Journal of Diabetes Research

Monitoring and Intervention Methods for the Management of Type 2 Diabetes

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

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Research Article

Ventricular Dysfunction in Obese and Nonobese Rats with Metabolic Syndrome

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Obesity and dyslipidemias are both signs of metabolic syndrome, usually associated with ventricular arrhythmias. Here, we tried to identify cardiac electrical alteration and biomarkers in nonobese rats with metabolic syndrome (MetS), and these findings might lead to more lethal arrhythmias than obese animals. The MetS model was developed in Wistar rats with high-sucrose diet (20%), and after twenty-eight weeks were obtained two subgroups: obese (OMetS) and nonobese (NOMetS). The electrocardiogram was used to measure the ventricular arrhythmias and changes in the heart rate variability. Also, we measured ventricular hypertrophy and its relationship with electrical activity alterations of both ventricles, using microelectrode and voltage clamp techniques. Also, we observed alterations in the contraction force of ventricles where a transducer was used to record mechanical and electrical papillary muscle, simultaneously. Despite both subgroups presenting long QT syndrome (0.66 ± 0.05 and 0.66 ± 0.07 ms with respect to the control 0.55 ± 0.1 ms), the changes in the heart rate variability were present only in OMetS, while the NOMetS subgroup presented changes in QT interval variability (NOMetS SD = 1.8, SD 2 = 2.8; SD1/SD2 = 0.75). Also, the NOMetS revealed tachycardia (10%; $p < 0.05$) with changes in action potential duration (63% in the right papillary and 50% in the left papillary) in the ventricular papillary which are correlated with certain alterations in the potassium currents and the force of contraction. The OMetS showed an increase in action potential duration and the force of contraction in both ventricles, which are explained as bradycardia. Our results revealed lethal arrhythmias in both MetS subgroups, irrespectively of the presence of obesity. Consequently, the NOMetS showed mechanical-electrical alterations regarding ventricle hypertrophy that should be at the NOMetS, leading to an increase of CV mortality.

1. Introduction

The MetS is known as a cluster of risk factors (impaired fasting glucose, insulin resistance, hypertension, dyslipidemias, and central obesity) [1–3] for type 2 diabetes mellitus and cardiovascular diseases, which occur together more often than by chance alone [4], that are associated with excess morbidity or/and mortality in humans [1, 5].

Obesity is a global epidemic for children [6] and adults, increasing the risk for cardiovascular morbidity and mortality, and the fact that obese along with overweight people are more prone to develop hypertension, hyperinsulinemia, dyslipidemias, and glucose homeostasis alteration [7–9].

The risk of development of cardiovascular diseases such as congestive heart failure, myocardial infarction, atrial fibrillation, and dilated cardiomyopathy is also increased [10, 11].

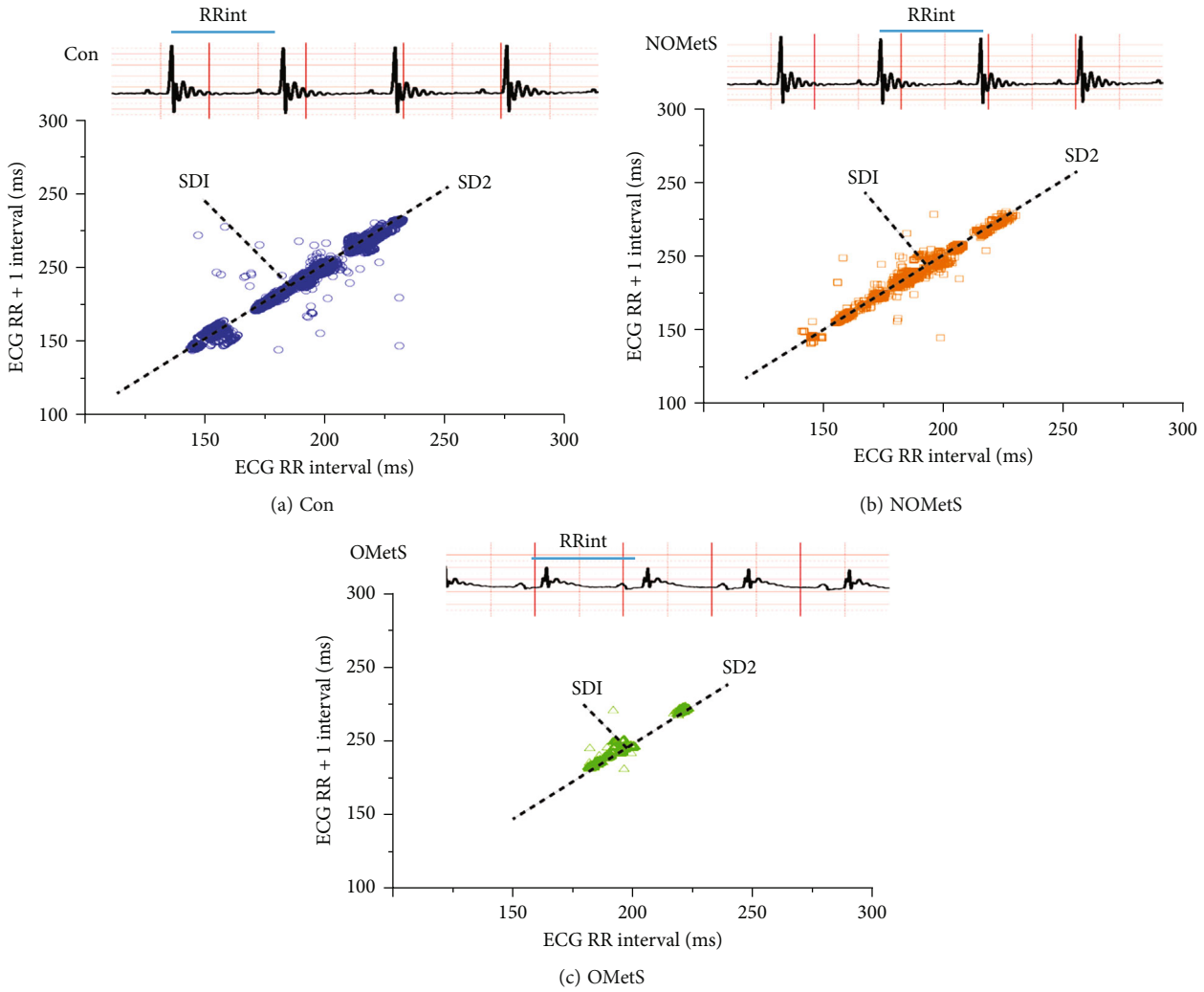

Figure 1

Generally, cardiomyopathy is commonly associated with electrocardiographic abnormalities [12], including impaired cardiac contractile [13]. Moreover, the long QT syndrome is related to alterations in ventricular electrical repolarization [14], and QT interval dispersion of ECG [15]. The alterations in beat-to-beat cardiac are clinically associated with cardiovascular risks [16]. One way of prognosis and diagnosis of cardiac diseases is to determine the heart rate variability (HRV) with the interval electrocardiogram (ECG) analysis [17]. On the other hand, the HRV is a noninvasive method that allows prognostic left ventricular dysfunction [18] and prolongation of QT intervals which are considered a factor risk to ventricular arrhythmias like Torsade de Pointes [17, 18]. Moreover, HRV is used to assess heart's health status at the prognosis, diagnosis of diabetes mellitus, hypertension, MetS, and obesity [19–21]. The relationship between lethal ventricular arrhythmias, cardiomyopathies, and LV dysfunction (associated with dyslipidemias and visceral fat) has been poorly identified and discussed in current literature [22, 23].

In this research, the MetS model gives us some information about the cardiomyopathies and lethal arrhythmias in obese and nonobese animal models. Our hypotheses is that the NOMetS could show higher probability of more lethal arrhythmias than in OMetS, the NOMetS is not usually considered as a cardiac critical problem due to clinically confuse it the obesity is condition to dyslipidemias and cardiac electrical alterations. Consequently, we suggest that the quantification of blood plasma biochemistry and electrocardiogram analysis allowed us to know more about arrhythmias more than waist measure in subjects with MetS.

2. Material and Methods

2.1. Animal Model. All animal procedures were performed according to the International Guiding Principles for Biomedical Research Involving Animals Council for the International Organization of Medical Science 2010, including the Animal Ethics Committee of the Internal Council and the Animal Care Committee of the Instituto de Fisiología Celular at the Universidad Nacional Autónoma de México. Twenty young adult male Wistar rodents (250-280 g) were kept in a 12 h light/dark cycle. MetS was induced by feeding with standard rat chow, composed by laboratory rodent diet (LABDIET 5001) with 28.5% of protein, 13.5% of fat, and

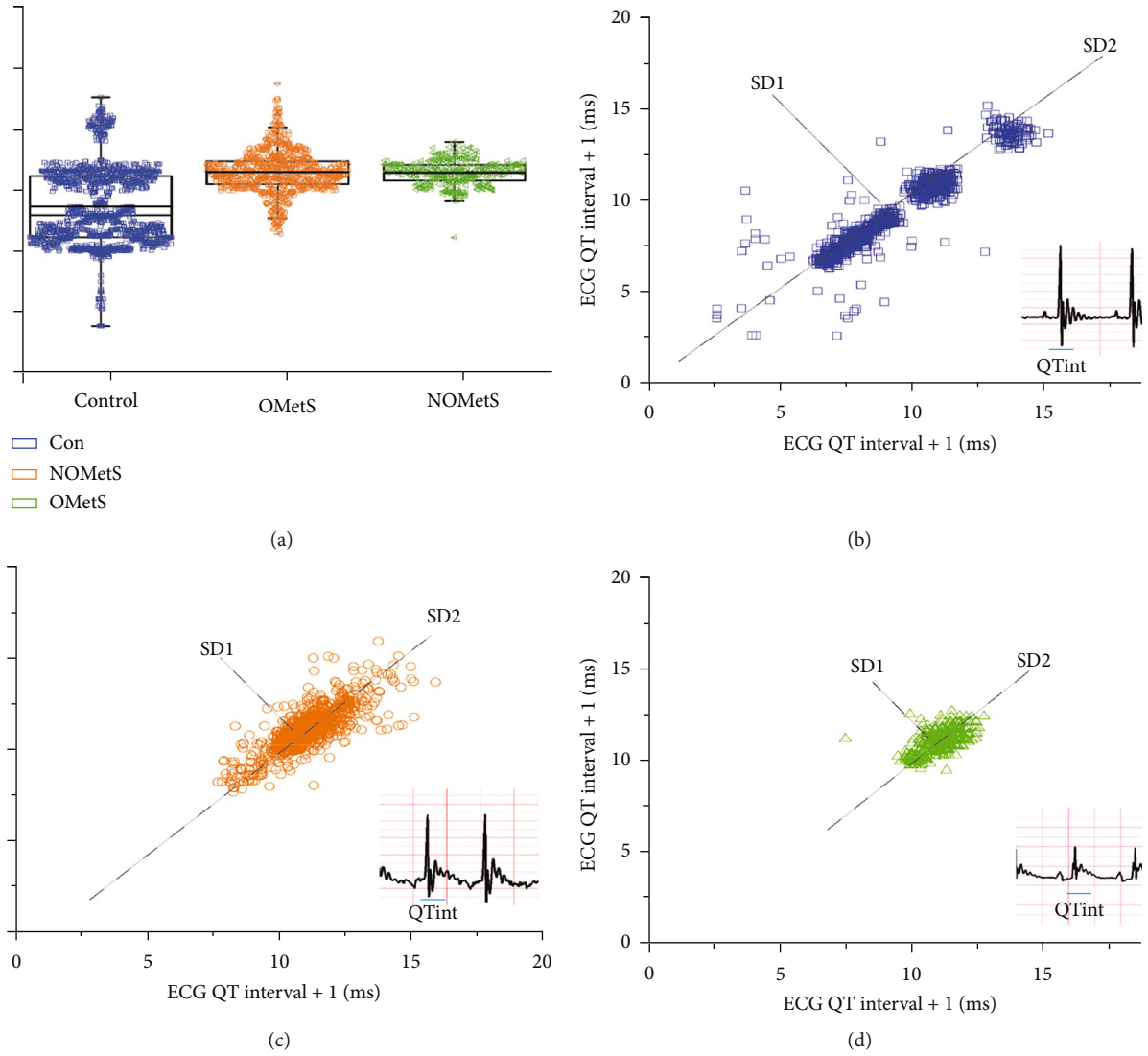

Figure 2

58% of carbohydrates [24]. Ad libitum tap water was provided to the control group and 20% (*w*/*v*) of sucrose solution to the experimental group for a twenty-eight-week treatment. The animals were anesthetized with an intraperitoneal sodium pentobarbital injection (40 mg/kg) [25]. The following measures were taken by abdominal circumference, epididymal fat, body weight, and body length. After, some blood peripancreatic and epididymal fat samples were taken, and then, the heart was removed. Finally, the animals were euthanized by cervical dislocation.

