in diastolic blood pressure was considered abnormal.

Age-specific reference values were applied [20]. The first two CARTs address parasympathetic function, and Vals evaluates both parasympathetic and sympathetic function, whereas OH assesses sympathetic integrity.

2.3. ^{123}I-MIBG Acquisition and Analysis. Within 1 month from performing the CARTs, patients were submitted to cardiac MIBG imaging. For this purpose, patients were treated with 130 mg potassium iodide orally for 3 days, starting on the day before tracer injection. There was no need to

withhold medications interfering with MIBG imaging, since all patients were receiving only insulin [9]. 185 MBq of ^{123}I-MIBG was administered slowly via a secured intravenous line in a peripheral vein.

Imaging was performed with a single-headed, large field of view ADAC Genesys SPECT gamma camera (ADAC Labs., Milpitas, CA, USA), equipped with a 3/8″ sodium iodide scintillation crystal and low-energy high-resolution collimator and interfaced with a Pegasys processing workstation. Anterior cardiac images were acquired at 15 minutes and 4 hours postinjection on a 256×256 matrix and an acquisition time of 600 sec, using a 15% energy window centered at the 159 keV photopeak of ^{123}I.

The heart-to-mediastinum (H/M) count density ratio was calculated in a standardized manner, using an elliptical region of interest (ROI) placed over the heart and a rectangular ROI drawn over the upper mediastinum. Both the acquisition and measurement methodology are in line with official recommendations and have been previously validated in our institution [21–23]. For the purposes of this study, the H/M at 4 hours postinjection (H/M_4) was used in analysis, and the cutoff point of abnormal values was set at H/M_4 < 1.80, based on the published data [22, 24].

2.4. ^{51}Cr-EDTA Glomerular Filtration Measurements. Within one month from the performance of CARTs, glomerular filtration rate (GFR) was measured in the morning, using the slope intercept and two sample technique, as described elsewhere [25]. In brief, blood samples were collected at 120 and 240 min after an intravenous injection of 3-4 MBq ^{51}Cr-EDTA. A scintillation well counter (Cobra II, Packard, Meriden, CT, USA) was set to count plasma ^{51}Cr, and the quadratic Brochner-Mortensen correction was applied in calculations [26]. GFR was measured with this technique because of the inaccuracy of various equations for the evaluation of GFR [27] and because CAN appears to predict the development of diabetic nephropathy [28].

2.5. Statistical Analysis. All data were analyzed with the statistical package SPSS (version 17.0; SPSS, Chicago, IL, USA). Data are presented as percentages for categorical variables and as mean and standard deviation for continuous variables. Differences between groups were assessed with paired or independent sample *t*-test, as appropriate, for continuous variables, and with chi-square test or Fisher's exact test, as appropriate, for categorical variables. One-way ANOVA was used when categorical or ordinal variables were compared between three or more groups, and Levene's test was used for evaluating the equality of variances. Stepwise backward binary logistic regression analysis was used to determine independent predictors, and a p value < 0.20 in univariate analysis was required for a variable to enter the multivariate model. A value of $p < 0.05$ was used to define statistical significance.

3. Results

Forty-nine patients with T1DM were enrolled in the study (29 males, mean age 36 ± 10 years (range, 19–62 years), mean DM duration 19 ± 6 years (range, 7–31 years)).

Table 1: Patients' characteristics.

Males (%)	59.2
Mean age (years)	36 ± 10
Mean duration of diabetes mellitus (years)	19 ± 6
Mean systolic blood pressure (mmHg)	115 ± 10
Mean diastolic blood pressure (mmHg)	78 ± 8
Mean body mass index (kg/m^2)	25.2 ± 3.1
Retinopathy (%)	20.4
Microalbuminuria (%)	12.2
Mean HbA_{1c} (%)	6.9 ± 1.5
Mean hematocrit (%)	42.1 ± 1.1
Mean aspartate transaminase (IU/L)	27.3 ± 1.9
Mean alanine transaminase (IU/L)	28.8 ± 1.3
Mean triglycerides (mg/dL)	117.8 ± 20.2
Mean total cholesterol (mg/dL)	159.4 ± 14.5
Mean high-density lipoprotein cholesterol (mg/dL)	48.1 ± 13.3

Patients' characteristics are shown in Table 1. HbA_{1c} was also measured within 1 month from CARTs. Blood samples from 37 patients were analyzed in our institution, whereas the rest were tested elsewhere because of temporary logistic constraints. As deviations in the latter HbA_{1c} measurements were strongly suspected subsequently, only results from our laboratory were taken into account in this study, which are presented in a supplementary analysis. Similarly, ^{51}Cr-EDTA GFR measurements within 1 month from CARTs were available for 46 patients, and thus renal function is analyzed separately, together with the 37 patients in whom HbA_{1c} was measured in our institution.

Twenty-nine patients (59%) had abnormal CARTs, and 37 patients (76%) had an H/M_4 < 1.80 ($p = 0.456$). MCR, PI, Vals, and OH were abnormal in 29 (59%), 8 (16%), 5 (10%), and 11 (22%) patients, respectively. The MIBG assessment of CAN as a function of the number of abnormal CARTs is presented in Table 2. Among the 36 patients with 0 or 1 abnormal CARTs, 24 patients (67%) also had sympathetic impairment in cardiac MIBG scintigraphy.

All 16 patients with only 1 abnormal CART had abnormal MCR. Abnormality in any one of the remaining CARTs was invariably accompanied by at least a second abnormal test. There were also 11 patients with abnormal OH test (H/M_4 = 1.50 ± 0.14), which were accompanied by 1 additional abnormal CART in 4 patients (H/M_4 = 1.45 ± 0.20) and 2 or more additional abnormal CARTs in 7 patients (H/M_4 = 1.53 ± 0.10). There was no significant difference between this latter group of 7 patients and the rest of the patients (1.66 ± 0.21, $p = 0.115$). When a more stringent cutoff point was used to assess advanced CAN, 5/7 (71%) patients with OH had an H/M_4 < 1.60 versus 19/42 patients (45%, $p = 0.247$).

In Table 3, the H/M_4 ratio is compared between patients divided according to the number of abnormal CARTs. Based on cardiac ^{123}I-MIBG, a threshold of ≥2 abnormal CARTs could determine significant differences in the assessment of CAN with cardiac MIBG imaging. When using H/M_4 < 1.80 as a reference standard, a cutoff point of

Table 2: Cardiac sympathetic innervation imaging with ^{123}I-metaiodobenzylguanidine results as a function of the number of abnormal cardiovascular autonomic reflex tests (CARTs).

	Number of abnormal CARTs					p
	0	1	2	3	4	
Patients with H/M_4 < 1.80	14/20 (70%)	10/16 (63%)	5/5 (100%)	6/6 (100%)	2/2 (100%)	0.198
H/M_4	1.68 ± 0.17	1.68 ± 0.26	1.51 ± 0.21	1.50 ± 0.08	1.64 ± 0.20	0.170

H/M_4: ratio of the heart to upper mediastinum count density at 4 hours postinjection.

Table 3: Comparison of cardiac sympathetic innervation imaging with ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) findings between groups of patients formed according to the number of abnormal cardiovascular autonomic tests (CARTs) versus those with fewer tests than the defined threshold of abnormality.

Number of abnormal CARTs	^{123}I-MIBG H/M_4		p
	Abnormal group	Normal group	
≥1	1.61 ± 0.22 ($n = 29$)	1.68 ± 0.17 ($n = 20$)	0.234
≥2	1.52 ± 0.14 ($n = 13$)	1.68 ± 0.21 ($n = 36$)	0.014
≥3	1.53 ± 0.09 ($n = 8$)	1.66 ± 0.21 ($n = 41$)	0.104
≥4	1.62 ± 0.11 ($n = 2$)	1.64 ± 0.21 ($n = 47$)	0.916

≥2 abnormal CARTs had a sensitivity of 100% but a specificity of 33% in determining CAN.

There was no difference between males and females in mean age (35 ± 8 versus 37 ± 11 years, resp.; $p = 0.356$), mean DM duration (18 ± 6 versus 19 ± 7 years, resp.; $p = 0.520$), number of abnormal CARTs ($p = 0.335$), H/M_4 (1.67 ± 0.23 versus 1.60 ± 0.17, resp.; $p = 0.296$), and ^{51}Cr-EDTA GFR (102 ± 18 versus 93 ± 15, resp.; $p = 0.086$), but males had lower HbA_{1c} than females (6.5% ± 1.4 versus 7.7% ± 2.0, resp.; $p = 0.038$).

Study participants with ≥2 abnormal CARTs were older (42 ± 11 versus 33 ± 8 years, $p = 0.003$) and had longer DM duration (25 ± 5 versus 16 ± 5 years, $p < 0.001$) than patients with <2 abnormal CARTs (Figure 1). In binary logistic regression analysis, only DM duration was a significant predictor of ≥2 abnormal CARTs in the entire study population (model's chi-square 21.010, $p < 0.001$).

In the 37 patients with available HbA_{1c} measurements, there were significant differences between those with ≥2 abnormal CARTs and those with 0-1 abnormal CARTs in HbA_{1c} (7.7% ± 2.3 versus 6.5% ± 1.2, resp.; $p = 0.044$), ^{51}Cr-EDTA GFR (86 ± 13 versus 101 ± 16 ml/min/1.73m^2, resp.; $p = 0.006$), age (42 ± 11 versus 35 ± 8 years, resp.; $p = 0.021$), DM duration (25 ± 17 versus 17 ± 5 years, resp.; $p < 0.001$), and H/M_4 (1.52 ± 0.15 versus 1.70 ± 0.22, resp.; $p = 0.012$). In binary logistic regression analysis, both age and HbA_{1c} were independent predictors of ≥2 abnormal CARTs (model's chi-square 30.541, $p < 0.001$).

Patients with H/M_4 < 1.80 ($n = 37$) were older than those with a normal MIBG study ($n = 12$) (38 ± 9 versus 30 ± 9 years, resp.; $p = 0.012$, Figure 2), but there were no differences between the two groups in gender (56.7 versus 66.7% males, resp.; $p = 0.544$), DM duration (20 ± 6 versus 16 ± 6 years, $p = 0.057$) and the number of abnormal CARTs (14/20, 10/16, 5/5, 6/6, and 2/2 abnormal H/M_4 ratios in patients with 0, 1, 2, 3, and 4 abnormal CARTs, resp., $p = 0.198$). In binary logistic regression analysis, only age was found to represent an independent predictor of H/M_4 < 1.80 (model's chi-square 6.959, $p = 0.008$).