2.2. Biochemical Measurements. Peripheral venous blood samples were used to quantify glucose, insulin, triglycerides, and total cholesterol using standard laboratory techniques in 8-hour-fasted rats [26]. The insulin resistance (IR) was quantified via the homeostasis model assessment of HOMA IR = serum insulin (uUI/ml) ∗ (blood glucose (mmol/L)/22.5 [27, 28].

2.3. Cardiac Function. The ECG was performed on anesthetized (0.5 mg/0.2 mg ketamine-xylazine/kg weight) rats. Bipolar ECGs were recorded using subcutaneous needle electrodes with Lead-I configuration; that signal was amplified in 700x and digitalized and captured to 10 kHz frequency for thirty minutes [25]. Data was stored in a personal computer and analyzed off-line, using Clampfit (molecular devices). All rats were continuously monitored to guarantee the right ventilation and temperature.

2.4. Ventricular Function. The heart was quickly removed and placed in a retrograde perfusion system, with Tyrode's solution at 36°C to wash the heart. In addition, excitation-contraction coupling was measured in the ventricular left and right papillary muscle isolates placed in a chamber to record simultaneously contraction force and action potential perfused with Tyrode's solution at 36°C, gassed with carbogen.

According to Frank-Starling's law [29], the contraction force was performed in the papillary muscles at Maxime

Table 1: Metabolic Characterization of animal model.

	Control ($n = 20$)	NOMeS ($n = 14$)	OMeS ($n = 6$)
Morphometric parameters			
Length (cm)	25.6 ± 0.2	25.7 ± 0.2	27.6 ± 0.5*
Waist (cm)	23.4 ± 0.7	24 ± 0.7	27.8 ± 0.6*
Weight (g)	518 ± 12	529 ± 5	725 ± 22*†
Epididymal fat (g)	4.6 ± 0.4	7.9 ± 0.5*	16.3 ± 2*†
Peripancreatic fat (g)	1.97 ± 0.5	2.1 ± 0.4	3.9 ± 0.8*†
BMI ($kg{\cdot}m^{-3}$)	31 ± 0.6	31 ± 0.6	36 ± 0.1*
Clinical biochemistry values			
Glucose ($mg{\cdot}dl^{-1}$)	90 ± 2	104 ± 3*	112 ± 7*
Urea	39 ± 10.6	29 ± 9*	33 ± 8*
Triglycerides ($mg{\cdot}dl^{-1}$)	75 ± 7	153 ± 33*	116 ± 22*
Insulin ($pM{\cdot}l^{-1}$)	26 ± 0.02	27 ± 0.03	22 ± 0.04
HDL-c ($mg{\cdot}dl^{-1}$)	15 ± 2	31 ± 7*	23 ± 5*†
LDL-c ($mg{\cdot}dl^{-1}$)	21 ± 4	47 ± 14*	23 ± 5†
HOMA-IR	0.8	1*	0.9*

Mean ± SD;$p \leq 0.05$, *vs control, †vs NOMetS.

longitude (Lmax). The action potential was recorded using the sharp microelectrodes of borosilicate (filled with 3 M KCl with a resistance of 25–35 MΩ). The signal was amplified with a WPI Duo 776 electrometer, digitalized (SCB-68 Quick references label, National Instruments), and analyzed using ClampFit (molecular devices) and Origin 7.0 (Southampton). The characterization of the action potential was measured with amplitude and the APD at 30 and 90% of the repolarization. The excitation-contraction coupling phenom was measured, using the time between the maximum voltage of the action potential and the maximum force of the contraction, including the delay between the start of the action potential with the start of the force of contraction [30].

2.5. Isolated Ventricular Cells from the Heart. Rat heart was cannulated through the aorta and perfused, according to the Langendorff method. Consequently, the heart was perfused in a recirculation mode for 8 minutes with collagenase, 28 mg/50 ml (Sigma Aldrich) and protease 1 mg/50 ml in solution in a Ca^{2+} free buffer. Finally, papillary muscle was mechanically dissociated in Kraft-Brüeh (KB) solution [31].

2.6. Alterations in Ventricular Electrical Activity. The patch-clamp technique was applied in the whole-cell configuration to record total currents; the patch pipettes had a resistance between 2 and 4 MOhm. The signal was captured at 5.4 kHz and amplified, digitalized (Heka), and stored on a personal computer. The cardiomyocytes were placed in a perfusion chamber in an inverted microscope (Nikon). Only Ca^{2+} tolerant rod-shaped right and left papillary cells were selected for this study.

Table 2: Heart rate variability.

	CON ($n = 20$)	NOMetS ($n = 14$)	OMetS ($n = 6$)
ECG RR interval			
SD1	1.03	1.04	0.51*†
SD2	1.31	1.53	0.74*†
SD1/SD2	0.79	0.68*	0.69*
ECG QT interval			
SD1	1.14	1.8*	1.5
SD2	1.5	2.8*	1.8
SD1/SD2	0.88	0.75*	0.89

Mean ± SD;$p \leq 0.05$, *vs control, †vs NOMetS.

2.7. Potassium Currents. The potassium current (I k) was elicited from a holding potential of -80 mV by a square voltage pulse of -40 mv at 5 ms, and depolarizing pulses to membrane potential from -40 mV to 50 mV for 500 ms were applied with 10 mV increments at 2 seconds intervals [31].

2.8. Solutions. The Clampex program of the pClamp software controlled the current- and voltage-clamp experimental protocols. The solution using during voltage clamp experiments was a normal external solution (in mM NaCl, 136; KCl, 5.4; $MgCl_2$, 1; HEPES-Na, 10; $CaCl_2$, 1.8; dextrose, 11; pH adjusted to 7.4 with NaOH). The potassium currents were recorded with extracellular solution that containing (in mM) choline chloride, 136; $MgCl_2$, 1; HEPES-K+, 4; HEPES, 6; N-Methyl-D-glucamine, 6; $CaCl_2$, 0.1; $CoCl_2$, 0.5; and dextrose, 11; and it was adjusted with KOH to pH 7.4. The KB solution Isenberg & Klockner 1982 is the following composition (in mM): taurine, 10; glutamic acid, 70; creatine, 0.5; succinic acid, 5; dextrose, 10; KH2PO_4, 10; KCl, 20; HEPES-K+, 10; and EGTA-K+, to adjust the pH to 7.4 with KOH [31]. The solutions to patch pipetes or internal solutions had the following composition (mM): 80 potassium aspartate, 10 KH_2PO_4, 1 $MgSO_4$,7 H_2O, 40 KCl, 10 HEPES, 10 EGTA, 3 Na_2ATP, and 0.2 NaGTP; pH was adjusted to 7.3 with KOH.

2.9. Cardiac Histopathologic Alterations. The ventricular muscle was embedded in paraffin for 5 μm, and cuts were done from the base to apex. After deparaffinization, the sections were stained with hematoxylin-eosin. The tissue samples were examined under a 40x microscope, and it was semiquantitatively analyzed with the Image J program.

2.10. Heart Rhythm Alterations. For analysis of the heart rate variability, we used the Poincare plot, in which the time series of ECG Intervals were plotted against the next value in a Cartesian coordinate system. In this research, the Poincare plots were constructed with RR and QT intervals of the recorded ECG, of all rat groups. The heart rate variability was quantified using the means of parameters SD1, SD2, and SD1/SD1 in each condition research [19].

2.11. Statistical Analysis. All data were analyzed using descriptive statics and expressed as means ± SD (Origin Pro 2017 and Clampfit 10.7). If the results presented a normal distribution and equal variance, Student's unpaired *t*

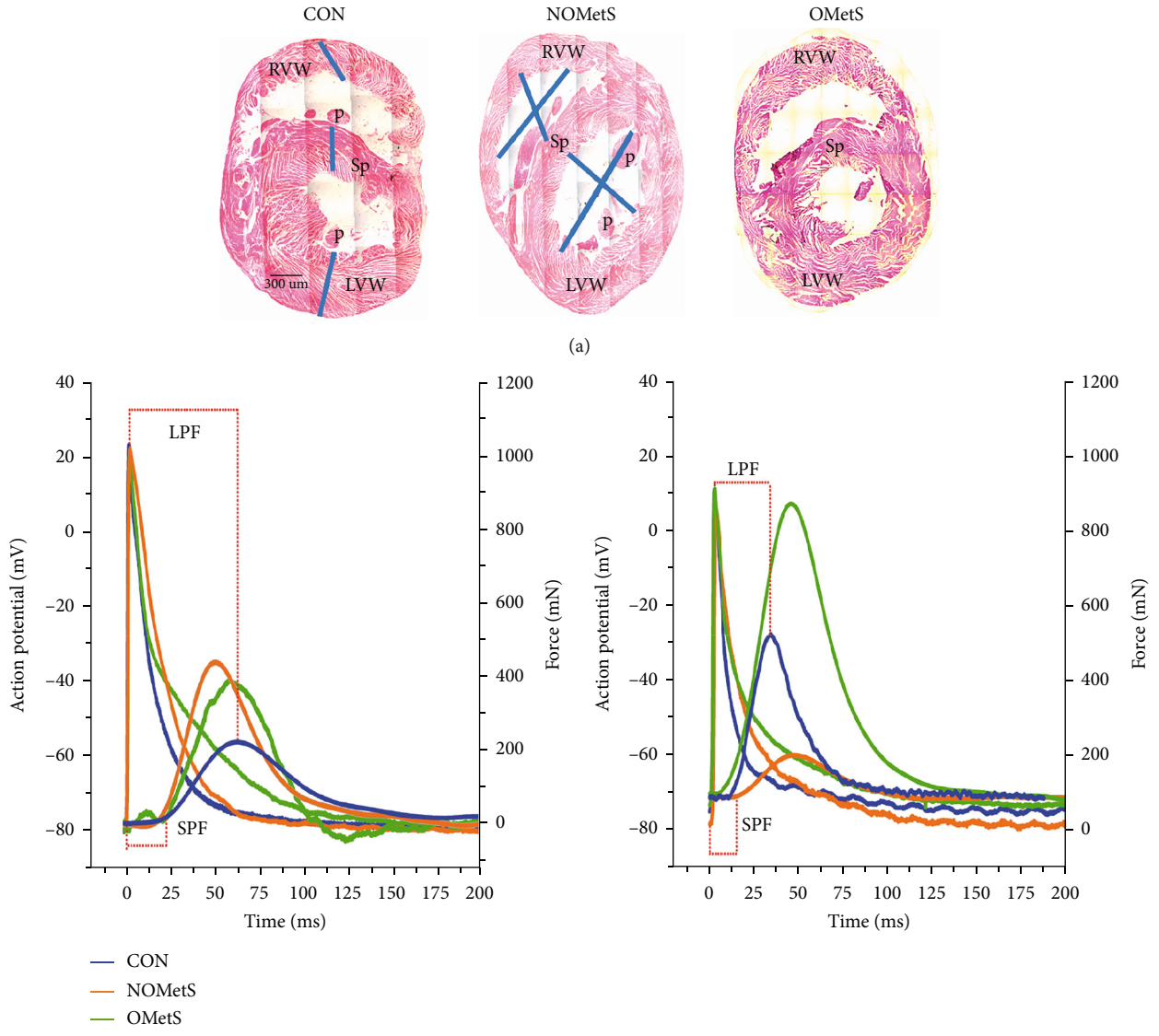

Figure 3

-test or one-way ANOVA was used by Dunnett's post hoc test. If the data presented a no-normal distribution or equal variance, the Mann–Whitney U test or Kruskal–Wallis test was performed. It was assumed to be a significant change if $p < 0.05$.

3. Results

3.1. Animal Model with Different Alterations in the Obesity of Metabolic Syndrome Profile. In the rodents, the high-sucrose diet by 24 weeks, developed MetS with three of the five metabolic (see Table 1) alterations as described by literature. In this model, seventy percent of those rats ($n = 14$) showed MetS with only a 2% body weight gain that is NOMetS, and this subgroup had the same abdominal circumference compared to the control group. However, these rats had 70% more epididymal fat than the control group. The remaining rats with MetS ($n = 6$) showed a 40% body-weight gain, and OMetS were named as subgroups.

In the abdominal circumference and epididymis fat, a significant increase of 18% was detected in the NOMetS subgroup, while the value in the OMetS subgroup was 300%, compared to control. All Wistar rats with MetS presented alterations of lipid metabolism. Although, the data of NOMetS rats showed a significant increase in triglycerides (TG) levels compared to OMetS, 153 mg/dl and 116 mg/dl, as well as in c-LDL 47 mg/dl and 23 mg/dl respectively, see Table 1.