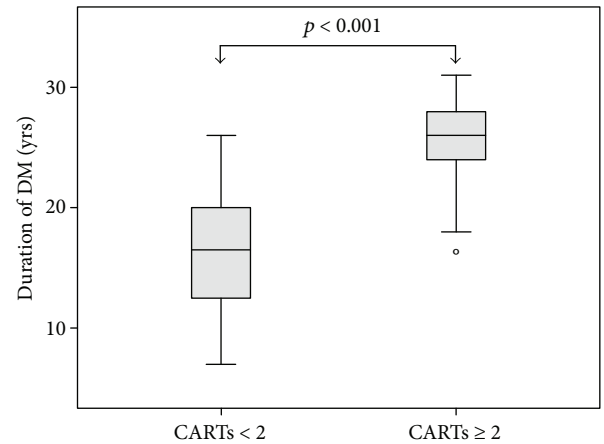


Figure 1: Box plots of the duration of type 1 diabetes mellitus in patients divided according to a cutoff point of ≥2 abnormal cardiovascular autonomic reflex tests (CARTs).

In the 37 patients with available HbA_{1c} measurements, patients with H/M_4 < 1.80 did not differ significantly from those with a normal ratio in age ($p = 0.085$), DM duration ($p = 0.196$), HbA_{1c} ($p = 0.435$), ^{51}Cr-EDTA GFR ($p = 0.253$), and the number of abnormal CARTs ($p = 0.186$). No significant predictors of H/M_4 < 1.80 were found when variables with a $p < 0.20$ were entered in binary logistic regression analysis.

4. Discussion

The present study compared clinical autonomic function testing and MIGB in T1DM patients with no overt complications or other comorbidities. Our findings suggest that clinical tests and MIBG measurements are not closely associated. A threshold of ≥2 abnormal CARTs determines significant changes in sympathetic integrity, as assessed with MIBG, and has high sensitivity but limited specificity to identify CAN. Among clinical variables, DM duration was identified as a significant determinant of ≥2 abnormal CARTs, whereas

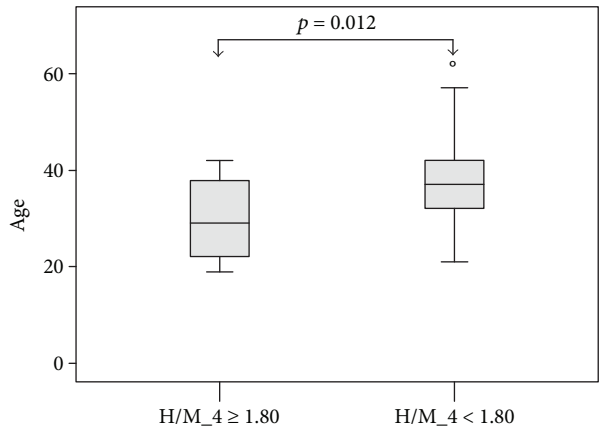


Figure 2: Box plots of age in patients divided according to a cutoff point of ratio of the heart to upper mediastinum count density at 4 hours postinjection (H/M_4) < 1.80 in cardiac ^{123}I-metaiodobenzylguanidine scintigraphy.

age was found to represent an independent predictor of abnormal MIBG findings.

The cardiovascular autonomic system plays a pivotal role in the regulation of heart rate, myocardial contractility, and blood pressure. Our group showed that the severity of CAN is associated with the severity of the left ventricular diastolic dysfunction in both patients with T1DM and type 2 DM [29, 30]. Indeed, CAN is a severe complication of DM with potentially deleterious effects on the outcome of these patients [3, 5, 6, 31]. Thus, early recognition of CAN may be crucial in the management of the disease because achieving normoglycemia may delay or prevent the onset of CAN in T1DM [32, 33]. Even though autonomic symptoms present more commonly in T1DM than in T2DM, these symptoms correlate weakly with deficits in early CAN [34]. The so-called Ewing battery of tests has been introduced many years ago to assess cardiovascular autonomic function and remains popular because these tests are noninvasive, simple, safe, reliable, reproducible, and standardized [7]. However, even though these tests are clinically relevant, they principally assess the parasympathetic system.

Myocardial ^{123}I-MIBG allows direct visualization and quantification of sympathetic presynaptic neuronal integrity [9]. However, it should be mentioned that uptake measurements are semiquantitative and not an index of absolute neuronal retention. Besides, the delivery of tracers is influenced by myocardial perfusion, and measurements may be influenced by body weight, a number of diseases, and certain medications [2].

The prevalence of CAN detected in our cohort with either CARTs or MIBG is within the range reported in the literature [35]. Our results also demonstrate that MCR abnormalities precede other tests' impairment, whilst MIBG abnormalities occur in the majority of diabetic patients with no abnormal CARTs. Moreover, DM duration and HbA_{1c} were significant predictors of the presence of ≥2 abnormal CARTs. Earlier work has shown inadequate glycemic control to be a leading cause for the development and progression of CAN and duration of the disease to be a significant risk factor [15, 36]. Previous data have also suggested that ^{123}I-MIBG scintigraphy may be more sensitive than heart rate variability for detection of CAN in diabetic patients [36, 37]. Furthermore, it has been reported that heart rate variability in deep breathing heralds subclinical CAN and is the most sensitive test to diagnose autonomic dysfunction [2, 35]. Particularly in patients with T1DM, significant correlations were observed between CAN and age, duration of DM, HbA_{1c}, retinopathy, microalbuminuria, hypoglycaemia, dyslipidemia, and diastolic blood pressure [14, 38].

Interestingly, since cardiac MIBG scintigraphy addresses the sympathetic autonomic system and CARTs principally assess parasympathetic function, our findings contradict the general notion that parasympathetic (vagal) impairment precedes sympathetic dysfunction during the natural course of CAN in diabetic patients [39]. As denervation occurs in an ascending length-dependent manner, the vagus nerve is usually affected first in CAN, resulting in a relative predominance of the sympathetic tone [35]. At an early stage, this leads to baroreceptor impairment and changes in heart rate variability, but at later stages of the disease, cardiac involvement may become evident. In this respect, however, it has been suggested that MIBG assesses specifically the sympathetic innervation of the heart whereas heart rate variability indices are better markers of systemic autonomic function [35, 39].

The Toronto Consensus Panel required ≥2 abnormal CARTs to define CAN, one abnormal CART for possible CAN, and considered OH with ≥2 abnormal CARTs as indicative of advanced CAN [8]. In the present study, the criterion of ≥2 abnormal CARTs could only discern significant abnormality in cardiac MIBG findings (Table 2). Literally, all patients with ≥2 abnormal CARTs had scintigraphic evidence of sympathetic dysfunction. However, the presence of ≥2 abnormal CARTs had limited specificity, because the majority of T1DM patients with none or only one abnormal CARTs also had sympathetic impairment. Similarly, one abnormal CART was associated with a moderate (63%) likelihood for MIBG abnormality. Regarding the case of postural hypotension (which assesses sympathetic function) plus ≥ 2 abnormal CARTs, in our patients, an abnormal OH invariably was accompanied by one or more additional abnormal CARTs. In these patients, the H/M_4 tended to be low, but no presence of advanced CAN could be confirmed, even after applying a H/M_4 threshold of <1.60.

Age was found to represent an independent predictor of H/M_4 < 1.80. Earlier studies have reported a decrease of cardiac MIBG uptake as a physiological consequence of advancing age [40]. However, more recent reports have challenged this notion and support stability of H/M measurements in patients without coronary heart disease, because changes of numerator parallel those of denominator during ageing [41]. Whatever may be the underlying cause (a consequence of ageing alone or an effect of DM), our data show that advanced age is a marker of CAN in T1DM patients, as demonstrated by a lower cardiac MIBG uptake.

Limitations of the present study include the relatively small sample size, which is particularly important for multivariate analyses. Although a rule does not exist, statistical

confidence is substantiated with larger numbers of cases. ^{123}I-MIBG measurements were based on an H/M ratio at 4 hours alone, excluding other scintigraphic indices. However, this was preferred for reasons of simplicity in presenting the results, whereas recent data report very similar values between early and late H/M ratios. Notably, although cardiac ^{123}I-MIBG testing offers improved sensitivity in detecting autonomic dysfunction and initiating timely therapeutic interventions, it is not known whether this type of assessment is accompanied with improved outcome compared to clinical autonomic testing alone.

Strengths of the present study include the enrollment of well-characterized and young patients with very good metabolic control, early CAN, long duration of DM, without cardiovascular disease, hypertension, or severe microvascular complications.

5. Conclusions

In T1DM patients with no complications or cardiovascular risk factors and treated with insulin alone, CAN is common and age is a strong predictor of its presence in cardiac ^{123}I-MIBG imaging. Cardiovascular autonomic reflex tests are not closely associated with ^{123}I-MIBG measurements, which can detect autonomic dysfunction more efficiently than the former. In comparison to the semiquantitative cardiac MIBG assessment, the recommended threshold of ≥ 2 abnormal tests is highly sensitive for identifying cardiovascular autonomic dysfunction but has limited specificity and is independently determined by the duration of T1DM. Therefore, MIBG might have to be used in combination with the CARTs for the diagnosis of cardiac autonomic neuropathy in patients with T1DM.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] G. Said, "Diabetic neuropathy," *Handbook of Clinical Neurology*, vol. 115, pp. 579–589, 2013.

[2] R. Pop-Busui, "What do we know and we do not know about cardiovascular autonomic neuropathy in diabetes," *Journal of Cardiovascular Translational Research*, vol. 5, no. 4, pp. 463–478, 2012.

[3] A. Verrotti, G. Prezioso, R. Scattoni, and F. Chiarelli, "Autonomic neuropathy in diabetes mellitus," *Frontiers in Endocrinology*, vol. 5, p. 205, 2014.

[4] S. Tesfaye, A. J. M. Boulton, P. J. Dyck et al., "Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments," *Diabetes Care*, vol. 33, no. 10, pp. 2285–2293, 2010.

[5] A. I. Vinik and D. Ziegler, "Diabetic cardiovascular autonomic neuropathy," *Circulation*, vol. 115, no. 3, pp. 387–397, 2007.

[6] J. Gerritsen, J. M. Dekker, B. J. Ten Voorde et al., "Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn study," *Diabetes Care*, vol. 24, no. 10, pp. 1793–1798, 2001.

[7] D. J. Ewing, C. N. Martyn, R. J. Young, and B. F. Clarke, "The value of cardiovascular autonomic function tests: 10 years experience in diabetes," *Diabetes Care*, vol. 8, no. 5, pp. 491–498, 1985.

[8] V. Spallone, D. Ziegler, R. Freeman et al., "Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management," *Diabetes/Metabolism Research and Reviews*, vol. 27, no. 7, pp. 639–653, 2011.

[9] M. I. Travin, "Cardiac autonomic imaging with SPECT tracers," *Journal of Nuclear Cardiology*, vol. 20, no. 1, pp. 128–143, 2013.

[10] G. Kreiner, M. Wolzt, P. Fasching et al., "Myocardial *m*-[^{123}I] iodobenzylguanidine scintigraphy for the assessment of adrenergic cardiac innervation in patients with IDDM. Comparison with cardiovascular reflex tests and relationship to left ventricular function," *Diabetes*, vol. 44, no. 5, pp. 543–549, 1995.

[11] A. K. Turpeinen, E. Vanninen, J. T. Kuikka, and M. I. Uusitupa, "Demonstration of regional sympathetic denervation of the heart in diabetes. Comparison between patients with NIDDM and IDDM," *Diabetes Care*, vol. 19, no. 10, pp. 1083–1090, 1996.

[12] D. D. Muhr-Becker, M. Weiss, K. Tatsch, G. Wolfram, E. Standl, and O. Schnell, "Scintigraphically assessed cardiac sympathetic dysinnervation in poorly controlled type 1 diabetes mellitus: one-year follow-up with improved metabolic control," *Experimental and Clinical Endocrinology & Diabetes*, vol. 107, no. 5, pp. 306–312, 1999.

[13] S. Nagamachi, S. Fujita, R. Nishii et al., "Prognostic value of cardiac I-123 metaiodobenzylguanidine imaging in patients with non-insulin-dependent diabetes mellitus," *Journal of Nuclear Cardiology*, vol. 13, no. 1, pp. 34–42, 2006.

[14] P. Kempler, S. Tesfaye, N. Chaturvedi et al., "Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM complications study," *Diabetic Medicine*, vol. 19, no. 11, pp. 900–909, 2002.

[15] O. Schnell, D. Muhr, S. Dresel, M. Weiss, M. Haslbeck, and E. Standl, "Partial restoration of scintigraphically assessed cardiac sympathetic denervation in newly diagnosed patients with insulin dependent (type 1) diabetes mellitus at one-year follow-up," *Diabetic Medicine*, vol. 14, no. 1, pp. 57–62, 1997.

[16] A. Giordano, M. L. Calcagni, A. Verrillo et al., "Assessment of sympathetic innervation of the heart in diabetes mellitus using 123I-MIBG," *Diabetes, Nutrition & Metabolism*, vol. 13, pp. 350–355, 2000.

[17] J. B. Buse, H. N. Ginsberg, G. L. Bakris et al., "Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association," *Circulation*, vol. 115, no. 1, pp. 114–126, 2007.

[18] American Diabetes Association, "Standards of medical care in diabetes," *Diabetes Care*, vol. 39, Supplement 1, pp. S1–S112, 2016.

[19] V. Spallone, F. Bellavere, L. Scionti et al., "Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 21, no. 1, pp. 69–78, 2011.

[20] J. Gerritsen, B. J. TenVoorde, J. M. Dekker et al., "Measures of cardiovascular autonomic nervous function: agreement, reproducibility, and reference values in middle age and elderly subjects," *Diabetologia*, vol. 46, no. 3, pp. 330–338, 2003.

[21] A. Flotats, I. Carrió, D. Agostini et al., "Proposal for standardization of ^{123}I-metaiodobenzylguanidine (MIBG) cardiac sympathetic imaging by the EANM Cardiovascular Committee and the European Council of Nuclear Cardiology," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 37, no. 9, pp. 1802–1812, 2010.

[22] H. J. Verberne, J. B. Habraken, B. L. van Eck-Smit, D. Agostini, and A. F. Jacobson, "Variations in ^{123}I-metaiodobenzylguanidine (MIBG) late heart mediastinal ratios in chronic heart failure: a need for standardisation and validation," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 35, no. 3, pp. 547–553, 2008.

[23] E. Papanastasiou, E. Moralidis, and A. Siountas, "The effect of scatter correction on planar and tomographic semiquantitative^{123}I cardiac imaging. A phantom study," *Hellenic Journal of Nuclear Medicine*, vol. 20, no. 2, pp. 154–159, 2017.

[24] K. Nakajima, "Normal values for nuclear cardiology: Japanese databases for myocardial perfusion, fatty acid and sympathetic imaging and left ventricular function," *Annals of Nuclear Medicine*, vol. 24, no. 3, pp. 125–135, 2010.

[25] G. Arsos, E. Moralidis, I. Tsechelidis, G. Sakagiannis, V. Sidiropoulou, and E. Psarouli, "Measurement of glomerular filtration rate with chromium-51 ethylene diamino tetraacetic acid in the presence of gallium-67 citrate," *Nuclear Medicine Communications*, vol. 32, no. 3, pp. 221–226, 2011.

[26] J. S. Fleming, M. A. Zivanovic, G. M. Blake, M. Burniston, and P. S. Cosgriff, "Guidelines for the measurement of glomerular filtration rate using plasma sampling," *Nuclear Medicine Communications*, vol. 25, no. 8, pp. 759–769, 2004.

[27] F. Iliadis, T. Didangelos, A. Ntemka et al., "Glomerular filtration rate estimation in patients with type 2 diabetes: creatinine- or cystatin C-based equations?," *Diabetologia*, vol. 54, no. 12, pp. 2987–2994, 2011.

[28] S. Orlov, D. Z. I. Cherney, R. Pop-Busui et al., "Cardiac autonomic neuropathy and early progressive renal decline in patients with nonmacroalbuminuric type 1 diabetes," *Clinical Journal of the American Society of Nephrology*, vol. 10, no. 7, pp. 1136–1144, 2015.

[29] T. P. Didangelos, G. A. Arsos, D. T. Karamitsos, V. G. Athyros, and N. D. Karatzas, "Left ventricular systolic and diastolic function in normotensive type-1 diabetic patients with or without autonomic neuropathy. A radionuclide ventriculography study," *Diabetes Care*, vol. 26, no. 7, pp. 1955–1960, 2003.

[30] T. P. Didangelos, G. Arsos, T. Karamitsos et al., "Left ventricular systolic and diastolic function in normotensive type 2 diabetic patients with or without autonomic neuropathy: a radionuclide ventriculography study," *Angiology*, vol. 65, no. 10, pp. 877–882, 2014.

[31] S. S. Soedamah-Muthu, N. Chaturvedi, D. R. Witte et al., "Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB prospective complications study (PCS)," *Diabetes Care*, vol. 31, no. 7, pp. 1360–1366, 2008.

[32] The Diabetes Control and Complications Trial Research Group, "The effect of intensive diabetes therapy on measures of autonomic nervous system function in the diabetes control and complications trial (DCCT)," *Diabetologia*, vol. 41, no. 4, pp. 416–423, 1998.

[33] R. Pop-Busui, P. A. Low, B. H. Waberski et al., "Effects of prior intensive insulin therapy on cardiac autonomic nervous system function in type 1 diabetes mellitus: the diabetes control and complications trial/epidemiology of diabetes interventions and complications study (DCCT/EDIC)," *Circulation*, vol. 119, no. 22, pp. 2886–2893, 2009.

[34] P. A. Low, L. M. Benrud-Larson, D. M. Sletten et al., "Autonomic symptoms and diabetic neuropathy: a population-based study," *Diabetes Care*, vol. 27, no. 12, pp. 2942–2947, 2004.

[35] V. L. Fisher and A. A. Tahrani, "Cardiac autonomic neuropathy in patients with diabetes mellitus: current perspectives," *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, vol. Volume 10, pp. 419–434, 2017.

[36] D. Ziegler, F. Weise, K. J. Langen et al., "Effect of glycaemic control on myocardial sympathetic innervation assessed by [123I] metaiodobenzylguanidine scintigraphy: a 4-year prospective study in IDDM patients," *Diabetologia*, vol. 41, no. 4, pp. 443–451, 1998.

[37] O. Schnell, K. Hammer, D. Muhr-Becker et al., "Cardiac sympathetic dysinnervation in type 2 diabetes mellitus with and without ECG-based cardiac autonomic neuropathy," *Journal of Diabetes and its Complications*, vol. 16, no. 3, pp. 220–227, 2002.

[38] M. Jaiswal, E. M. Urbina, R. P. Wadwa et al., "Reduced heart rate variability among youth with type 1 diabetes: the SEARCH CVD study," *Diabetes Care*, vol. 36, no. 1, pp. 157–162, 2012.

[39] G. Jermendy, "Clinical consequences of cardiovascular autonomic neuropathy in diabetic patients," *Acta Diabetologica*, vol. 40, pp. s370–s374, 2003.

[40] M. Estorch, I. Carrio, L. Berna, J. Lopez-Pousa, and G. Torres, "Myocardial iodine-labeled metaiodobenzylguanidine 123 uptake relates to age," *Journal of Nuclear Cardiology*, vol. 2, no. 2, pp. 126–132, 1995.

[41] A. F. Jacobson, J. Chen, L. Verdes, R. D. Folks, D. N. Manatunga, and E. V. Garcia, "Impact of age on myocardial uptake of ^{123}I-mIBG in older adult subjects without coronary heart disease," *Journal of Nuclear Cardiology*, vol. 20, no. 3, pp. 406–414, 2013.

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 9808295, 7 pages
https://doi.org/10.1155/2018/9808295

Research Article

Establishing Differences in Thermographic Patterns between the Various Complications in Diabetic Foot Disease

Alfred Gatt,[1] **Owen Falzon**,[2] **Kevin Cassar**,[3] **Christian Ellul**,[1] **Kenneth P. Camilleri**,[2,4] **Jean Gauci**,[2] **Stephen Mizzi**,[1] **Anabelle Mizzi**,[1] **Cassandra Sturgeon**,[3] **Liberato Camilleri**,[5] **Nachiappan Chockalingam**,[6] **and Cynthia Formosa**[1]

[1]*Faculty of Health Sciences, University of Malta, Msida, Malta*
[2]*Centre for Biomedical Cybernetics, Faculty of Engineering, University of Malta, Msida, Malta*
[3]*Faculty of Medicine and Surgery, University of Malta, Msida, Malta*
[4]*Department of Systems & Control Engineering, University of Malta, Msida, Malta*
[5]*Department of Statistics and Operations Research, Faculty of Science, University of Malta, Msida, Malta*
[6]*Faculty of Health Science, Staffordshire University, Stoke-on-Trent, UK*

Correspondence should be addressed to Alfred Gatt; alfred.gatt@um.edu.mt

Received 29 September 2017; Accepted 3 January 2018; Published 12 March 2018

Academic Editor: Nikolaos Papanas

Aim. To evaluate the potential of thermography as an assessment tool for the detection of foot complications by understanding the variations in temperature that occur in type 2 diabetes mellitus (DM). *Methods*. Participants were categorized according to a medical examination, ankle brachial index, doppler waveform analysis, and 10-gram monofilament testing into five groups: healthy adult, DM with no complications, DM with peripheral neuropathy, DM with neuroischaemia, and DM with peripheral arterial disease (PAD) groups. Thermographic imaging of the toes and forefeet was performed. *Results*. 43 neuroischaemic feet, 41 neuropathic feet, 58 PAD feet, 21 DM feet without complications, and 126 healthy feet were analyzed. The temperatures of the feet and toes were significantly higher in the complications group when compared to the healthy adult and DM healthy groups. The higher the temperatures of the foot in DM, the higher the probability that it is affected by neuropathy, neuroischaemia, or PAD. *Conclusions*. Significant differences in mean temperatures exist between participants who were healthy and those with DM with no known complications when compared to participants with neuroischaemia, neuropathy, or PAD. As foot temperature rises, so does the probability of the presence of complications of neuropathy, neuroischaemia, or peripheral arterial disease.