3.2. Effect of Obesity Profile on Heart Rhythm. The OMetS animal model presented a decrease of 308 ± 0.9 bpm in heart rate, while in the NOMetS subgroup, there was an increase of 330 ± 1 bpm compared to the control group which had 317 ± 0.5 bpm. Interestingly, the effect of obesity profile on the electrocardiogram record, in both cases NOMetS and

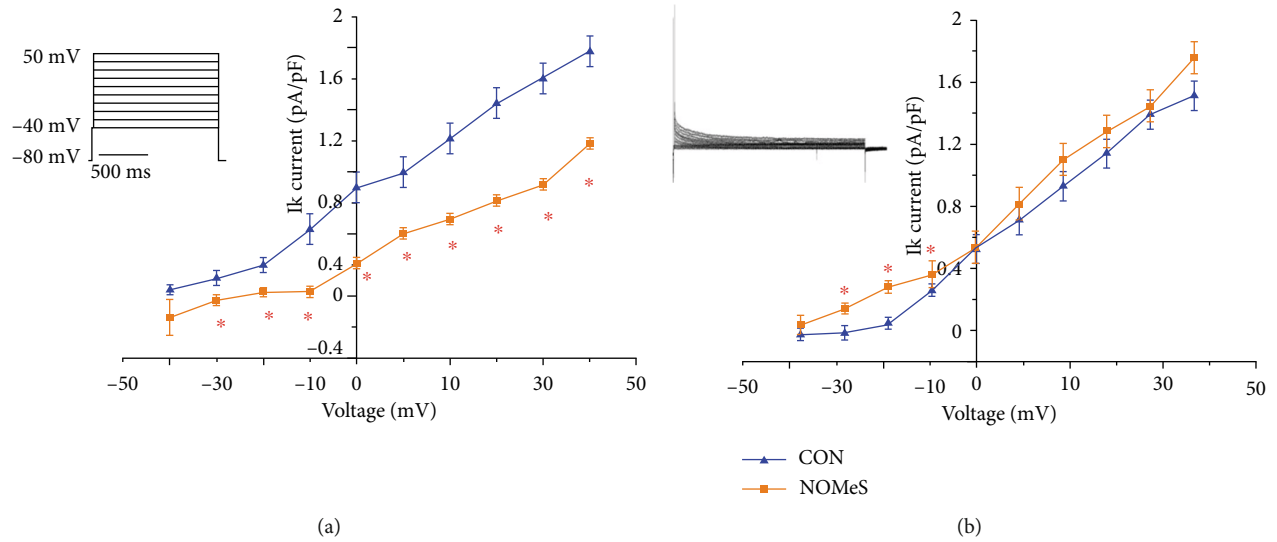

Figure 4

Table 3: Electrical and mechanical ventricular function.

	Control ($n = 20$)		NOMetS ($n = 14$)		OMetS ($n = 6$)	
	RPM	LPM	RPM	LPM	RPM	LPM
Amplitude (mV)	96 ± 2.4	87 ± 2	97 ± 1.6	91 ± 9	93 ± 2	79 ± 2*
APD 30% (ms)	6 ± 0.4	8 ± 0.8	6 ± 0.4	5.8 ± 0.4	7 ± 0.3*	7.4 ± 0.5
APD 90% (ms)	41 ± 1.8	38 ± 3	67 ± 4.4*	57 ± 2*	47 ± 2.7*†	70 ± 5.2*
LPF (ms)	57	73	56	50*	46*	49*
SPF (ms)	14	17	12	15*	12	10*
Force (mN)	311	477†	519*	205*	615*	839*†

APD: action potential duration; LPM: left papillary muscle; RPM: right papillary muscle; LPF: latency period force; SPF: start period force. Mean ± SD;$p \leq 0.05$, *vs control, †vs NOMetS.

OMetS, was observed as in long QT syndrome; hence, the QTc values were longer 0.66 ± 0.05 and 0.66 ± 0.07 ms, respectively, than the control group, 0.55 ± 0.1 ms (Figures 1 and 2).

3.3. The Heart Rate Variability by Obesity. The cardiac health was evaluated with heart rate variability using Poincare plots, and as expected, the control group revealed an ellipse shape behavior (Figure 1(a)), with a variability of SD1 = 1.03, SD2 = 1.31, and index SD1/SD2 = 0.79 see Table 2. Together, in the data of heart rate variability of NOMetS rats, the ellipse shape is like the control group. Above the line identity of SD2 are registered the 80% of RR intervals, and SD1/SD2 index is decreased (Figure 1(b)). In the plot of OMetS subgroup, a decrease was observed in the heart rate variability in the SD1, SD2, and index SD1/SD2 biomarkers (see Figure 1(c)) compared with the control set (Table 2).

The beat-to-beat variations are also extremely sensitive to small fluctuations in several levels (Figure 2(a)). Thus, the variability in the QT intervals was quantified, which tends to behave in typical ellipse shape in the Poincare plots of RR interval (Figures 2(b)–2(d)). The quantitative analysis of the QT interval showed changes in the three biomarkers, only in the OMetS subgroup (Table 2). The variability of the NOMetS subgroup had an increase in SD1 = 1.8, SD2 = 2.8, and the SD1/SD2 = 0.75 index (see Figure 2(c) and Table 2), meanwhile in the OMetS heart rate variability also increased, but not showing significant differences between aggregate sets (see Figure 2(d)).

3.4. Alterations in Ventricular Function Related to Obesity. Our results indicated that obesity profile altered the ventricular function, which was measured with excitation-contraction (E-C) coupling phenom. In the OMetS subgroup, the force contraction and action potential duration (APD) were higher in the left papillary ventricle than the right one. Consequently, E-C coupling was the desired outcome for ventricular function in the control rats (see Figure 3(b), Table 3). The NOMetS model showed alterations in the electrical and mechanical mechanisms of the E-C coupling. The contraction force increased 66 percent in the right and decreased 57 percent in the left papillary muscle while in relationship with the APD had an increased 61 percent and decreased 40 percent, respectively.

However, the left papillary muscle showed a decoupling which was related to the reduction in the latency period

(LPF) and the start period (SPF) of the contraction force (see Figure 3(b) and Table 3). The OMetS subgroup also presented an increase in the mechanisms of E-C coupling, showing a decoupled left papillary muscle of 30% and 40% in LPF and SPF, respectively, (Figures 3(a) and 3(b) and Table 3). The force was almost 2-fold than the control group.

High-sucrose diet-induced obesity is associated with hypertrophic cardiomyopathy [32]. The Hematoxylin-eosin staining (Figure 3(a)) suggests a ventricular hypertrophy in the NOMetS subgroup. The ventricles of the heart had a perimeter of 7.5 ± 0.5 mm, 6.5 ± 0.3 mm, and 4.6 ± 0.3 mm for NOMetS, OMetS, and control, respectively (Figure 3(a)). The MetS do not alter the ventricular-wall size, and the data showed 3.3 ± 1, 2.8 ± 0.8 and 3.1 mm for NOMetS, OMetS, and control. Of note, there was no significant difference in heart weight, and the data were 2.1, 2.3 and 1.98 g for NOMetS, OMets, and control, respectively.

3.5. The Obesity Profile Is Associated with Alterations in the Activity of Potassium Currents. The increase in APD of ventricles in NOMetS rats allowed indirectly induced changes in the potassium total currents. For this reason, we recorded potassium currents in isolated myocytes and the data showed a decrease in the amplitude of potassium current (Ito) in the right papillary muscle [33] (Figure 4(a)). Also, we measured the potassium current in myocytes of left papillary muscle; these currents showed an increase at only negative voltages (Figure 4(b)). Furthermore, the amplitude of each current component, obtained from the fitting, was normalized to cell capacitance to compare current densities from cells of different sizes. The NOMetS subgroup did not affect the resting membrane potential of ventricular papillary muscle (see Figure 4).

4. Discussion and Conclusions

This study proposed to identify cardiac electrical disease by electrical and metabolic biomarkers, using heart rate variability of RR, QT intervals, and blood plasma biochemistry to improve the diagnostic and prognosis of cardiometabolic diseases. Recent evidence suggests that genetic and environmental factors contribute to the MetS development; such factors are high-carbohydrates, high-fat diets, and lack of physical activity [24]. These factors promote insulin resistance, impaired fasting glucose, alteration of lipids metabolism, and a chronic inflammatory state and visceral obesity [34]. In this study, *Wistar* rats are not genetically susceptible to develop obesity [24]; though, a high-sucrose diet in drinking water like environmental factors produced MetS with resistance to insulin and dyslipidemia and impaired fasting glucose after 2 months of treatment [24]. In this model, after this twenty-eight-week high-sucrose diet, the rats presented MetS with and without obesity [35]. During a period of high-sucrose diet intake in the animal model, the liver tissue converted glucose into fatty acids and stored them in the adipose tissue [8, 36]. Consequently, in this study, both subgroups had increased in the distribution of epididymal fat weight by 0.7 and 2.5 times for NOMetS and OMetS, respectively (see Table 1). The NOMets rats were showing insulin resistance, and this data indicated in the rats a behavior of excess nutrients in their metabolism. This suggests an imbalance in the storage and synthesis of lipids in the liver, and the TG concentration in plasma is higher than control and OMetS [37]. Also, the NOMetS group had c-LDL and c-HDL plasma concentration higher than OMetS, and both proteins respond to the mechanism of high blood lipid metabolism and low reservoir in fat tissue [5]. These results explain that the rats with obesity only had the mechanism to reverse cholesterol transport [5].

The excess of fatty acids in plasma and insulin resistance has been proposed as a key driver for accumulation in obese individuals [2, 38]. In the MetS, the overweight model or OMetS under the higher-sucrose diet was enough to have an excess of triglycerides in plasma but c-HDL and insulin, similarly, in the control set. Furthermore, this model had alterations in HOMA-IR index [27] and the peripancreatic and epididymal fat increasing in 3 times and 85%, respectively, more than the control group. In general, obesity has been associated with the left ventricular dysfunction and cardiomyopathies by ventricular hypertrophy [39, 40].

However, the OMetS subjects only showed the electromechanical alterations in the function of both ventricles due to obesity. The outcomes revealed long QT syndrome related to alterations in the duration of AP ventricular and changes in the dispersion in RR. Also, the OMetS subgroup had alterations in the function of both ventricles (Figures 3(a) and 3(b). Table 3) and bradycardia, and the ventricle force augmented without alterations in ventricular morphology (Figure 3(b)).

On the other hand, the NOMetS subgroup presented syndrome long-QT with an increase in APD 90% in both chambers even without obesity, and it is worth mentioning that the clinical biomarkers of dyslipidemia and obesity were higher than that of the obese subgroup. The beginning of the QRS complex has been defined by the onset of ventricular electrical activity; the NOMetS. Also, this subgroup showed dispersion in QT intervals. This is related to susceptibility to reentry ventricular tachyarrhythmias and, revealed dysfunction in both ventricles, the contraction force was reduced in left papillary muscle, and the right papillary was enhanced, these alterations are caused by the changes to the electrical activity. Furthermore, the dispersion in electrical activity, at the start, the repolarization of action potential and the electro-mechanical changes allowed us to provoke the long QT syndrome in these animals [41].

On the other hand, the excitation-contraction decoupling was associated with hypertrophy [42]. In this model, the animals were fed with high sucrose diet and only the NOMetS subgroup presented eccentric hypertrophy associated with over ventricular dysfunction and fat tissue (see Table 1). These were related to the release of catecholamines in the heart [43]. Additionally, in NOMetS subgroup, tachycardia was observed, which is related to a parasympathetic innervation increase of pacemaker tissue [25].

The events that occur in E-C coupling of cardiac muscle depend on calcium concentration and the duration of action potential [44]. The lethal arrhythmia as the long QTc syndrome is correlated with APD prolongation, and the main cause is the reduction in the densities of K repolarizing

currents [45]. In the NOMetS animals, the alterations in the start and latency periods are presented, and an increase of APD associated with IK current decrease in the right ventricle [46] (see Figure 4).

In humans, the common central mechanisms modulated by both sympathetic and parasympathetic cardiovascular modulation were measured with HRV [47]. This biomarker is used to prognostic and diagnose health of the heart in metabolic diseases [48]. In this work, the HRV was impaired according to the levels of body fat, and the OMetS animals showed a decreased standard deviation in both mechanisms of modulation of the autonomic nervous system. The NOMetS animals presented an increase in the variability, only in QT intervals and the participation of the parasympathetic system (Figure 1(b)), raising the likelihood of lethal arrhythmias as long QT (Figure 2(a)), and the increase of modulation by the sympathetic and parasympathetic system on electrical properties of the ventricle (Figure 2(c)).

In this study, the c-HDL is high in both subgroups in which the protection was conferred to cardiovascular diseases [49] and attached to atherosclerosis [26]. However, both subgroups had high probability for lethal arrhythmias due to long QT syndrome and dyslipidemias (Table 1).

Additionally, the ventricular ejection force was measured with strain papillary muscle [42]; this data allowed indirectly to quantify the peripheral blood flow and ventricular chamber volumes [50]. The outcomes of the E-C coupling suggest that the left and right ventricles reflected alterations like cardiomyopathy in both MetS subgroups. The MetS with obesity did not alter morphologically the ventricles (Figure 3(a) and Table 3). Meanwhile, the H-E staining data revealed that the nonobese group exhibited a dilated cardiomyopathy [51]. The data showed the ventricular arrhythmias were produced by the MetS.

Finally, the type of ventricular arrhythmia depends on whether obesity is present or not. The outcomes suggest that the MetS without obesity promotes a poor prognosis of cardiomyopathies, and these alterations could be measured for prognostic and diagnostic purposes for the heart rate variability of ECG. The OMetS rats presented alterations of HRV with decreasing of asymmetry Poincare of RR interval, associated with increased contraction force in both ventricles and electrical alterations. However, the NOMetS animals had higher alterations in the metabolism, and the ventricular electrical activity are strongly correlated to long-QT syndrome, QT variability, and hypertrophic ventricular.

4.1. Statement Experimental Protocols. All animal procedures were performed in accordance with "International Guiding Principles for Biomedical Research Involving Animals", Council for International Organization of Medical science 2010. The protocol was approved by the ethics committee (CICCUAL-PROYECTO-00365) of the Benemérita Universidad Autónoma de Puebla.

Data Availability

The data sets used and/or analyzed during the current study are available from all authors on reasonable request.

Conflicts of Interest

The authors declare no competing interests.

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Research Article

Response to 1-Year Fixed-Regimen Bevacizumab Therapy in Treatment-Naïve DME Patients: Assessment by OCT Angiography

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Purpose. To evaluate the effectiveness of intravitreal bevacizumab treatment in patients with diabetic macular edema (DME) by assessing retinal changes using optical coherence tomography angiography (OCT-A). *Methods.* This prospective study was performed in patients with treatment-naïve DME. The eyes of patients were imaged using a swept-source OCT system with a scan area of 6×6 mm. The DME patients with a central macular thickness (CMT) of $\geq 300\,\mu$m received nine bevacizumab injections within 12 months. The demographic, systemic, and ocular parameters, including the best-corrected visual acuity (BCVA), CMT, microaneurysm (MA) count, and foveal avascular zone (FAZ) area in both superficial capillary plexus (SCP) and deep capillary plexus (DCP), as well as vessel density in SCP, were assessed in the patients. In addition, the response (good or poor) of the DME eyes to bevacizumab treatment and the final visual acuity (BCVA of 75 letters) were analyzed. *Results.* Seventy-seven eyes of DME patients were subjected to the final analysis. Bevacizumab treatment reduced CMT from 425.06 μm (±77.15) to 350.25 μm (±82.04) and improved BCVA by about 8.61 letters (from 64.73 to 73.34) in the patients. The mean number of MAs in SCP decreased from 3.51 ± 2.07 to 2.31 ± 1.15 ($p < 0.001$) and in DCP from 17.12 ± 11.56 to 12.21 ± 6.99 ($p < 0.001$), whereas the area of FAZ increased in SCP from 328.22 ± 131.38 to 399.70 ± 156.98 ($p < 0.001$) and in DCP from 571.13 ± 396.01 to 665.89 ± 412.77 ($p = 0.001$). The final BCVA letter score and CMT were statistically significant in both poor and good responders, as well as in BCVA < 75 and BCVA ≥ 75 groups. *Conclusion.* The fixed-regimen intravitreal bevacizumab therapy was effective in treating DME. Apart from noninvasive visualization of microvascular damage, OCT-A showed limited usefulness in predicting treatment response. Although the study showed that the number of MAs was significantly reduced during treatment, which is an OCT-A predictor of a good response to bevacizumab treatment at a 12-month visit, commonly observed artifacts may reduce the usefulness of OCT-A.

1. Introduction

Diabetic retinopathy (DR) is identified as one of the leading causes of preventable visual impairment and blindness worldwide [1–3]. It is estimated that the number of patients with diabetes mellitus will reach 429 million by 2030 [4] and increase further to 642 million by 2040 [5].

In the early stages of DR, microvascular damage, including the loss of pericytes and proliferation of endothelial cells, weakens the vascular walls, resulting in the formation of microaneurysms (MAs) and increasing the vascular permeability and pathologic neovascularization [6, 7]. The breakdown of the blood-retinal barrier caused by a high concentration of inflammatory mediators and the leakage of MAs lead to the development of diabetic macular edema (DME), which is identified as a leading cause of vision impairment in patients with diabetes mellitus type 2 [1, 8–11]. Because vascular endothelial growth factor (VEGF)

is the dominant factor of retinal vascular hyperpermeability, DME is mainly treated using anti-VEGF inhibitors [3, 9, 12, 13]. Bevacizumab is a monoclonal, humanized VEGF-inhibiting antibody which is a common, off-label medication used for the treatment of DME [14–16] [17]. Although it is not approved for ophthalmology [16, 17], bevacizumab is often used as a first-line treatment in many countries. As the efficacy and safety of this drug have been less documented than approved drugs, even small-scale prospective studies may broaden the knowledge about optimal treatment regimens with bevacizumab for DME.

Currently, fluorescein angiography (FA) is the diagnostic gold standard for investigating the stages of DR [18]. By contrast, optical coherence tomography angiography (OCT-A) allows depth-resolved visualization of each retinal capillary layer without the need for dye injection [1, 12, 19–22]. The main advantage of this technique is that it enables three-dimensional imaging of retinal layers (superficial capillary plexus (SCP) and deep capillary plexus (DCP)) [3, 23].

In diabetic patients, OCT-A can reveal the enlargement of foveal avascular zone (FAZ), abnormalities in capillary flow density, and MAs, larger nonperfused areas, and neovascularization compared with controls. Studies have demonstrated more severe microvascular damage in DCP than in SCP in patients with DR [6, 8, 12, 24]. However, OCT-A artifacts are common and often observed as motion or doubling artifacts in the deeper layers, due to shadows in moving blood cells in the overlying retinal vessels [18, 24, 25].

This study is aimed at determining whether OCT-A can be a valuable tool for monitoring treatment with VEGF inhibitors in patients with DME. In addition, it also attempted to analyze whether predictive factors can be distinguished among the OCT-A parameters and characterize the dynamics of changes in the macular vascular network during bevacizumab treatment.

2. Materials and Methods

This prospective study was conducted among patients recruited from the ophthalmological outpatient clinic of the Clinical Department of Ophthalmology at the Faculty of Medical Sciences in the Medical University of Silesia, during 2018–2020. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical University of Silesia (KNW/0022/KB1/126/I/18/19). All the included patients were clearly informed about the study, especially its purpose, protocol, and benefits as well as possible risks. Furthermore, written informed consent was obtained from all the participants.

The inclusion criteria for the study included the following: (1) patients with type 1 or type 2 diabetes mellitus, (2) age ≥ 18 years, (3) diagnosis of nonproliferative DR, (4) diagnosis of DME with a central macular thickness (CMT) of $\geq 300\,\mu$m, (5) naïve to intravitreal treatment, and (6) best-corrected visual acuity (BCVA) of 24–78 ETDRS (Early Treatment Diabetic Retinopathy Study) letters. The exclusion criteria were as follows: (1) history of any retinal surgery; (2) previous intravitreal injections of anti-VEGF agents or steroids; (3) macular, focal, or pan-retinal laser photocoagulation; (4) eye conditions that interfere with imaging and affect visual acuity (e.g., cataract and cornea abnormalities); (5) diagnosis of glaucoma; (6) presence of epiretinal membrane, vitreoretinal traction in the macula, or other types of maculopathy unrelated to diabetes mellitus (e.g., age-related macular degeneration); (7) proliferative DR; and (8) unwilling to cooperate with OCT-A imaging.

All the participants were initially interviewed and then examined during the routine ophthalmologic visit. The following data were collected from them: age, sex, height, weight, concomitant medications and duration of diabetes mellitus, concomitant systemic diseases (e.g., hypertension, history of heart incidents and stroke, and chronic kidney disease), and serum level of glycated hemoglobin (HbA1c).

The participants were treated with intravitreal injections of bevacizumab. Anti-VEGF inhibitors were administered by an ophthalmologist in the ophthalmological outpatient clinic of the Clinical Department of Ophthalmology at the Faculty of Medical Sciences in the Medical University of Silesia. In each studied eye, nine injections of 1.25 mg/0.05 ml bevacizumab (Avastin) were administered over 12 months. The first five injections were given every month, and the subsequent four injections were administered every 2 months.

Before every injection, the patients were subjected to BCVA test, slit-lamp examination, and OCT and OCT-A analyses. The pupils of patients' eyes were dilated with 1% tropicamide before the OCT imaging. OCT-A was performed using a swept-source OCT (SS-OCT) system (DRI OCT Triton; Topcon, Inc., Tokyo, Japan) with a wavelength of 1050 nm at a speed of 100,000 A-scans per second (each 512×512 mm) [1, 3, 8]. Two fovea-centered OCT-A scans were taken with an area of 6×6 mm at baseline (up to 4 weeks before the first injection), during every visit up to 4 hours before intravitreal bevacizumab injection and up to 4 weeks after the last anti-VEGF injection. Only one OCT-A scan of better quality was assessed for every visit. The built-in IMAGEnet6 software (version 1.26.16898) was used for automated layer segmentation of retinal vasculature (SCP and DCP) [1, 3, 8]. The following boundaries were defined in segmentation: for SCP—2.6 mm below the internal limiting membrane to 15.6 mm below the junction between the inner plexiform and the inner nuclear layers; for DCP—15.6 mm below the inner plexiform and the inner nuclear layers to 70.2 mm below them [3, 23]. All the images were analyzed by two separate readers, and if the automated positioning or segmentation was recognized as inaccurate, manual corrections such as centration or propriete delineation of layers of the scans were made. The FAZ profile in the SCP and DPC was manually outlined using the freehand selection tool and was calculated by the build-in Topcon software [26]. Automated analysis by the build-in software was made for density, but microaneurysms were counted manually.

OCT-A scans within the area of 6×6 mm were assessed in quantitative and qualitative analyses. Quantitative

analysis included the evaluation of vessel density in SCP, FAZ area, and the number of MAs in SCP and DCP [23]. The exclusion criteria for quantitative and qualitative analyses were as follows: (1) quality score of <40, (2) motion artifacts, (3) blurry images, (4) poor centration, (5) signal loss, and (6) images with segmentation error [1, 3]. Artifacts that prevented a reliable assessment were excluded.

In this study, DME was defined as a CMT of >300 μm. Based on the response (poor and good) to anti-VEGF therapy, the study participants were divided into two groups. A good response to bevacizumab was defined as a reduction in the CMT of DME eyes by ≥10% after nine consecutive anti-VEGF injections compared to the initial value.

An additional analysis was also performed by dividing the patients into two groups: those who achieved a final visual acuity of ≥75 ETDRS letters and patients with a worse result.

2.1. Statistical Analysis. The changes in the OCT parameters were estimated using a mathematical equation derived for this purpose. Then, the relative FAZ area in SCP and DCP, relative MAs in SCP and DCP, relative CMT, relative BCVA (in EDTRS score), and all the analyzed densities were calculated according to the following equation to assess the effects of the therapy:

$$\text{Parameter}_{\text{relative}} = \frac{\text{Parameter}_{\text{after therapy}} - \text{Paremter}_{\text{before therapy}}}{\text{Parameter}_{\text{before therapy}}} * 100\%. \quad (1)$$

Categorical variables were analyzed using the chi-squared test. The normality of the data was assessed using the Shapiro–Wilk test. Continuous variables measured during the 1-year period of the study were evaluated using repeated-measures analysis of variance (ANOVA). The association between continuous variables was investigated using Pearson's correlation or the Mann–Whitney test if applicable. Qualitative variables were assessed using the Spearman test followed by regression analysis. p values of <0.05 were considered significant. All analyses were performed using Statistica 13.3 (Tibco, Palo Alto, CA, USA).

3. Results

A total of 116 eligible eyes of 112 patients with DME were analyzed in the baseline examination. We excluded 39 eyes (33.62%) as they were deemed unsuitable for OCT-A due to a low-quality score (14 eyes), blurry images (10 eyes), motion artifacts (7 eyes), poor centration (3 eyes), or signal loss (5 eyes). Finally, 77 eyes with DME were included in the final analysis (33 male (42.86%) and 44 female). The mean age of patients included in the study was 68.50 ± 8.53 years (47 to 84 years), and the mean HbA1c level was 7.06 ± 0.95% (5.50-9.40%). Patients had DM on average for 12.65 ± 7.74 (4.00-31.00 years). The mean BMI was 26.23 ± 3.98 (19.38-39.79). IOP was on average 15.74 ± 1.98 (12.00-21.00), while AL was 23.37 ± 0.68 mm(21.53-24.97) and the qualitative data are provided in Table 1. We performed our analysis in two stages. Firstly, we divided the study eyes based on the response to bevacizumab: poor response—25 eyes and good response—52 eyes. Secondly, we divided all the study participants based on the final BCVA of 75 ETDRS letters. Forty eyes achieved a final BCVA of ≥75 letters in the ETDRS chart, while 37 eyes showed a BCVA of <75 letters. If both eyes were eligible for the study, we selected the one with more severe retinal edema. In 8% of patients, the OCT-A images were manually corrected with inaccurate automated positioning and segmentation of OCT-A scans. OCT-A images of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) with structural OCT B-scans before and after the intravitreal treatment of bevacizumab are presented in Figures 1(a)–1(c) and 2(a)–2(c).