1. Introduction

The diabetic foot is characterized by the presence of various complications that typically tend to develop as a result of poor glycaemic control. The main complications include neuropathy, peripheral arterial disease (PAD), and neuroischaemia, amongst others. These complications are amongst the most serious and costly as they often lead to amputation. In many cases, development of diabetic foot complications can be avoided or substantially delayed with timely assessment, diagnosis, and treatment provided at an early stage of the disease. Prophylactic foot care has been shown to decrease patient morbidity [1].

Common diabetic foot complications such as ischaemia and neuropathy have an effect on the temperature of the foot [2]. It is postulated that changes in temperature of the foot may be indicative of the presence of such complications.

Thermography or medical infrared imaging may be used to detect temperature changes. This technique is deemed safe since it is noncontact, noninvasive, and nonirradiant and has been utilized in a number of medical applications including

imaging of the breast [3], skin [4] and foot vascular complications, and ulceration in diabetes [5–7].

The current clinical practice of temperature assessment is mainly by manual palpation of foot temperature. However, a gradual increase in foot temperature may be too subtle to be detected only by the hand, making the timely and early detection of underlying pathology difficult. It has been established that increased temperatures in the foot may be present up to a week before a foot ulcer occurs [8], thus making it important for any variations in temperature to be detected promptly.

Thermal imaging offers an excellent means of making a quantitative determination of surface temperature and can offer an alternative mode of detection of major foot complications since it has been reported that monitoring of skin temperature reduces the risk of diabetic foot ulceration in high-risk patients [9].

Although Nagase et al. had looked at variations in plantar thermographic patterns in normal controls and nonulcer diabetic patients, it has not been fully elucidated to what extent the individual variations of the plantar thermographic patterns show different trends between these two cohorts [10].

Presently, little is known about the range of abnormal thermoregulation in those patients with diabetes presenting both for screening and management [8]. The aim of this study was to evaluate the potential of thermography as an assessment tool for the detection of type 2 diabetic foot disease by assessing the variations in temperature that occur in the diabetic foot before ulceration appears. Objectives were to find a possible correlation between temperature readings of the plantar foot and toes in diabetic foot complications to reduce the risk of foot ulceration by early detection of pathologies.

2. Method

This study employed medical infrared imaging to visualize the temperature distribution of the feet of participants living with DM with or without complications and healthy controls. Following ethical approval from the University Ethics Committee, initially, healthy adult participants were recruited, medically examined, and imaged as reported elsewhere [11] whilst participants with type 2 diabetes mellitus were recruited from the patient list of a vascular surgeon. All participants underwent a thorough clinical examination that included validated tests for neuropathy [12] and peripheral arterial disease [13].

Participants with DM were categorized into 4 groups based on the medical examination and testing: a heathy DM group (i.e., presenting with DM but no significant medical comorbidities and/or complications), a PAD group (presenting with ABPI < 0.6 and monophasic Doppler spectral waveforms at the ankles, but no neuropathy), a neuropathic group (presenting with positive 10-gram monofilament at any one of 10 tested sites and/or reduced vibration perception threshold as measured with a tuning fork and an ABPI between 0.9 and 1.3), and a neuroischaemic group (presenting with ABPI < 0.9 and neuropathy).

Participants who presented with active ulceration or other significant comorbidities that could affect the distribution of thermographic patterns, such as rheumatoid arthritis or Raynaud's phenomenon, were excluded.

Following a 15-minute acclimatization period, all participants rested in a supine position on a couch in a room which was temperature controlled at 23°C.

An ABPI was obtained [14, 15], according to standard clinical practice utilizing a Huntleigh (Cardiff, Wales) Dopplex Assist. A cuff was applied proximal to the ankle, and an 8 Mhz doppler probe was applied at the posterior tibial artery and the dorsalis paedis artery. The probe was held at an angle of 45° against blood flow while the cuff was inflated until the doppler signal was cut off. Then the cuff pressure was slowly released. Once the signal was reobtained, the systolic pressure of the particular artery was noted.

This process was repeated for the brachial artery; the cuff was applied above the elbow and the doppler probe was held in order to obtain its systolic pressure. The ABPI was calculated with the higher of the posterior tibial and dorsalis paedis pressures being taken into consideration. Normal ABPI values ranged from 0.9 to 1.3.

Spectral Doppler waveform analysis was also employed to classify the recorded waveform as being triphasic, biphasic, or monophasic [16]. A triphasic waveform is indicative of normal arterial perfusion, whilst the other two classifications are indicative of PAD, with the monophasic waveform denoting a more severe form of the condition. Only those participants with monophasic waveforms and an ABPI < 0.6 were included to ensure an unequivocal diagnosis of PAD.

Testing for neuropathy involved the use of a 10 g Semmes Weinstein monofilament administered at 10 sites on each foot. In this validated method, exactly 10 g of force was applied before the monofilament bent, thus ensuring that exactly the same amount of force is applied. Neuropathy was diagnosed if at least one site was not felt by the participant. All the above measurements were carried out by the same experienced clinician in order to ensure consistency.

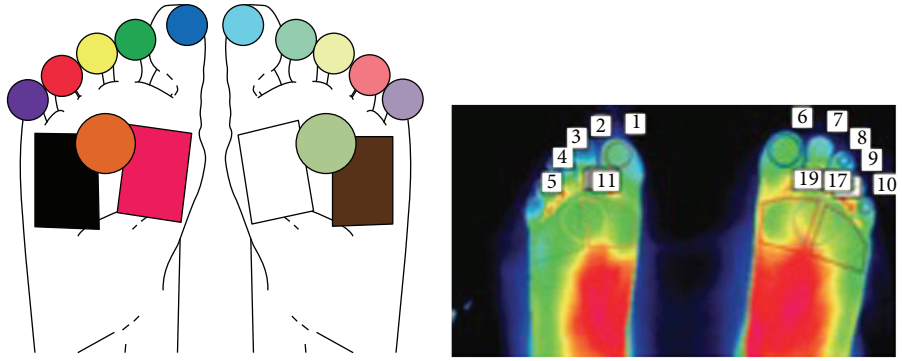
3. Image Acquisition, Segmentation, Data Extraction, and Analysis

A FLIR SC7200 infrared camera with a spatial resolution of 320×256 pixels and a temperature resolution of 20 mK was used for the acquisition of thermal images. The protocol for obtaining thermal images followed the recommendations of the American Academy of Thermology [17]. The camera was placed on a tripod 1.5 m from the subject and perpendicular to the body part that was being photographed [11]. Images of the plantar aspect of the feet were recorded for later analysis.

Thermal images obtained of the feet were divided into regions so that temperature data could be extracted (Figures 1(a) and 1(b)) [11].

4. Results

Thermographic images from 43 neuroischaemic limbs (from 30 subjects), 41 neuropathic limbs (from 32 subjects),



(a) (b)

Figure 1: (a) Diagram showing the foot regions considered for temperature extraction. (b) An actual thermal image and the corresponding regions of interest. The temperatures from the toe regions and forefoot regions were considered for further analysis.

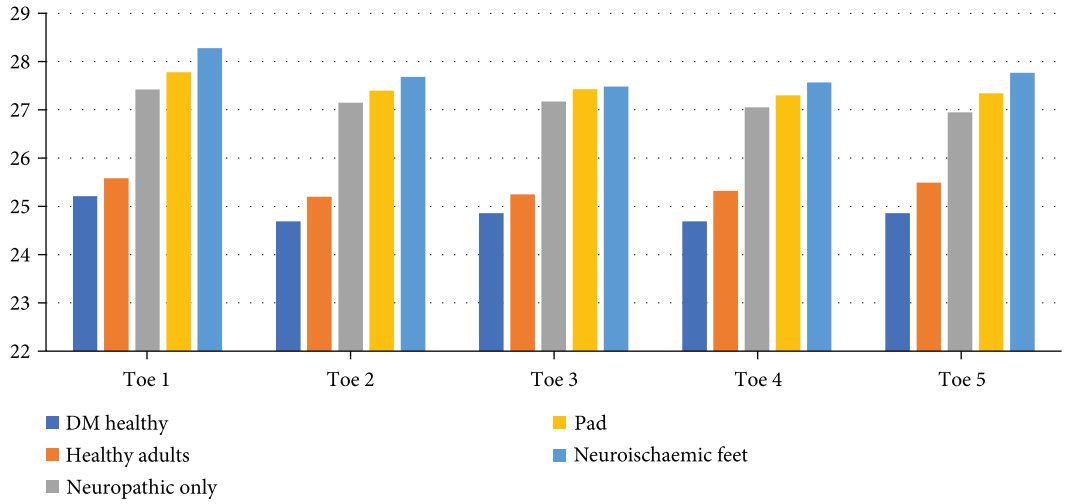


Figure 2: Toe temperature distribution.

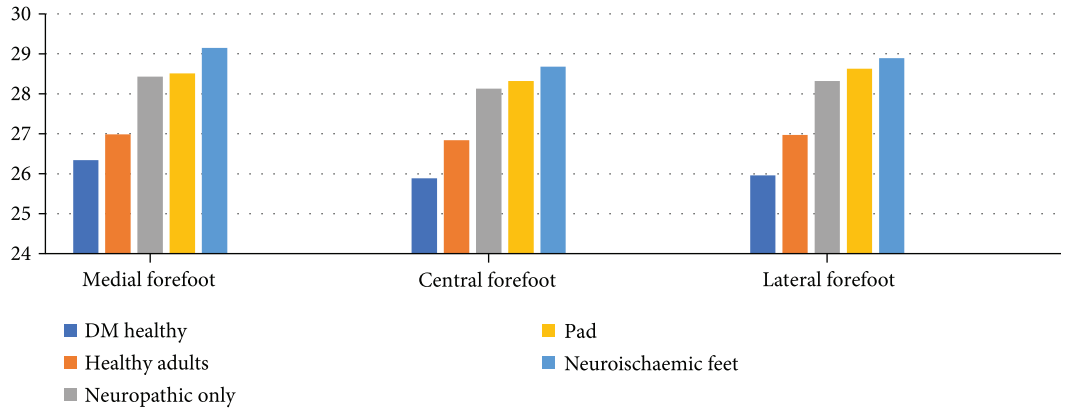


Figure 3: Forefoot temperature distribution.