The data collected from medical history showed no significant difference between the patients with a BCVA of <75 letters and those with a BCVA of ≥75 letters in terms of general medical and ophthalmological variables. Similarly, no difference was observed between good and poor responders (Table 1). In addition, patients from the BCVA ≥ 75 group were significantly older 70.8 ± 9.59 vs. 66.03 ± 6.47 years ($p = 0.019$), and their diabetes was significantly shorter 10.85 ± 6.44 vs. 14.59 ± 8.61 years ($p = 0.034$). Furthermore, both baseline CMT 365.78 ± 70.86 vs. 463.51 ± 62.33 μm ($p < 0.001$) and ETDRS 69.85 ± 5.75 vs. 59.19 ± 4.83 ($p < 0.001$) and final CMT 280.5 ± 47.7 vs. 384.03 ± 66.88 μm ($p < 0.001$) and final ETDRS score 67.08 ± 4.04 ($p < 0.001$) values differed significantly between the BCVA > 75 and the BCVA < 75, respectively. The only significant differences between the good and poor responders were found in the final values of CMT 317.83 ± 55.76 vs. 417.68 ± 55.76 ($p < 0.001$) and ETDRS 73.71 ± 7.98 vs. 72.56 ± 7.98 ($p < 0.02$).

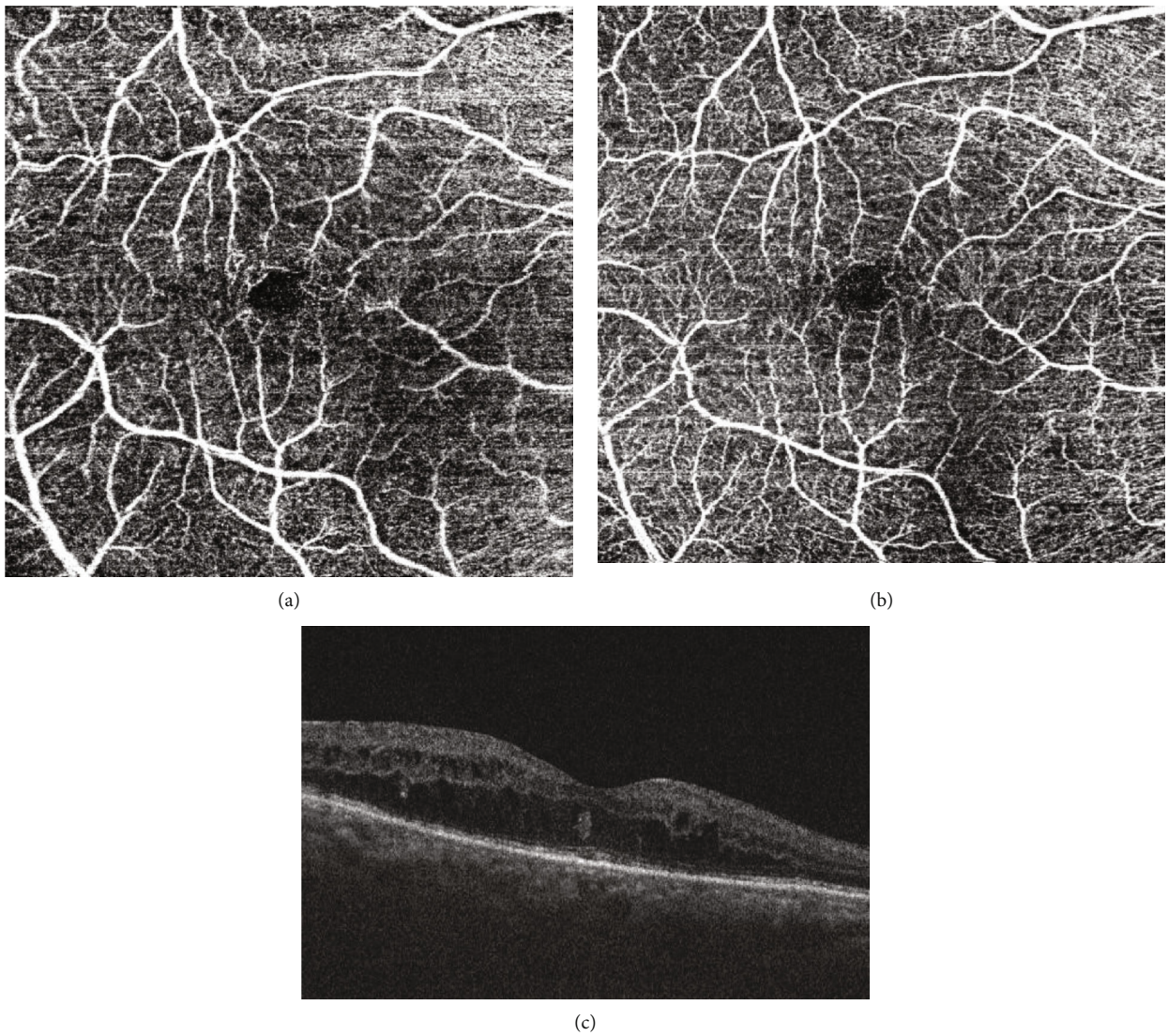
The OCT-A parameters were compared between the DME eyes that responded well and the DME eyes that responded poorly to anti-VEGF inhibitors. In both groups, the mean area of FAZ and the mean number of MAs were observed to be higher in DCP compared to SCP. Furthermore, independent of the response to bevacizumab treatment, the mean number of MAs was decreased in SCP from 3.51 ± 2.07 to 2.31 ± 1.15 ($p < 0.001$) and in DCP from 17.12 ± 11.56 to 12.21 ± 6.99 ($p < 0.001$) in both groups. The FAZ area increased in SCP from 328.22 ± 131.38 to 399.70 ± 156.98 ($p < 0.001$) and in DCP from 571.13 ± 396.01 to 665.89 ± 412.77 ($p = 0.001$).

A significant reduction in the number of MAs in SCP and DCP was observed after fixed-regimen bevacizumab therapy, which confirmed that DR did not progress in the treated DME patients (with the simultaneous absence of other markers of progression such as hemorrhages and IRMA not found in the fundus images).

3.1. Repeated-Measures ANOVA in All Participants. Repeated-measures ANOVA revealed that a range of ocular biometric parameters (BCVA: $p < 0.001$, FAZ area in SCP: $p < 0.001$, and FAZ area in DCP: $p < 0.001$) showed significant changes over 1 year of the study (Figures 1 and 2). Moreover, vascular density average was found to be decreased significantly during the treatment ($p < 0.001$; nasal

Table 1: Qualitative data of patients described as poor (CMT reduction < 10%, $N = 52$) and good (CMT reduction > 10%, $N = 25$) responders and in the BCVA < 75 ($N = 37$) and BCVA ≥ 75 ($N = 40$) groups receiving intravitreal bevacizumab treatment. The data were compared using the chi-squared (χ^2) test. Statistical significance was set at $p < 0.05$.

	Response < 75	Response > 75	Row	χ^2	p	Responders good	Responders poor	Row	χ^2	p
Per os treatment										
Absent	13	19	32			21	11	32		
Present	24	21	45	1.21	$p = 0.27134$	31	14	45	0.09	$p = 0.76308$
Totals	37	40	77			52	25	77		
Insulin										
Absent	30	31	61			41	20	61		
Present	7	9	16	0.15	$p = 0.69879$	11	5	16	0.01	$p = 0.90698$
Totals	37	40	77			52	25	77		
Gender										
Male	15	18	33			21	12	33		
Female	22	22	44	0.16	$p = 0.69279$	31	13	44	0.40	$p = 0.52719$
Totals	37	40	77			52	25	77		
Chronic kidney disease										
Absent	34	36	70			46	24	70		
Present	3	4	7	0.08	$p = 0.77295$	6	1	7	1.16	$p = 0.28127$
Totals	37	40	77			52	25	77		
Hypertension										
Absent	13	16	29			20	9	29		
Present	24	24	48	0.19	$p = 0.65981$	32	16	48	0.04	$p = 0.83465$
Totals	37	40	77			52	25	48		
Ischemic heart disease										
Absent	28	34	62			45	17	62		
Present	9	6	15	1.07	$p = 0.30199$	7	8	15	3.70	$p = 0.05444$
Totals	37	40	77			52	25	77		
Myocardial infarction										
Absent	33	35	68			48	20	68		
Present	4	5	9	0.05	$p = 0.81770$	4	5	9	2.48	$p = 0.11548$
Totals	37	40	77			52	25	77		
Brain stroke										
Absent	34	39	73			50	23	73		
Present	3	1	4	1.23	$p = 0.26790$	2	2	4	0.59	$p = 0.44184$
Totals	37	40	77			52	25	77		
Lens status (phakic/pseudophakia)										
Phakic	24	25	49			31	18	49		
Pseudophakia	13	15	28	0.05	$p = 0.82936$	21	7	28	1.12	$p = 0.28474$
Totals	37	40	77			52	25	77		
Laterality										
OD	19	23	42			25	17	42		
OS	18	17	35	0.29	$p = 0.58825$	27	8	35	2.70	$p = 0.10017$
Totals	37	40	77			52	25	77		
Combined treatment										
Absent	31	30	61			42	19	61		
Present	6	10	16	0.90	$p = 0.34255$	10	6	16	0.23	$p = 0.62910$
	37	40	77			52	25	77		



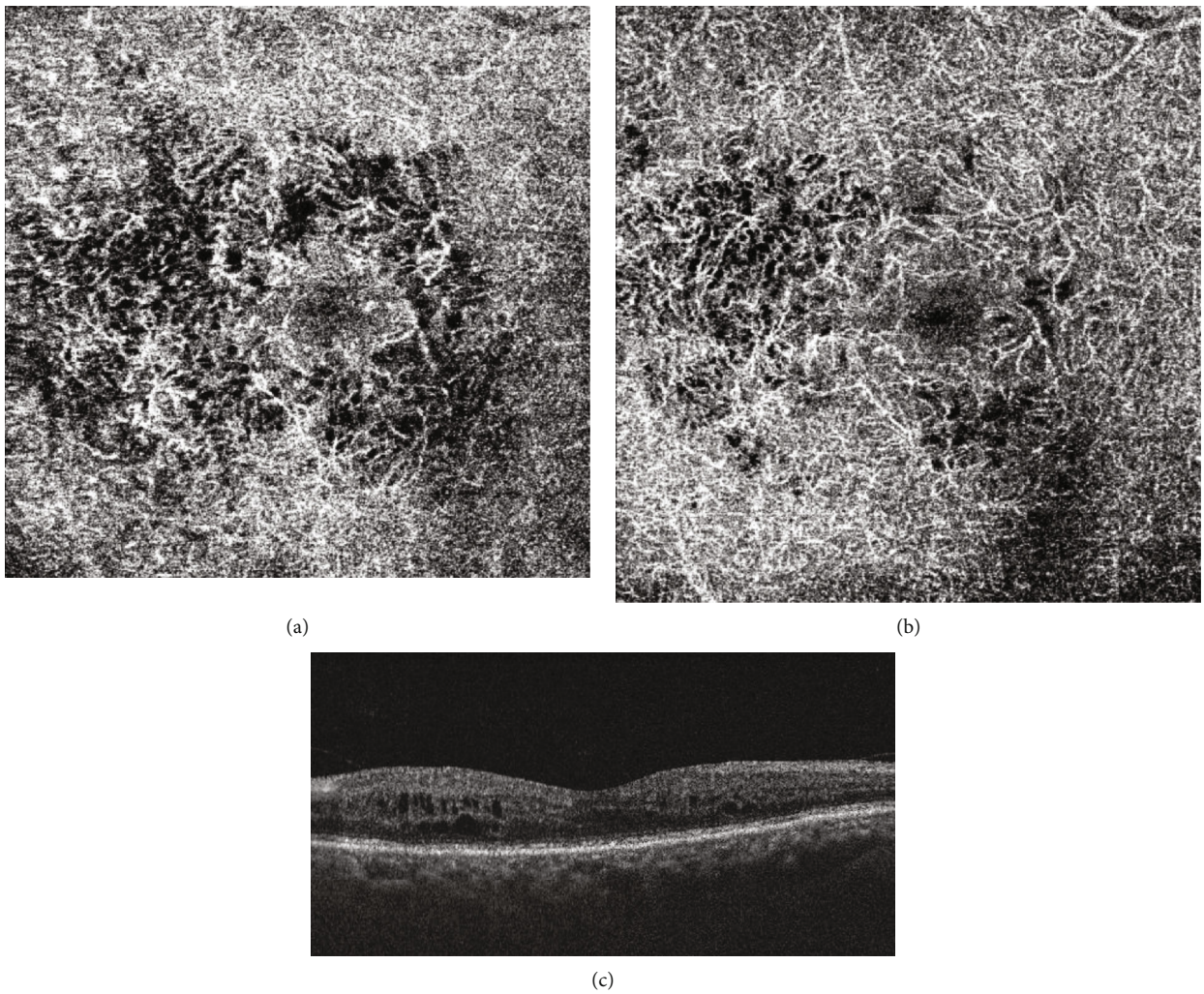
(a) (b) (c)

Figure 1: (a) Superficial capillary plexus of study patient before intravitreal treatment of bevacizumab. (b) Superficial capillary plexus of study patient after intravitreal treatment of bevacizumab. (c) Structural OCT B-scan before intravitreal treatment of bevacizumab.

density: $p = 0.003$, superior density: $p < 0.001$, and temporal density: $p < 0.001$) (Figure 3). However, neither total nor inferior density exhibited any significant interaction ($p = 0.79$ and $p = 0.84$, respectively). In addition, repeated-measures ANOVA revealed that the injections significantly reduced the number of MAs in SCP and DCP (Figure 4). Similarly, a significant reduction was noted in CMT and ETDRS in both groups after intravitreal anti-VEGF treatment (Figure 1). The changes observed in FAZ areas are displayed in Figure 3 and density in Figure 5. The variations found in MAs and FAZ area in the SCP and DCP, as well as in nasal, temporal, and superior vessel density in the SCP, indicated significant changes in the macular vascular network during the treatment.

3.2. Repeated-Measures ANOVA in All Participants. To measure the changes during treatment between two groups, a repeated measurement ANOVA was conducted. Repeated-measures ANOVA revealed that a range of ocular biometric parameters (BCVA: $p < 0.001$, FAZ area in SCP: $p < 0.001$, and FAZ area in DCP: $p < 0.001$) showed a significant increase over 1 year of the study (Figure 3). Moreover, vascular density average was found to be decreased significantly during the treatment ($p < 0.001$; nasal density: $p = 0.003$, superior density: $p < 0.001$, and temporal density: $p < 0.001$).

However, neither total nor inferior density exhibited any significant interaction ($p = 0.79$ and $p = 0.84$, respectively). In addition, repeated-measures ANOVA revealed that the injections significantly reduced the number of MAs in SCP and DCP (Figure 4). Similarly, a significant reduction was noted in CMT and ETDRS in both groups after intravitreal anti-VEGF treatment. The decrease found in MAs and FAZ area in the SCP and DCP, as well as in nasal, temporal, and superior vessel density in the SCP, indicated significant changes in the macular vascular network during the treatment.



(a) (b) (c)

Figure 2: (a) Deep capillary plexus of study patient before intravitreal treatment of bevacizumab. (b) Deep capillary plexus of study patient after intravitreal treatment of bevacizumab. (c) Structural OCT B-scan after intravitreal treatment of bevacizumab.

3.3. Repeated-Measures ANOVA between the Groups. A significant increase in the poor responders compared to a decrease in good responders was noted in the FAZ area in DCP ($p < 0.001$), while the FAZ area in SCP did not differ between the groups ($p = 0.51$). Both FAZ areas in DCP and SCP were measured consecutively during the study that decreased significantly between BCVA < 75 and BCVA ≥ 75 groups ($p = 0.007$ and $p = 0.044$ for SCP and DCP, respectively) (Figure 3).

Furthermore, vascular density superior was found to significantly decrease weaker in the BCVA < 75 group than in the BCVA ≥ 75 group ($p = 0.03$) (Figure 5). All other vascular densities did not differ significantly.

On the other hand, an opposite trend was noticed between the good and poor responders, where changes in nasal density were not found to be significant.

3.4. Correlations. We also investigated the correlations in the course of bevacizumab treatment and at the end of the study.

The analysis of variables measured in all the included patients showed that relative BCVA strongly negatively correlated with CMT ($r = –0.41$, $p < 0.001$) in good responders, while in poor responders, the correlation was very weak ($r = –0.01$, $p < 0.001$). On the other hand, a positive correlation was found between the FAZ area and MAs measured in SCP ($r = 0.44$).

A positive linear correlation between relative BCVA (a percentage change between the EDTRS score at the last visit and at baseline) and relative FAZ area (a percentage change between the size of FAZ at the final visit and at baseline) in DCP was indicated. Our analysis revealed that for each 20% increase in the relative FAZ area, a 0.8% decrease in relative BCVA could be expected. In addition, we found that HbA1c% negatively correlated with oral treatment ($r = –0.41$) and relative BCVA ($r = –0.23$), while positively correlated with hypertension ($r = 0.48$) and relative density superior ($r = 0.28$). Relative CMT correlated with other parameters, and the number of MAs in DCP was associated with the baseline ETDRS score ($r = –0.30$, $p = 0.018$) and the MA count in the posterior zone (average correlation, $r = –0.377$, $p < 0.001$). No other correlations were found between the diabetes parameters and OCT-A findings, except for the

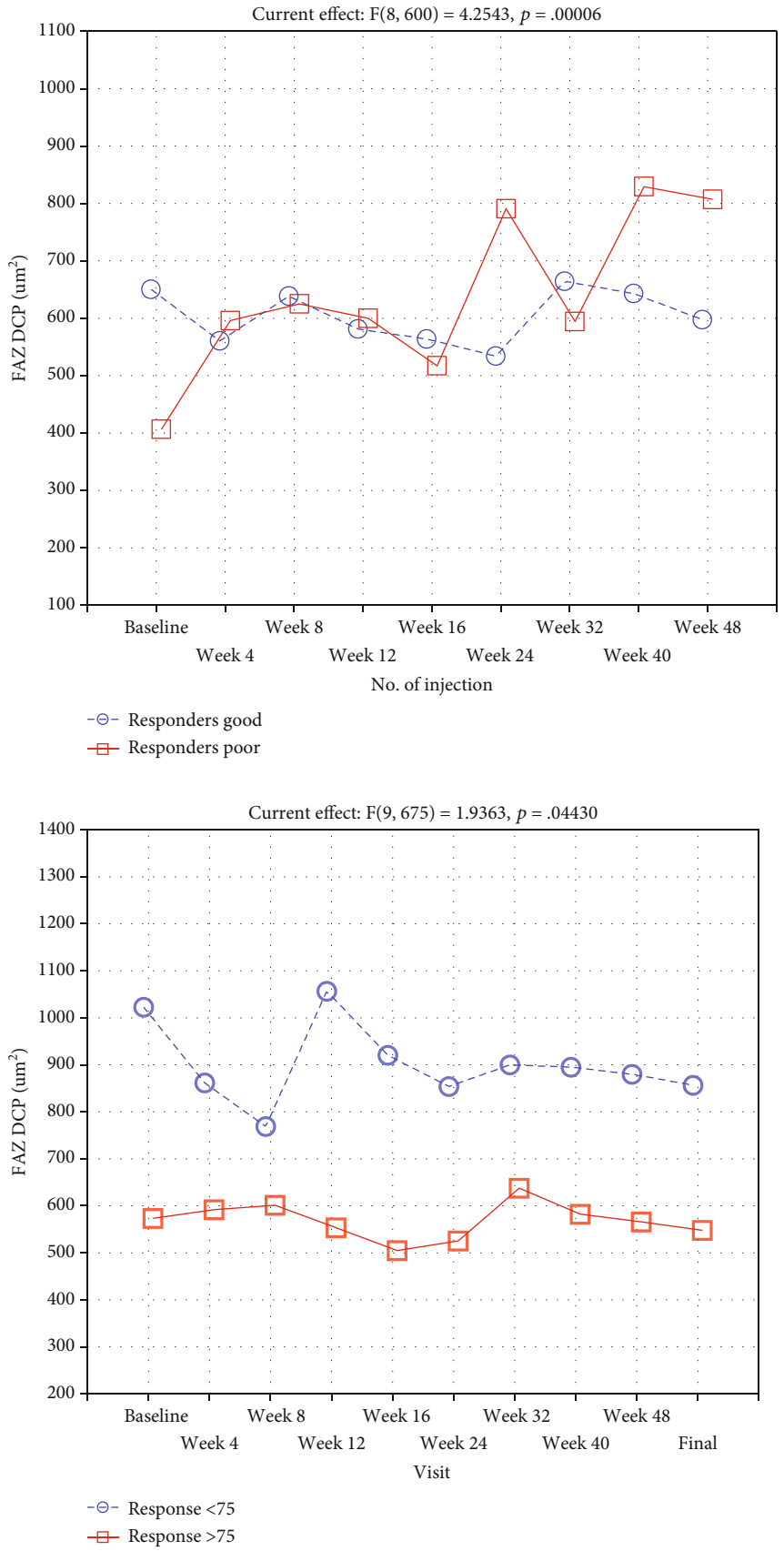


Figure 3: Size of FAZ in DCP in the BCVA < 75 ($N = 37$) and BCVA ≥ 75 ($N = 40$) groups and in the DME patients described as poor (CMT reduction < 10%, $N = 52$) or good (CMT reduction > 10%, $N = 25$) responders during subsequent intravitreal bevacizumab injections.

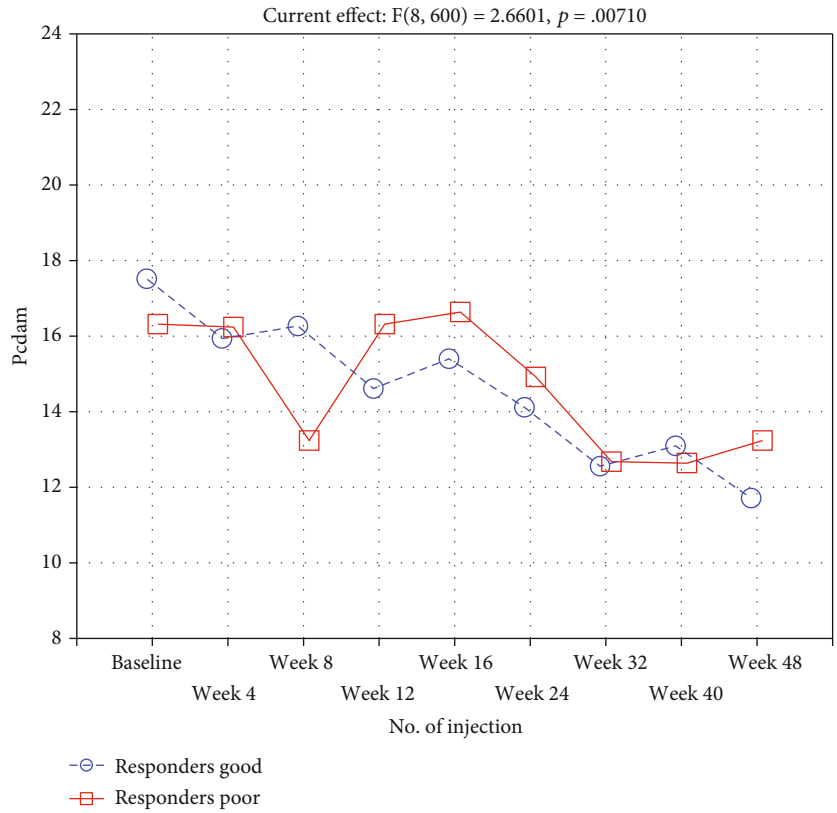


Figure 4: Number of MAs in the DCP group and in the DME patients described as poor (CMT reduction < 10%, $N = 52$) or good (CMT reduction > 10%, $N = 25$) responders during subsequent intravitreal bevacizumab injections.

correlation between HBA1c% and relative density superior and relative density inferior ($r = 0.24$, $p = 0.034$ and $r = 0.28$, $p = 0.016$, respectively). We also found no relationships between oral treatment, gender, insulin treatment, combined treatment, hypertension, ischemic heart disease, brain stroke or lens status, and the OCT-A parameters, apart from the associations between insulin treatment and relative MAs in DCP ($r = 0.23$, $p < 0.05$) and lens status ($r = -0.25$) and between relative MAs in SCP, lens status, and superior density ($r = 0.33$). Relative MA count in DCP was strongly correlated with relative FAZ area in DCP in the BCVA < 75 group ($r = 0.46$), whereas in the BCVA ≥ 75 group, the correlation was weak ($r = -0.07$). Furthermore, the correlation between the relative MAs in SCP and the relative FAZ area in SCP was strong in poor responders in comparison to good responders.

A regression analysis was performed to identify the factors affecting the changes in MA distribution in DCP.

In the BCVA ≥ 75 group, for every 1% increase in relative FAZ area in SCP, a 0.23% increase in DCP MAs was expected, while a 1% increase in total relative density was predicted to lead to a 0.30% decrease in DCP MAs. In the case of the BCVA < 75 group, for every 0.58% increase in relative MAs in DCP, a 1% increase in relative CMT was expected, while a 1.53% decrease was predicted to lead to a 1% increase in relative density in the inferior quadrant (Table 2).