58 PAD limbs (from 42 subjects), 21 DM healthy limbs (15 subjects,), and 126 healthy limbs (from 63 subjects) were analyzed.

When analyzing the mean temperature data in all 5 toes and 3 plantar regions of the forefoot (medial, central, and lateral regions) (Figures 2 and 3), there are significant

Table 1: Tukey test to compare mean temperatures between the two groups.

		Sample size	Mean	Std. deviation	p value
Toe 1	Neuropathic, neuroischaemic, and PAD	121	27.83	2.637	0.000
	Healthy and DM healthy	123	25.52	3.473	
Toe 2	Neuropathic, neuroischaemic, and PAD	128	27.38	2.899	0.000
	Healthy and DM healthy	122	25.12	3.452	
Toe 3	Neuropathic, neuroischaemic, and PAD	131	27.33	2.886	0.000
	Healthy and DM healthy	123	25.19	3.476	
Toe 4	Neuropathic, neuroischaemic, and PAD	125	27.27	2.926	0.000
	Healthy and DM healthy	123	25.21	3.317	
Toe 5	Neuropathic, neuroischaemic, and PAD	125	27.29	2.852	0.000
	Healthy and DM healthy	123	25.38	3.238	
Medial forefoot	Neuropathic, neuroischaemic, and PAD	135	28.67	2.454	0.000
	Healthy and DM healthy	123	26.88	2.874	
Central forefoot	Neuropathic, neuroischaemic, and PAD	134	28.35	2.474	0.000
	Healthy and DM healthy	123	26.68	2.835	
Lateral forefoot	Neuropathic, neuroischaemic, and PAD	130	28.58	2.374	0.000
	Healthy and DM healthy	123	26.80	2.861	

differences in mean temperatures between the five groups of patients, as demonstrated by the Tukey test (Table 1). This test clusters these five groups into two groups where the mean temperatures of diabetic participants with peripheral arterial disease, diabetes patients with neuropathy, and diabetes patients with neuroischaemia are significantly higher than the mean temperatures of healthy adults and diabetes patients with no known complication. Thus, for further statistical comparison, these five categories were divided into a "healthy group" (comprised of healthy adults and DM participants with no known complications) and a "complications group" (comprised of neuropathic, neuroischaemic, and PAD participants).

5. Logistic Regression Analysis

In the first logistic regression model fit, we relate the health status (neuropathic, neuroischaemic, or PAD; healthy or DM healthy) to two predictors, which include toe temperature and toe location. As indicated in Table 2, a binomial distribution is assumed since the dependent variable has two categories, while a logit link function is used since this is the canonical link for the binomial distribution.

Toe location was not found to be a significant predictor since the p value (0.901) exceeds the 0.05 level of significance. A backward procedure was used to fit the parsimonious model, which identified temperature as a sole significant predictor.

As shown in Table 3, the regression coefficient of temperature (0.220) is positive indicating that the toe temperature of neuropathic, neuroischaemic, or PAD participants is expected to be higher than that of healthy or DM healthy patients. The odds ratio indicates that the odds that the participant has neuropathy, neuroischaemia, or PAD rather than being healthy increases by 24.7% for every 1°C increase in toe temperature. This odds ratio ranges from 19.8% to 29.7% assuming a 95% confidence level. The logistic

Table 2: Likelihood ratio tests (model 1).

Effect	Likelihood ratio tests			
	Model fitting criteria −2 log likelihood	Likelihood ratio tests Chi-square	df	p value
Intercept	1495.659	0.000	0	.
Temperature	1634.676	139.02	1	0.000
Toe location	1496.714	1.055	4	0.901

regression model that yields the probability that a patient has neuropathy, neuroischaemia, or PAD given the toe temperature is given by

$$\log_e\left(\frac{p}{1-p}\right) = -5.786 + 0.220 \text{ temperature}, \quad (1)$$

where p is the probability that the participant has neuropathy, neuroischaemia, or PAD and $1-p$ is the probability that the patient is healthy. The probability curves displayed in Figure 4 clearly show that the likelihood of neuropathy, neuroischaemia, or PAD increases as the toe temperature increases.

In the second logistic regression model fit, we relate the health status (neuropathic, neuroischaemic, or PAD; healthy or DM healthy) to two predictors, which include temperature and forefoot location, whether medial, central, or lateral. A binomial distribution and a logit link function are again assumed.

Plantar location was not found to be a significant predictor since the p value (0.912) exceeds the 0.05 level of significance. A backward procedure was used to fit the parsimonious model, which identified temperature as a sole significant predictor (Table 4).

The results in Table 5 indicate that the regression coefficient for the plantar forefoot temperature (0.254) is positive

Table 3: Parameter estimates of the logistic regression model for toe temperatures.

Effect	B	Std. error	Wald	p value	Odds ratio	95% CI for odds ratio	
						Lower bound	Upper bound
Intercept	−5.786	0.534	117.363	0.000			
Temperature	0.220	0.020	119.693	0.000	1.247	1.198	1.297

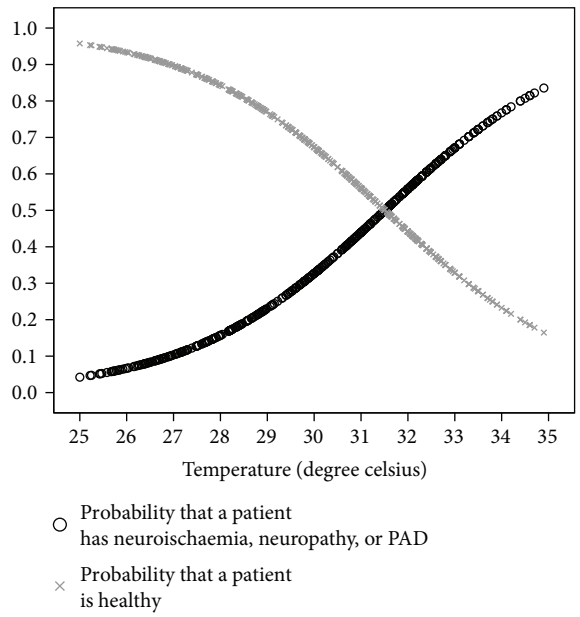


Figure 4: Logistical regression curves of toe temperatures.

Table 4: Likelihood ratio tests (model 2).

Effect	Likelihood ratio tests			
	Model fitting criteria −2 log likelihood	Likelihood ratio tests Chi-square	df	p value
Intercept	924.056	0.000	0	.
Temperature	1004.520	80.464	1	0.000
Plantar forefoot location	924.240	0.184	2	0.912

indicating that the plantar forefoot temperature of neuropathic, neuroischaemic, or PAD patients is expected to be higher than that of healthy or DM healthy patients. The odds ratio indicates that the odds that the participant has neuropathy, neuroischaemia, or PAD rather than being healthy increases by 28.9% for every 1°C increase in plantar temperature. This odds ratio ranges from 19.8% to 29.7% assuming a 95% confidence level. The logistic regression model that yields the probability that a patient has neuropathy, neuroischaemia, or PAD given the plantar temperature is given by

$$\log_e\left(\frac{p}{1-p}\right) = -6.949 + 0.254 \ \text{temperature}, \qquad (2)$$

where p is the probability that the patient has neuropathy, neuroischaemia, or PAD and $1-p$ is the probability that the patient is healthy. The probability curves displayed in Figure 5 clearly show that the likelihood of neuropathy, neuroischaemia, or PAD increases as the plantar forefoot temperature increases.

6. Discussion

The results of this thermographic study demonstrate three main inferences: (i) that there are no significant differences in mean temperatures of the toes and forefoot between healthy subjects and patients with diabetes showing no complications; (ii) that there are no significant differences in mean temperatures of these same areas between participants with complications of neuropathy, neuroischaemia, and PAD; (iii) that there are significantly higher mean temperatures in these latter group of subjects when compared to both healthy and DM participants with no complications.

This increase in temperature is further confirmed by the logistic regression models of both toe and forefoot areas, which establish temperature as being the sole significant predictor of complications. These models demonstrate that the probability of complications of PAD, neuropathy, and/or neuroischaemia being present increases as the temperature of these regions rises.

This study is the first of its kind to report temperature differences between possible categories of complications of DM relative to healthy adults. The authors recommend that these findings and thermographic techniques should be considered for further clinical investigations of the DM patient. These results imply that should a rise in temperature be detected in the diabetic foot, there is a higher likelihood that diabetic foot complications have set in, as further reported by Sun et al. [8] who state that thermographic patterns may change as early as one week prior to ulceration. The findings of the study indicate that an increase in temperature may not necessarily imply impending ulceration, but simply the development of peripheral neuropathy, ischaemia, or both.

Further research is warranted to establish whether the inclusion of thermography into screening protocols could help detect the development of diabetic foot complications earlier so that appropriate prompt preventative measures may be taken to avoid unnecessary complications.

Whilst neuropathic feet have been previously reported as being warmer than healthy feet, we can now confirm that even neuroischaemic and ischaemic feet exhibit the same trend. This may be due to altered thermoregulatory mechanisms of the feet, which can be affected by both neuropathy and PAD. Local ischaemia may lead to disruption of sympathetically mediated noradrenergic vasoconstriction which leads to increased flow to the cutaneous vessels rather than through the deeper nutritive vessels which in turn leads to

Table 5: Parameter estimates of the logistic regression model for forefoot temperatures.

Effect	B	Std. error	Wald	p value	Odds ratio	95% CI for odds ratio	
						Lower bound	Upper bound
Intercept	−6.949	0.846	67.395	0.000			
Temperature	0.254	0.030	69.481	0.000	1.289	1.214	1.368

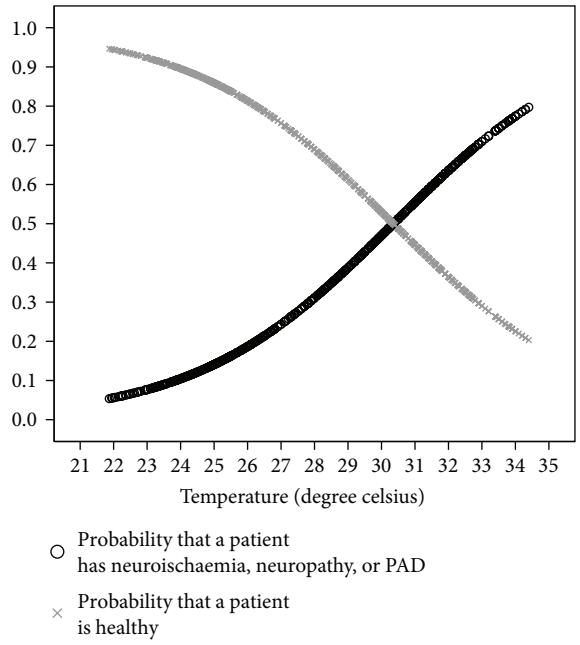


Figure 5: Logistical regression curves of forefoot temperatures.

higher heat emissivity. Arteriovenous anastamoses (AVA) which are thick-walled, low resistance conduits allow high-flow rates directly from arterioles to venules. AVA are numerous and richly innervated by sympathetic vasoconstrictor nerves. Substantial changes occur in blood flow depending on whether AVAs are closed or open [18].