4. Discussion

Our study aimed to demonstrate the effectiveness of 12-month intravitreal bevacizumab therapy in patients with DME and their response to the treatment. The effectiveness of the anti-VEGF treatment was evaluated morphologically by performing OCT-A and SS-OCT. The results showed that intravitreal bevacizumab therapy effectively reduced DME. A significant improvement in BCVA and reduction in CMT were found after fixed-protocol intravitreal bevacizumab injections. In addition, no progression of DR was noticed in patients with DME after the treatment. The mean number of MAs in both SCP and DCP was also decreased in patients regardless of their classification.

Our study showed that the fixed protocol of treatment with 9 bevacizumab injections within 12 months was helpful in the improvement of visual acuity and in reduction of central macular thickness in the study group of patients with diagnosed DME. Other studies have reported that intravitreal anti-VEGF injections can reduce microvascular damage caused by diabetes mellitus. The Diabetic Retinopathy Clinical Research Network qualified 660 patients with DME and randomly assigned them to treatment with anti-VEGF agents such as aflibercept, bevacizumab, or ranibizumab. At baseline, the mean visual acuity letter score of the patients was determined at 64.8 ± 11.3, and the mean central subfield retinal thickness at 412 ± 130 μm. The bevacizumab group

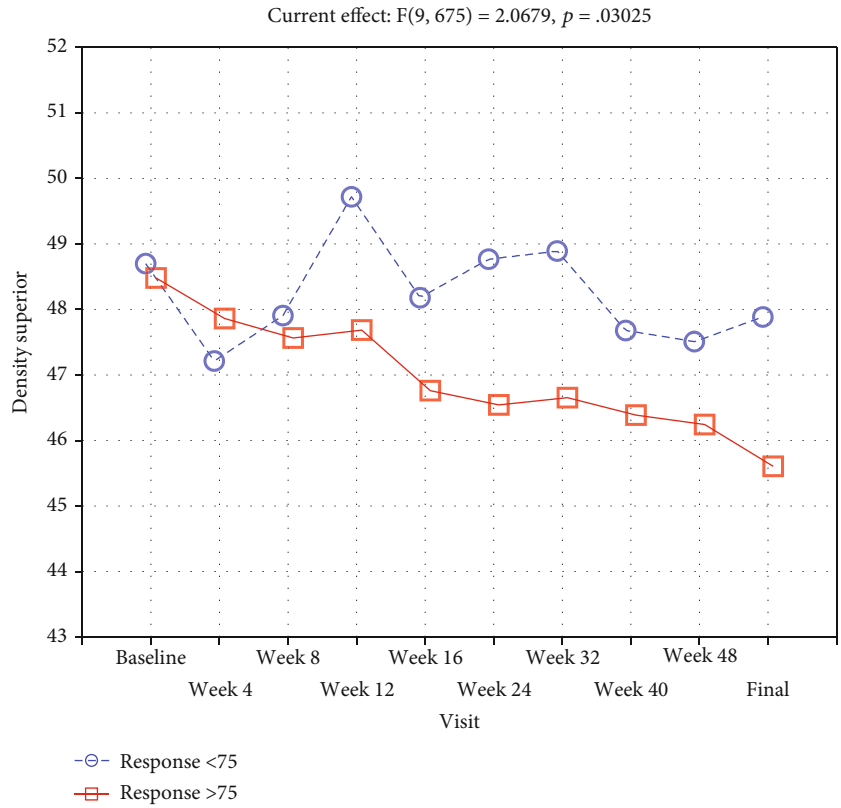


Figure 5: Density in the superior, quadrant of DME patients during subsequent intravitreal bevacizumab injections.

received 10 injections on average within 1 year of the study with improvement of 9.7 in the BCVA letter score, and the mean CMT reduction was 101 ± 121 μm [14]. These findings do not differ significantly from that of our study, especially the improvement in the visual acuity letter score. However, the studies differ in the type of anti-VEGF treatment applied and the number of injections administered and it is difficult to compare the two studies. On the one hand, Sarda et al. reported that the mean visual gain observed in their study was +10.1 ETDRS letters and reduction in CMT was 65.1 μm after 5 aflibercept or ranibizumab intravitreal injections [27]. On the other hand, Călugăru et al. demonstrated that the mean BCVA improved and CMT decreased significantly compared to the baseline values after anti-VEGF therapy in anatomic nonresponders and responders [28]. As in other studies, Vujosevic et al. observed the significant improvement in BCVA with mean change of 13 ± 10 ETDRS letters after treatment. In that study, naïve patients were treated either single DEX-I 0.7 mg (Ozurdex, Alergan, Inc., Irvine, California, USA) or 3 monthly IVR 0.5 mg (Lucentis, Novartis, Genetech, San Francisco, USA) [26].

MAs are visible lesions often observed in the early stages of DR that could be visualized using OCT-A. Moreover, these lesions can be located precisely within the retinal vasculature by OCT-A [18, 29]. Our results showed that, regardless of division into groups, in all patients, the mean number of MAs was higher in DCP than in SCP, and MA count was decreased after intravitreal bevacizumab treatment in comparison to baseline. Moreover, we noted that poor-responding DME eyes had a higher number of MAs in DCP than good responders after treatment. Similar observations were reported by Lee et al. who observed that the number of MAs was increased to a great extent in DCP in comparison to SCP after three consecutive injections of different kinds of anti-VEGF agents. Division into poor and good responders was based on the reduction in CMT by >50 μm after the treatment and also compared their results with a group of control eyes. However, no significant differences were noticed between good and poor responders in the SCP parameters [12, 18]. In our study, we found that the mean number of MAs decreased in both SCP and DCP, apart from the response to bevacizumab treatment. Hasegawa et al. observed 77.3 ± 8.1% of MAs in DCP in DME eyes, whereas in DME eyes with a CMT of >400 μm, 91.3 ± 9.1% of MAs were located in DCP [13].

Pongsachareonnont et al. demonstrated that the number of MAs was reduced in SCP and DCP (40% relative to baseline) after a single anti-VEGF injection of aflibercept, bevacizumab, or ranibizumab and ranibizumab was the most effective among the three studied drugs. Anti-VEGF agents helped decrease the MA count in association with a reduction of CMT and improvement of BCVA [8]. Ho et al. observed that MAs were more clearly delineated in the 6 × 6 mm scans compared with 3 × 3 mm scans. Discrepancies can be found among studies regarding the detectability of MAs between FA and OCT-A images, and it was pointed out that not all MAs visualized on FA images were identified on OCT-A images [18]. Salz et al. showed that SS-OCT-A

Table 2: Regression summary for dependent variable: relative MAs in DCP for BCVA ≥ 75 and BCVA < 75 groups.

(a)

	Response ≤ 75 $R = 0.77058035$; $R^2 = 0.59379408$; adjusted $R^2 = 0.45839211$ $F(9, 27) = 4.3854$; $p < 0.00132$; Std. error of estimate: 23.721					
	b^*	Std.Err.	b	Std.Err.	t (27)	p value
Intercept			-5.02	10.76	-0.47	0.64
CMT relative	0.13	0.29	0.29	0.66	0.44	0.67
ETDRS %	-0.21	0.23	-0.94	1.04	-0.90	0.37
FAZ SCP %	0.34	0.15	0.23	0.11	2.20	0.04
FAZ DCP %	0.12	0.19	0.06	0.10	0.61	0.54
Density %	-0.32	0.15	-0.30	0.14	-2.09	0.05
Density sup %	0.17	0.19	0.79	0.87	0.90	0.37
Density inf %	-0.45	0.21	-1.31	0.59	-2.21	0.04
Density nasal%	0.18	0.16	1.03	0.91	1.13	0.27
Density temporal%	0.13	0.18	0.59	0.87	0.68	0.50

(b)

	Response ≥ 75 $R = 0.61835710$; $R^2 = 0.38236551$; adjusted $R^2 = 0.19707516$ $F(9, 30) = 2.0636$; $p < 0.06629$; Std. error of estimate: 29.124					
	b^*	Std.Err.	b	Std.Err.	t (30)	p value
Intercept			1.24	10.34	0.12	0.91
CMT relative	0.58	0.24	1.03	0.43	2.37	0.02
ETDRS %	0.11	0.23	0.36	0.77	0.47	0.64
FAZ SCP %	-0.14	0.19	-0.10	0.14	-0.72	0.48
FAZ DCP %	-0.36	0.24	-0.20	0.13	-1.50	0.14
Density %	-0.12	0.18	-0.41	0.63	-0.65	0.52
Density sup %	0.26	0.19	0.90	0.65	1.39	0.17
Density inf %	-0.45	0.20	-1.53	0.67	-2.28	0.03
Density nasal%	0.17	0.28	1.05	1.69	0.62	0.54
Density temporal%	-0.27	0.25	-1.78	1.65	-1.08	0.29

had a sensitivity of 85% and a specificity of 75% compared to FA [18, 30].

Furthermore, Falavarjani et al. reported no significant difference in the FAZ area and vessel density in patients with macular edema after a single intravitreal anti-VEGF injection. However, they indicated that more injections should be conducted to confirm their results [18, 31]. In our study, we found that the FAZ area was larger in DCP than in SCP, apart from the response to the treatment, which is also in line with the results of Lee et al. Moreover, poor responders showed a larger FAZ area in DCP compared to good responders. After bevacizumab treatment, an increase in the FAZ area was noted in both SCP and DCP compared to baseline, which contradicts the results of Pongsachareonnont et al., who reported a significant reduction in the FAZ area in both plexuses after injection of anti-VEGF agents [8].

Another parameter assessed in OCT-A images is the vascular perfusion density at the macula in eyes with DR. Vessel density is the proportion of blood vessel area and total scanned area [29]. Our study showed that total vessel density in SCP decreased after bevacizumab treatment. According to Sorour et al., the values of macular vessel density in SCP, DCP, and total capillary plexus did not significantly differ between baseline and after one, two, or three injections of anti-VEGF agents in both 3 × 3 and 6 × 6 mm scans [9].

OCT-A can quickly illustrate the microvascular abnormalities in both SCP and DCP in diabetic patients in a noninvasive way. Unfortunately, artifacts are a major limitation of the OCT-A examination. Our study noted motion artifacts, blurry images, low-quality scores, and images with a segmentation error. Other researchers have shown that motion and doubling artifacts were relatively high in both 3 × 3 and 6 × 6 mm scans [25].

We also analyzed the ocular, systemic, and demographic parameters of participants in our study. The patients in the BCVA ≥ 75 group were significantly older, and their diabetes was significantly shorter. No other correlations were found between the diabetes parameters and the OCT-A findings except that between HBA1c% and density superior and density inferior, which may be a random observation, considering the number of analyzed parameters. In addition, no relationship between gender, type of treatment, systemic factors, or lens status and the OCT-A parameters was found, apart from the associations between insulin treatment and relative MAs in DCP and lens status, and between relative MAs in SCP, lens status, and superior density. Tang et al. analyzed 434 OCT-A images of SCP obtained in patients with different stages of DR to assess the biomarkers of DR. They found that OCT-A metrics were related to the severity of DR but not to the presence of DME. Increased FAZ area was associated with a shorter axial length and decreased CMT. Nevertheless, OCT-A parameters were not correlated with age, duration of diabetes, and systemic factors (e.g., blood pressure, lipids, estimated glomerular filtration rate, and body mass index) [1].

We conducted a 12-month prospective study on DME patients who were treated with fixed-regimen intravitreal bevacizumab injections. Only treatment-naïve patients were included, and all of them received nine bevacizumab injections, while in many other studies, patients received fewer injections of anti-VEGF inhibitors [12, 18, 27, 28]. In addition, both the number of injections and follow-up period and the number of assessed parameters were lower in other studies compared to our study. Moreover, we obtained OCT-A images in every visit of patients. Usually, OCT-A scans with an area of 3 × 3 mm are used for analysis, while we used 6 × 6 mm scans in our study. However, a relatively high percentage of inaccurate images was obtained which did not allow us to perform reliable examinations as the

scans had projection and motion artifacts and images obtained from the DME patients were of lower quality.

5. Study Limitations

The study has several noticeable limitations. First, the number of participants was significantly reduced due to the low quality of the obtained OCT-A images. Second, we measured OCT-A parameters in SCP and DCP only in 6×6 mm scan frame and not in other frames. Third, we only included the eyes for which good-quality images and good fixation were achieved, and thus, the generalizability of our findings was limited [3]. Fourth, quantitative evaluation of the MA count and FAZ area was a subjective analysis because no reliable software is currently available for this purpose [12].