It is reported that the application of clinical examination or nerve conduction studies alone is not adequate in screening diabetic at-risk feet at early stage [8]; thus, the use of an adjunct method such as thermography may prove useful.

Further research into thermographic patterns of patients with diabetes and with active ulcers may help elucidate the natural history of development of ulceration.

7. Conclusions

This study has confirmed that the mean temperatures of the toes and forefeet of the complications group exhibit significantly higher temperatures than those of the healthy group, whilst each group presents with comparable temperatures within themselves. These results indicate that thermography demonstrates potential as a screening or clinical investigation tool, although more research in the area is warranted.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank all the participants who consented to take part in this trial. This research project was financed by the Malta Council for Science and Technology through the National Research & Innovation Programme 2013.

References

[1] M. S. Pinzur, M. P. Slovenkai, E. Trepman, N. N. Shields, and Diabetes Committee of American Orthopaedic Foot and Ankle Society, "Guidelines for diabetic foot care: recommendations endorsed by the Diabetes Committee of the American Orthopaedic Foot and Ankle Society," *Foot & Ankle International*, vol. 26, no. 1, pp. 113–119, 2005.

[2] S. Bagavathiappan, J. Philip, T. Jayakumar et al., "Correlation between plantar foot temperature and diabetic neuropathy: a case study by using an infrared thermal imaging technique," *Journal of Diabetes Science and Technology*, vol. 4, no. 6, pp. 1386–1392, 2010.

[3] B. B. Lahiri, S. Bagavathiappan, T. Jayakumar, and J. Philip, "Medical applications of infrared thermography: a review," *Infrared Physics & Technology*, vol. 55, no. 4, pp. 221–235, 2012.

[4] K. Otsuka, S. Okada, M. Hassan, and T. Togawa, "Imaging of skin thermal properties with estimation of ambient radiation temperature," *IEEE Engineering in Medicine and Biology Magazine*, vol. 21, no. 6, pp. 49–55, 2002.

[5] H. Wang, D. R. Wade Jr., and J. Kam, "IR imaging of blood circulation of patients with vascular disease," *Proc. SPIE*, D. D. Burleigh, K. E. Cramer and G. R. Peacock, Eds., vol. 5405, pp. 115–123, 2004.

[6] E. Ring and K. Ammer, "The technique of infrared imaging in medicine," *Thermology International*, vol. 10, pp. 7–14, 2000.

[7] D. G. Armstrong and L. A. Lavery, "Monitoring healing of acute Charcot's arthropathy with infrared dermal thermometry," *Journal of Rehabilitation Research and Development*, vol. 34, no. 3, pp. 317–321, 1997.

[8] P.-C. Sun, H.-D. Lin, S.-H. E. Jao, Y.-C. Ku, R.-C. Chan, and C.-K. Cheng, "Relationship of skin temperature to sympathetic dysfunction in diabetic at-risk feet," *Diabetes Research and Clinical Practice*, vol. 73, no. 1, pp. 41–46, 2006.

[9] D. G. Armstrong, K. Holtz-Neiderer, C. Wendel, M. J. Mohler, H. R. Kimbriel, and L. A. Lavery, "Skin temperature monitoring reduces the risk for diabetic foot ulceration in high-risk

patients," *The American Journal of Medicine*, vol. 120, no. 12, pp. 1042–1046, 2007.

[10] T. Nagase, H. Sanada, K. Takehara et al., "Variations of plantar thermographic patterns in normal controls and non-ulcer diabetic patients: novel classification using angiosome concept," *Journal of Plastic, Reconstructive & Aesthetic Surgery*, vol. 64, no. 7, pp. 860–866, 2011.

[11] A. Gatt, C. Formosa, K. Cassar et al., "Thermographic patterns of the upper and lower limbs: baseline data," *International Journal of Vascular Medicine*, vol. 2015, Article ID 831369, 9 pages, 2015.

[12] S. Baraz, K. Zarea, H. B. Shahbazian, and S. M. Latifi, "Comparison of the accuracy of monofilament testing at various points of feet in peripheral diabetic neuropathy screening," *Journal of Diabetes and Metabolic Disorders*, vol. 13, no. 1, p. 19, 2014.

[13] C. Formosa, A. Gatt, and N. Chockalingam, "Screening for peripheral vascular disease in patients with type 2 diabetes in Malta in a primary care setting," *Quality in Primary Care*, vol. 20, no. 6, pp. 409–414, 2013.

[14] C. Formosa, K. Cassar, A. Gatt et al., "Hidden dangers revealed by misdiagnosed peripheral arterial disease using ABPI measurement," *Diabetes Research and Clinical Practice*, vol. 102, no. 2, pp. 112–116, 2013.

[15] A. T. Hirsch, Z. J. Haskal, N. R. Hertzer et al., "ACC/AHA 2005 practice guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic)," *Circulation*, vol. 113, no. 11, pp. e463–e465, 2006.

[16] L. Norgren, W. R. Hiatt, J. A. Dormandy et al., "Inter-society consensus for the management of peripheral arterial disease (TASC II)," *International Angiology*, vol. 45, no. 1, pp. S5–S67, 2007.

[17] M. Bharara, J. E. Cobb, and D. J. Claremont, "Thermography and thermometry in the assessment of diabetic neuropathic foot: a case for furthering the role of thermal techniques," *The International Journal of Lower Extremity Wounds*, vol. 5, no. 4, pp. 250–260, 2006.

[18] K. Lossius, M. Eriksen, and L. Walløe, "Fluctuations in blood flow to acral skin in humans: connection with heart rate and blood pressure variability," *The Journal of Physiology*, vol. 460, no. 1, pp. 641–655, 1993.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 2452853, 6 pages
https://doi.org/10.1155/2017/2452853

Review Article

Perspectives on Peripheral Neuropathy as a Consequence of Metformin-Induced Vitamin B12 Deficiency in T2DM

Marwan A. Ahmed,[1] George L. Muntingh,[1] and Paul Rheeder[2]

[1]*Department of Pharmacology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa*
[2]*Department of Internal Medicine, Steve Biko Academic Hospital, University of Pretoria, Pretoria, South Africa*

Correspondence should be addressed to Marwan A. Ahmed; mrwnwd@yahoo.com

Received 8 May 2017; Accepted 31 July 2017; Published 27 August 2017

Academic Editor: Ilias Migdalis

Peripheral neuropathy (PN) is a primary complication of type 2 diabetes mellitus (T2DM) and a direct manifestation of vitamin B12 deficiency. Examining the effects of metformin use on PN status became imperative following clinical studies that showed the vitamin B12-lowering effect of the medication. The complexity of the topic and the inconsistency of the results warrant consideration of topic-specific perspectives for better understanding of the available evidence and more appropriate design of future studies.

1. Introduction

T2DM is a metabolic disorder that is increasingly becoming a public health concern. The disease is associated with a variety of systemic macrovascular and microvascular complications. Diabetic peripheral neuropathy (DPN) is the most common complication, and it may eventually develop in up to 50% of patients [1]. DPN is associated with ulcerations, recurrent foot infections, Charcot's joints, foot or ankle fractures, amputations, and depression. As a result, DPN can cause physical limitations, increased utilization of health care, and diminished work productivity [2]. Gordois et al. estimated the annual expenditure on DPN in the United States to be between 4.6 and 13.7 billion dollars [3]. In addition, 27% of diabetes-related medical costs are attributable to DPN [3].

Both American and European guidelines recommend metformin as the first-line agent for the pharmacological management of T2DM. Accumulating evidence suggests that long-term use of metformin is associated with low vitamin B12 levels, and findings from both observational and interventional studies have confirmed this association [4–8]. Recent American Diabetes Association (ADA) guidelines recommend periodic testing of vitamin B12 in metformin-treated patients, especially in those with peripheral neuropathy [9].

Since vitamin B12 is essential for the remethylation of homocysteine to methionine, metformin-induced vitamin B12 deficiency could be associated with hyperhomocysteinemia, a condition with a questionable detrimental impact on macrovascular disease in T2DM patients [10]. The clinical presentation of vitamin B12 deficiency generally includes haematological and neurological manifestations. Neuropathy can be the only manifestation of the deficiency, without a haematologic presentation [11]. Over the last few decades, the clinical manifestations of vitamin B12 deficiency have shown notable trends towards neurological signs and symptoms [12]. PN resulting from vitamin B12 deficiency is clinically indistinguishable from DPN [13]. Such PN neuropathy can be asymptomatic [12] and could likely worsen DPN in diabetic patients. It is reasonable to assume that PN resulting from metformin-associated vitamin B12 deficiency has a significant risk of being misdiagnosed as DPN. Long-term metformin use can theoretically cause or worsen PN in diabetes patients, adding to its considerable burden.

The potential for metformin-induced vitamin B12 deficiency to cause or worsen DPN has recently been investigated by several studies with conflicting findings. This article provides new perspectives that may assist in interpreting the available evidence and give a deeper understanding of the topic for the design of future research.

2. The Current Evidence

The potential association between metformin use and vitamin B12 deficiency reported by interventional and observational studies has enabled researchers to question whether such an association can have clinical implications. Recently, the topic has attracted attention, and several studies were conducted to investigate the potential for metformin-induced vitamin B12 deficiency to cause, or worsen, PN in T2DM patients.

The insidious nature of neuropathy makes clinical trials and cohort studies impractical to investigate the possible relationship between metformin use and worsening of PN in T2DM patients [14]. Ethical, sample size, and study duration considerations oppose utilizing clinical trials to answer the question [14]. Despite their questionable validity and known limitations, cross-sectional and case-control studies seem to be the most convenient for studying the relationship. Thus, all of the available evidence comes from such studies. The results were conflicting and studies have shown differences in designs and settings (Table 1). Neuropathy was assessed by different tools with various degrees of subjectivity, and all studies had relatively small sample sizes (Table 1).

3. Antineuropathic and Neuroprotective Effects of Metformin

Animal studies have recently shown that metformin can exert neuroprotective and antineuropathic activities that are independent of its euglycemic effect. Metformin protects against numbness and neuropathic pain induced by chemotherapy in mice [21]. The sensory symptoms of pain, dysaesthesia, and paraesthesia characterize both diabetic PN and chemotherapy-induced PN, signifying the impact of these findings. Animal studies have also reported that metformin abolished pain resulting from the activation of sensory neurons [22] as well as resolved neuropathic allodynia [23], protected against ethanol-induced neuronal apoptosis [24], and enhanced neurogenesis [25]. It also suppressed cortical neuronal apoptosis [26] and exerted neuroprotective effects in Parkinson's disease [27]. Clinically, a retrospective chart review also reported the association between metformin use and a decrease in lumbar radiculopathy pain [28]. Antineuropathic effects of metformin may be mediated by $5'$ adenosine monophosphate-activated protein kinase (AMPK) activation [29]. Impaired AMPK signaling was linked to PN in animals [30].

The glycemic control-independent neuroprotective effect of metformin may add a new perspective to PN due to metformin-induced vitamin B12 deficiency [14]. Given the above evidence, metformin can influence PN in two ways, excluding that related to glycemic control. It may exert a positive neuroprotective effect, or it may enhance PN by inducing vitamin B12 deficiency. The results showing no association between vitamin B12 and PN do not preclude the ability of metformin to exert negative effects on PN status by lowering vitamin B12 levels. The vitamin B12 deficiency-mediated effects may blunt the antineuropathic, neuroprotective, and euglycemic actions, which partly blunt the medication's additional beneficial actions in PN.

The theory may provide deeper insight into the contradicting results of studies investigating PN in metformin-treated T2DM patients. The inconsistency of results may be attributed to variation in the individual weights of metformin's direct impact on the neurons, its metabolic euglycemic effect, and its vitamin B12 deficiency-mediated neuropathic effect. It can be hypothesized that the findings in each study reflect the dominant action of metformin in that particular population. The sample and study characteristics, such as the mean age, duration of diabetes, metformin dose, and duration as well as race and comorbidities, may contribute to the dominance of certain axes and to the net impact of metformin on PN.

4. Study Design and Methodological Issues

Factors within clinical practice might have methodological impacts that deserve consideration when interpreting the evidence on PN resulting from metformin-associated vitamin B12 deficiency [14]. Both American and European guidelines recommend using metformin as a first-line medication for T2DM. Encountering T2DM patients who are not on metformin is uncommon. Metformin users and nonusers are anticipated to be inherently different in observational studies that compare the two groups. Obtaining similar study groups can become unattainable by having one group with the "abnormality" of not taking metformin. The highly probable and adherent imbalance between the two comparison groups makes it unclear whether metformin use has played the primary role in the outcome and worsening of PN. In other words, factors distributed differentially between the two groups can provide alternative explanations for the findings, thus threatening the study validity. A control group may discredit the validity in studies aiming at investigating the effect of metformin-induced vitamin B12 deficiency on PN. Even comparison groups can only be attained by randomization in controlled trials, and such study designs are impractical to answer this question.

Considering the antineuropathic and neuroprotective actions of metformin also gives us a different view regarding the designs of studies aiming at exploring the relationship between medication use and PN. In the metformin group, there is a possibility that the medication's antineuropathic and neuroprotective effects predominate or dilute its vitamin B12 deficiency-mediated neuropathic impact. Thus, comparing PN in the metformin and nonmetformin groups could produce distorted conclusions that do not reflect the real contribution of metformin-induced vitamin B12 deficiency to PN. This perspective adds strength to the studies that compared vitamin B12-normal and -deficient patients using metformin and discredits the classical designs comparing metformin users and nonusers.

5. An Emerging Theory on Metformin-Induced Vitamin B12 Deficiency

Vitamin B12 is involved in both methylmalonyl-CoA mutase and methionine synthase intracellular pathways. Vitamin B12 deficiency interferes with the two pathways and causes increased levels of methylmalonic acid and homocysteine,

Table 1: Settings, designs, and results of studies that investigated the impact of metformin-induced low vitamin B12 on peripheral neuropathy in T2DM patients.

Study	Setting	Design	Results
Wile and Toth [15]	Neuromuscular clinic at a university hospital, Canada	Case-control study. Cases were T2DM patients on metformin with primary diagnosis of PN (59 participants). Controls were T2DM patients not taking metformin with primary diagnosis of PN (63 participants).	The metformin group had more severe PN (assessed by TCSS and NIS). Electrophysiological markers showed no significant difference between the two groups. Cumulative metformin dose showed a significant positive correlation with TCSS scores (rho = 0.80) and NIS scores (rho = 0.79).
Singh et al. [16]	Internal medicine clinic in a tertiary hospital, India	Cross-sectional study. Randomly selected T2DM patients were divided into metformin users (84 participants) and nonusers (52 participants).	The metformin group had more severe PN (assessed by TCSS). Cumulative metformin dose revealed a significant positive correlation with TCSS (rho = 0.53).
de Groot-Kamphuis et al. [17]	Secondary care outpatient diabetes clinic, the Netherlands	Cross-sectional study. Randomly selected T2DM patients were divided into metformin users (164 participants) and nonusers (134 participants).	Prevalence of neuropathy (obtained from records) was significantly lower in the metformin group.
Chen et al. [13]	Diabetes clinic of a tertiary hospital, UK	Cross-sectional study. Randomly selected T2DM patients were divided into metformin users (152 participants) and nonusers (50 participants).	All PN-assessing tools (monofilament, neurothesiometry, NTSS-6, and s-LANSS) showed no significant differences between the two groups.
Biemans et al. [18]	Four primary care centers, the Netherlands	Cross-sectional study. Metformin-treated T2DM patients were divided into the vitamin B12-deficient (126 participants) and normal (322 participants) groups.	There were no significant differences in PN (assessed by MNSI and extracted from records) between the two groups.
Russo et al. [19]	Diabetes clinic of a university hospital, Italy	Cross-sectional study. T2DM patients were divided into metformin users (124 participants) and nonusers (139 participants).	There was no significant difference in prevalence of PN between the two groups. PN was suspected based on abnormalities of certain evaluations and confirmed by NCVs.
Roy et al. [20]	Tertiary Hospital, India	Cross-sectional study. T2DM patients were divided into (1) the metformin group (35 participants), (2) the metformin + other antihyperglycemic group (20 participants), and (3) the nonmetformin group (35participants).	Neuropathy (assessed by NCVs) did not differ significantly between the groups.
Ahmed et al. [14]	Diabetes clinics of two tertiary hospitals, South Africa	Cross-sectional study. Metformin-treated T2DM patients were divided into the vitamin B12-deficient (34 participants) and normal (87 participants) groups.	There was no difference in the presence of PN (assessed by NTSS-6) between the two groups. Levels of vitamin B12 and NTSS-6 scores were not correlated.

MNSI: Michigan Neuropathy Screening Instrument; NCVs: nerve conduction velocities; NIS: Neuropathy Impairment Score; NTSS-6: Neuropathy Total Symptom Score-6; PN: peripheral neuropathy; rho: Spearman's rank correlation coefficient; s-LANSS: Self-administered Leeds Assessment of Neuropathic Symptoms and Signs; TCSS: Toronto Clinical Scoring System.

which are also considered as biochemical indicators of cellular (metabolic) vitamin B12 deficiency [31]. The increase in homocysteine concentration can also be a result of folic acid deficiency [31].

The theory that metformin only lowers the circulating vitamin B12 without affecting the intracellular vitamin levels was recently introduced [32]. This theory is based on the fact that low vitamin B12 status should result in increased levels

of the biochemical indicators of metabolic deficiency. Clinical studies, which reported nonelevated concentrations of methylmalonic acid and homocysteine among metformin-treated patients, constitute the framework of the theory. Reinstatler et al. found that despite their significantly lower levels of vitamin B12, metformin users had lower concentrations of homocysteine when compared to nonusers [33]. As the study was conducted after the commencement of the folic acid fortification in the United States, homocysteine levels carried more diagnostic specificity in assessing vitamin B12 status. The theory also relies on the controlled trial of de Jager et al. which found no significant increase in homocysteine concentrations despite low vitamin B12 levels in the metformin group when studied over a period of 4.3 years [4]. Greibe et al. reported that a six-month treatment with metformin resulted in lower serum vitamin B12 without an impact on MMA in women with polycystic ovary syndrome [34]. The authors took a step further and measured vitamin B12 bound to haptocorrin, a transporter that binds 70% to 80% of the circulating vitamin forming a metabolically inert complex. The results showed a reduction in haptocorrin-bound vitamin levels in the metformin group. They concluded that metformin-induced low vitamin B12 levels resulted from the decrease in B12-haptocorrin fraction and did not reflect a true metabolic deficiency. An animal study has also reported increased liver accumulation and decreased circulating concentrations of vitamin B12 in rats following subcutaneous administration of metformin [35]. The authors proposed that metformin enhanced redistribution of vitamin B12 rather than affecting its cellular status.

Adopting the above theory would mean that metformin does not cause or worsen PN in diabetic patients. However, substantial evidence contradicts the theory. The difference in homocysteine levels in the metformin and nonmetformin groups was not statistically significant in the study of Reinstatler et al. [33]. The trial of de Jager et al. indeed reported that metformin caused a slight increase of 5% in homocysteine levels. The p value was 0.09, which may indicate a borderline significant trend. The trial's authors attributed the nonsignificance to the relatively low numbers of patients with vitamin B12 deficiency, and they expected the homocysteine levels to show further increases with a longer treatment duration [4]. Moreover, the six-month period of metformin use in a study by Greibe et al. did not seem to be sufficient to deplete vitamin B12 stores and consequently result in elevated MMA or homocysteine levels. Most importantly, the association between metformin use and the increased levels of cellular vitamin B12 biomarkers was reported by many studies [15, 20, 36–38]. Moving to clinical practice, the aim of clinical research, accepting the theory that metformin only reduces circulating B12 and not its intracellular levels, may take us to another level and raises a crucial question on the validity of the current vitamin B12 serum test. Adopting this theory dictates a revolutionary approach in testing B12 in clinical settings. The theory, however, raises the importance of showing the potential of metformin to cause cellular vitamin B12 deficiency as an achievable initial goal of future research.

6. Other Perspectives

The clinical trial of de Jager et al. has shown that long-term metformin treatment gradually lowered the levels of vitamin B12 [4]. Thus, the possible development of PN due to metformin-induced vitamin B12 deficiency is anticipated to be insidious and progressive. Long periods of treatment with metformin in studies with sufficiently large sample sizes may be required to reveal a detectable and statistically significant PN.

Vitamin B12 levels may differ in various ethnic groups. Several studies reported higher concentrations of vitamin B12 in black individuals when compared to white individuals [39–41]. This is attributed to higher levels of vitamin B12-binding proteins in the black populations [42]. We have recently reported higher concentrations of the vitamin among black South African T2DM patients on metformin [14]. To our knowledge, the impact of ethnicity on the cellular status of the vitamin is not yet investigated. Currently, utilized cutoff points and definitions of vitamin B12 deficiency do not consider the possible effects of ethnicity. Ethnicity should be taken into consideration as a contributing factor when studying PN as a clinical consequence of metformin-induced vitamin B12 deficiency.

7. Conclusion

The conflicting results of the available evidence reflect the complexity of linking metformin-induced vitamin B12 deficiency and PN in T2DM patients, where the disease state also induces neuropathies. Different perspectives can be considered in interpreting and designing studies attempting to explore the triangle of metformin, vitamin B12, and PN in T2DM. The glycemic control-independent neuroprotective and antineuropathic effects of metformin recently reported in animal studies may explain the contradicting nature of the obtained results and enhance understanding of the topic. Obtaining similar study groups is probably unachievable in observational studies that compare metformin users and nonusers, which blunts their validity and raises questions about most of the available evidence.

Future research should probably first focus on confirming metformin's potential to cause vitamin B12 metabolic deficiency. Studies with sufficiently large sample sizes and proper designs that utilize more objective, and conventional, PN-assessing tools are required to investigate the relationship between metformin use and PN in T2DM patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] S. Tesfaye, A. J. Boulton, P. J. Dyck et al., "Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments," *Diabetes Care*, vol. 33, no. 10, pp. 2285–2293, 2010.

[2] S. D. Candrilli, K. L. Davis, H. J. Kan, M. A. Lucero, and M. D. Rousculp, "Prevalence and the associated burden of illness of

symptoms of diabetic peripheral neuropathy and diabetic retinopathy," *Journal of Diabetes and Its Complications*, vol. 21, no. 5, pp. 306–314, 2007.

[3] A. Gordois, P. Scuffham, A. Shearer, A. Oglesby, and J. A. Tobian, "The health care costs of diabetic peripheral neuropathy in the US," *Diabetes Care*, vol. 26, no. 6, pp. 1790–1795, 2003.

[4] J. de Jager, A. Kooy, P. Lehert et al., "Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial," *British Medical Journal*, vol. 340, article c2181, 2010.

[5] R. Z. Ting, C. C. Szeto, M. H. Chan, K. K. Ma, and K. M. Chow, "Risk factors of vitamin B12 deficiency in patients receiving metformin," *Archives of Internal Medicine*, vol. 166, no. 18, pp. 1975–1979, 2006.

[6] M. Nervo, A. Lubini, F. V. Raimundo et al., "Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil," *Revista da Associação Médica Brasileira*, vol. 57, no. 1, pp. 46–49, 2011.

[7] L. S. Hermann, B. Nilsson, and S. Wettre, "Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study," *British Journal of Diabetes and Vascular Disease*, vol. 4, pp. 401–404, 2004.

[8] M. C. Pflipsen, R. C. Oh, A. Saguil, D. A. Seehusen, D. Seaquist, and R. Topolski, "The prevalence of vitamin B_{12} deficiency in patients with type 2 diabetes: a cross-sectional study," *Journal of American Board of Family Medicine*, vol. 22, no. 5, pp. 528–534, 2009.

[9] American Diabetes Association, "8. Pharmacologic approaches to glycemic treatment," *Diabetes Care*, vol. 40, Supplement 1, pp. S64–S74, 2017.

[10] G. T. Russo, A. Di Benedetto, D. Magazzu et al., "Mild hyperhomocysteinemia, $C6_{77}T$ polymorphism on methylenetetrahydrofolate reductase gene and the risk of macroangiopathy in type 2 diabetes: a prospective study," *Acta Diabetologica*, vol. 48, no. 2, pp. 95–101, 2011.

[11] E. B. Healton, D. G. Savage, J. C. Brust, T. J. Garrett, and J. Lindenbaum, "Neurologic aspects of cobalamin deficiency," *Medicine (Baltimore)*, vol. 70, no. 4, pp. 229–245, 1991.

[12] C. Briani, C. Dalla Torre, V. Citton et al., "Cobalamin deficiency: clinical picture and radiological findings," *Nutrients*, vol. 5, no. 11, pp. 4521–4539, 2013.

[13] S. Chen, A. J. Lansdown, S. J. Moat et al., "An observational study of the effect of metformin on B12 status and peripheral neuropathy," *British Journal of Diabetes and Vascular Disease*, vol. 12, pp. 189–193, 2012.

[14] M. A. Ahmed, G. Muntingh, and P. Rheeder, "Vitamin B12 deficiency in metformin-treated type-2 diabetes patients, prevalence and association with peripheral neuropathy," *BMC Pharmacology and Toxicology*, vol. 17, no. 1, p. 44, 2016.

[15] D. J. Wile and C. Toth, "Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy," *Diabetes Care*, vol. 33, no. 1, pp. 156–161, 2010.

[16] A. K. Singh, A. Kumar, D. Karmakar, and R. K. Jha, "Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients," *Journal of Postgraduate Medicine*, vol. 59, no. 4, pp. 253–257, 2013.

[17] D. M. de Groot-Kamphuis, P. R. van Dijk, K. H. Groenier, S. T. Houweling, H. J. Bilo, and N. Kleefstra, "Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin," *The Netherlands Journal of Medicine*, vol. 71, no. 7, pp. 386–390, 2013.

[18] E. Biemans, H. E. Hart, G. E. Rutten, V. G. Cuellar Renteria, A. M. Kooijman-Buiting, and J. W. Beulens, "Cobalamin status and its relation with depression, cognition and neuropathy in patients with type 2 diabetes mellitus using metformin," *Acta Diabetologica*, vol. 52, no. 2, pp. 383–393, 2014.

[19] G. T. Russo, A. Giandalia, E. L. Romeo et al., "Diabetic neuropathy is not associated with homocysteine, folate, vitamin B_{12} levels, and MTHFR $C6_{77}T$ mutation in type 2 diabetic outpatients taking metformin," *Journal of Endocrinological Investigation*, vol. 39, no. 3, pp. 305–314, 2016.

[20] R. P. Roy, K. Ghosh, M. Ghosh et al., "Study of vitamin B_{12} deficiency and peripheral neuropathy in metformin-treated early type 2 diabetes mellitus," *Indian Journal of Endocrinology and Metabolism*, vol. 20, no. 5, pp. 631–637, 2016.

[21] Q. L. Mao-Ying, A. Kavelaars, K. Krukowski et al., "The anti-diabetic drug metformin protects against chemotherapy-induced peripheral neuropathy in a mouse model," *PLoS One*, vol. 9, no. 6, article e100701, 2014.

[22] O. K. Melemedjian, A. Khoutorsky, R. E. Sorge et al., "mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK," *Pain*, vol. 154, no. 7, pp. 1080–1091, 2013.

[23] O. K. Melemedjian, M. N. Asiedu, D. V. Tillu et al., "Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain," *Molecular Pain*, vol. 7, p. 70, 2011.

[24] I. Ullah, N. Ullah, M. I. Naseer, H. Y. Lee, and M. O. Kim, "Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons," *BMC Neuroscience*, vol. 13, p. 11, 2012.

[25] J. Wang, D. Gallagher, L. M. DeVito et al., "Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation," *Cell Stem Cell*, vol. 11, no. 1, pp. 23–35, 2012.

[26] M. Y. El-Mir, D. Detaille, G. R-Villanueva et al., "Neuroprotective role of antidiabetic drug metformin against apoptotic cell death in primary cortical neurons," *Journal of Molecular Neuroscience*, vol. 34, no. 1, pp. 77–87, 2008.

[27] S. P. Patil, P. D. Jain, P. J. Ghumatkar, R. Tambe, and S. Sathaye, "Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice," *Neuroscience*, vol. 277, pp. 747–754, 2014.

[28] A. Taylor, A. H. Westveld, M. Szkudlinska et al., "The use of metformin is associated with decreased lumbar radiculopathy pain," *Journal of Pain Research*, vol. 6, pp. 755–763, 2013.

[29] J. Ma, H. Yu, J. Liu, Y. Chen, Q. Wang, and L. Xiang, "Metformin attenuates hyperalgesia and allodynia in rats with painful diabetic neuropathy induced by streptozotocin," *European Journal of Pharmacology*, vol. 764, pp. 599–606, 2015.

[30] S. K. Roy Chowdhury, D. R. Smith, A. Saleh et al., "Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes," *Brain*, vol. 135, Part 6, pp. 1751–1766, 2012.

[31] A. L. Bjorke Monsen and P. M. Ueland, "Homocysteine and methylmalonic acid in diagnosis and risk assessment from

infancy to adolescence," *The American Journal of Clinical Nutrition*, vol. 78, no. 1, pp. 7–21, 2003.

[32] R. Obeid, "Metformin causing vitamin B12 deficiency: a guilty verdict without sufficient evidence," *Diabetes Care*, vol. 37, no. 2, pp. e22-e23, 2014.

[33] L. Reinstatler, Y. P. Qi, R. S. Williamson, J. V. Garn, and G. P. Oakley Jr., "Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: the National Health and Nutrition Examination Survey, 1999–2006," *Diabetes Care*, vol. 35, no. 2, pp. 327–333, 2012.

[34] E. Greibe, B. Trolle, M. V. Bor, F. F. Lauszus, and E. Nexo, "Metformin lowers serum cobalamin without changing other markers of cobalamin status: a study on women with polycystic ovary syndrome," *Nutrients*, vol. 5, no. 7, pp. 2475–2482, 2013.

[35] E. Greibe, J. W. Miller, S. H. Foutouhi, R. Green, and E. Nexo, "Metformin increases liver accumulation of vitamin B12-an experimental study in rats," *Biochimie*, vol. 95, no. 5, pp. 1062–1065, 2013.

[36] M. G. Wulffele, A. Kooy, P. Lehert et al., "Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial," *Journal of Internal Medicine*, vol. 254, no. 5, pp. 455–463, 2003.

[37] Y. Sato, K. Ouchi, Y. Funase, K. Yamauchi, and T. Aizawa, "Relationship between metformin use, vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes," *Endocrine Journal*, vol. 60, no. 12, pp. 1275–1280, 2013.

[38] E. K. Hoogeveen, P. J. Kostense, C. Jakobs, L. M. Bouter, R. J. Heine, and C. D. Stehouwer, "Does metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus?," *Journal of Internal Medicine*, vol. 242, no. 5, pp. 389–394, 1997.

[39] S. P. Stabler, R. H. Allen, L. P. Fried et al., "Racial differences in prevalence of cobalamin and folate deficiencies in disabled elderly women," *The American Journal of Clinical Nutrition*, vol. 70, no. 5, pp. 911–919, 1999.

[40] S. Saxena and R. Carmel, "Racial differences in vitamin B12 levels in the United States," *American Journal of Clinical Pathology*, vol. 88, no. 1, pp. 95–97, 1987.

[41] H. G. Kwee, H. S. Bowman, and L. W. Wells, "A racial difference in serum vitamin B12 levels," *Journal of Nuclear Medicine*, vol. 26, no. 7, pp. 790–792, 1985.

[42] F. Fernandes-Costa and J. Metz, "A comparison of serum transcobalamin levels in white and black subjects," *The American Journal of Clinical Nutrition*, vol. 35, no. 1, pp. 83–86, 1982.