6. Conclusions

This study analyzed whether fixed-protocol intravitreal bevacizumab therapy is effective in treating DME and whether OCT-A is a useful tool to observe the changes in the clinical features of DR. Although OCT-A allows noninvasive monitoring of changes in the vascular network of the macula, it has limited usefulness in predicting the response to treatment. A more effective reduction in the MA count during treatment is an OCT-A predictor of a good response of DME eyes to bevacizumab. However, artifacts are very common in the OCT-A images of DME patients and can significantly reduce the usefulness of this modality. The factors that contribute to achieving a satisfactory visual acuity (>75 ETDRS letters) are higher baseline BCVA and less severe macular edema, as well as older age and longer duration of the disease. These could indicate that DME is less aggressive in form, given that only previously untreated patients are included in the study.

Data Availability

Data are available upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Safety Assessment of Glucose-Lowering Drugs and Importance of Structured Education during Ramadan: A Systematic Review and Meta-Analysis

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Background. Ramadan is the sacred month of the Islamic Hijri (lunar) calendar, and during this entire month, healthy adult Muslims abstain from eating and drinking from dawn to sunset. Muslims with Type 2 Diabetes Mellitus (T2DM) who choose to fast during Ramadan encounter major risks such as hypoglycemia, hyperglycemia, diabetic ketoacidosis, dehydration, and thrombosis. Although patients with poor glycemic control and on multiple insulin injections are at high risk and exempt from fasting, many still insist on it. Thus, healthcare professionals play a pivotal role in managing diabetes-related complications in patients who fast during Ramadan. However, there is a lack of standard guidelines to be followed in association with structured education and administration of drugs and dosage. Therefore, we performed a systematic review and meta-analysis of the literature to determine the safety and efficacy of different classes of drugs and the importance of structured education during Ramadan. *Methods*. In this review, an extensive PubMed search was performed to obtain literature on T2DM patients who fast during the month of Ramadan until the year 2020. Preference was given to fully downloadable articles. The articles were extracted based on the eligibility criteria. The extracted data were analyzed using Review Manager software version 5.3. *Results*. A total of 32 articles were included for the review and 7 studies for meta-analysis. Majority of the studies demonstrated the importance of structured education either as a group session or as a one-on-one session with the healthcare professionals in preventing diabetes-related risks during Ramadan. As far as glucose-lowering drugs are concerned, DPP-4 inhibitor combined with metformin remains the drug of choice for T2DM patients who fast during Ramadan. The newer class of glucose-lowering agents appear to lower the risk of hypoglycemia in comparison with sulphonylureas, while among sulphonylureas gliclazide is relatively safe. The meta-analysis indicates that DPP-4 inhibitors would significantly reduce the risk of hypoglycemia as compared to sulphonylurea (odds ratio = 0.38, 95% CI: 0.26 to 0.55, $p < 0.00001$). *Conclusion*. The results of our systematic review show that structured education and counselling by healthcare professionals can be an effective tool in preventing complications associated with fasting during Ramadan in people with T2DM. Additionally, the safest class of oral glucose-lowering drugs preferred during Ramadan fasting in T2DM patients is DPP-4 inhibitors.

1. Introduction

Among the religions in the world, Islam accounts for approximately 22% of the world's population. As of 2010, the total number of Muslims was estimated to be over 1.5 billion [1]. Though Muslims are found in all five inhabited continents, more than 60% of the global Muslim population predominantly belong to Asia while about 20% is in the

Middle East and North Africa (MENA). In the European continent, Muslims comprise approximately 7% of the overall population [1, 2]. Early Islam emerged in the Arabian Peninsula region which justifies the fact that this region has the highest concentration of Muslims. The total population of MENA region grew drastically from 3 million in 1870 to 8 million in 1950 and to 85 million by 2020 [3].

According to the International Diabetes Federation Atlas 2019, diabetes has emerged as one of the rapidly increasing global health emergencies. By the end of 2019, around 463 million people were predicted to have diabetes in the world. This number is estimated to reach 578 million by the end of 2030 and 700 million by 2045. A similar trend is estimated in the MENA region; a whopping 96% increase in the diabetes population is predicted in this region by 2045 in comparison with the population data of 2019. In the Arab world, the prevalence of Type 2 Diabetes Mellitus (T2DM) in adults (20-79 years) is reported to range from 4% to 22% [4]. A host of factors including dietary and lifestyle changes brought about by rapid economic development, increased urbanization, and the transition to a sedentary lifestyle led to a rise in the prevalence of T2DM [5]. This was also found to be significantly associated with higher Gross Domestic Product (GDP) ($p = 0.020$) and energy consumption in diet ($p = 0.017$) [6].

Muslims follow the Islamic lunar calendar, and Ramadan falls in the ninth month of this calendar [7]. Muslims across the globe observe the holy month of Ramadan with high reverence as it is one of the five pillars of Islam and forms an inherent part of the Muslim faith [1]. During this period, healthy adult Muslims refrain from any form of food or drink orally or parenterally from dawn to dusk for hours ranging from 10 to 20 hours according to geographical location of the region and seasons with an exception to exempt categories such as the sick, travelers, prepubertal children, pregnant, nursing mothers, and women during their menstruation [8–12]. Despite their health constrain, many such people insist and observe fast during this period [13]. Fasting Muslims usually consume two main meals during Ramadan, one before sunrise, known in Arabic as "Suhoor", and the other after sunset, known as "Iftar" [7, 14].

People with T2DM who intend to fast in Ramadan are encountered with major risks such as hypoglycemia, hyperglycemia, diabetic ketoacidosis, dehydration, and thrombosis due to an elevation in adrenaline, noradrenaline, and cortisol hormones [15, 16]. Throughout the period of fasting, there is a restricted fluid intake which results in higher chances of dehydration and could further deteriorate with a long duration of fasting in hot and humid climatic conditions [15].

Although, globally, a large number of Muslims fast during Ramadan, there is no clear scientific agreement on the guidelines or advice that should be followed by patients who are fasting. The information on the safety and efficacy of glucose-lowering drugs during Ramadan fasting is still scanty [17–19]. The EPIDIAR study was the very first study to provide information on fasting-related issues in diabetic patients during the month of Ramadan. This study consequently led to various management strategies including Ramadan-related education, change of medication with meals, and recommendations for this area in diabetes [8]. The IDF-DAR (International Diabetes Federation-Diabetes and Ramadan) Practical Guidelines provide healthcare professionals with consistent background information and practical recommendations which are aimed at delivering the best possible care and supporting to patients with diabetes during Ramadan, while minimizing the risk of complications [13]. Recently, American Diabetes Association (ADA) and European Association for the study of Diabetes (EASD) published new consensus statement for the management of diabetes [19]. Following this statement, recommendations for management during Ramadan were prepared by an expert group (Ibrahim et al.). The objective of the present paper is to systematically review and investigate the safety of glucose-lowering drugs administered, structured education, and lifestyle changes in people with diabetes during Ramadan fasting.

2. Materials and Methods

A thorough electronic search on PubMed was performed from the year 1950 to June 2020. The search is specifically aimed at retrieving publications which reported the effect of various classes of glucose-lowering drugs during Ramadan on health outcomes of people with T2DM. All possible sources were used to download the full-length article. The key words for search were "Fasting" [MeSH Terms] AND "Diabetes Mellitus" [MeSH Terms]. With Ramadan as MeSH Terms, the list of searched items was minimal. Thus, Ramadan articles were sorted out from the fasting list of articles. The title and abstract of the studies were evaluated for eligibility. All studies, in which diabetes mellitus and Ramadan fasting were discussed, were considered and determined for the eligibility as per the inclusion criteria. In studies with mixed patient populations [Type 1 DM (T1DM) and T2DM], data on the T2DM group were included and extracted. Cross-reference from the selected articles was also searched. Finally, a total of 248 full length articles were considered. The numbers of studies included in qualitative and quantitative synthesis (meta-analysis) were 32 and 7, respectively. The design of the systemic review search was based on Population Intervention Comparison Outcome Study Design (PICOS). The population for the review included patients with age greater than or equal to 18 years, male and female with T2DM for more than one year and fasting during Ramadan. Studies on children, gestational diabetes, pregnancy, T1DM, and nondiabetic patients were excluded. The outcome for this systematic review was to determine the effect of glucose-lowering drugs and effect of structured education during Ramadan.

The study quality was assessed in accordance with the guidelines for systematic review by Cochrane collaboration. The criteria included adequate sample size, detailed eligibility criteria, description of the population, description of the intervention, control or comparison, outcome assessment, well-defined statistical measurements, accounting for confounders, discussion, and conclusion supported by findings. Data were extracted from the eligible articles using predefined template of data extraction. The PRISMA flow chart

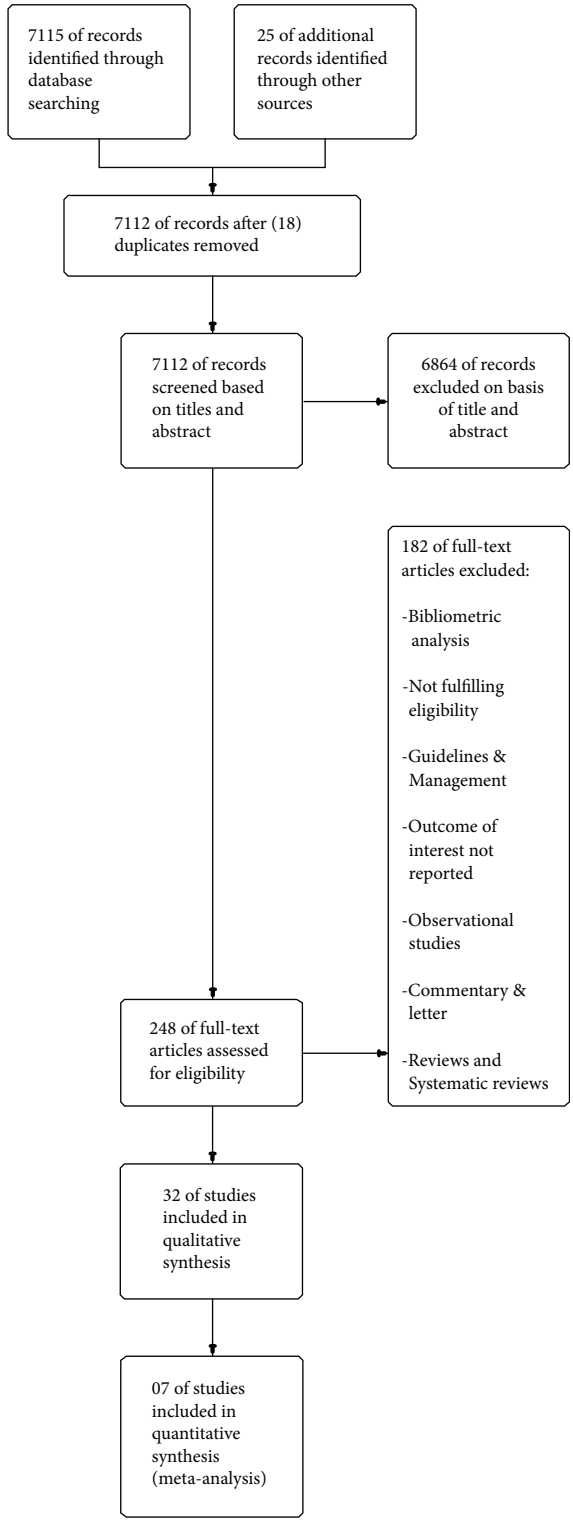

Figure 1: PRISMA flow chart.

(Figure 1) depicts the summary of literature search showcasing the reason for exclusion, characteristics of included studies, participants, interventions, and outcomes.

The End Note X7 software was used to compile and store all the related references. All study designs such as epidemiological studies, observational studies, surveys, prospective studies, cross-sectional studies, randomized control trials, open-label studies, comparative studies, and questionnaires were included in the meta-analysis. Review Manager version 5.3 was used for meta-analysis of DPP-4 inhibitors versus sulphonylurea safety profile. A random effects model was used for pooled data analysis. Heterogeneity and effect size were measured.

3. Results and Discussion

3.1. Effect of Structured Education. Structured education plays a key role in preventing hypoglycemia and other complications in people with diabetes who fast during the month of Ramadan. However, the majority of T2DM patients who fast do not consult their healthcare professionals before the commencement of Ramadan nor they monitor blood glucose level systematically throughout the month of Ramadan [20]. In regions with Muslims in minority, education of physicians on dose adjustment and patient risk stratification for fasting is uncertain. Also, physicians of such regions often lack sufficient knowledge about cultural aspects of fasting to provide adequate support to their patients. Eventually, patients seek advice of their relatives and religious scholars from the religious perspective [21]. In both scenarios, patients are at risk as neither physicians know the importance of religious fasting nor relatives or religious scholars know about potential medical complications of fasting. Physicians, religious scholars, and patients should work hand in hand for a safe Ramadan fast. One-on-one dietary educational session reduced the number of adverse events of fasting [22, 23]. Uysal et al. concluded that fasting is not contradictory to T2DM patients if appropriate advice about meals and use of glucose-lowering drugs are provided [24]. Most of the studies emphasized the importance of counselling before Ramadan fasting in terms of nutritional issues, timing and dosage of glucose-lowering medication, importance of self-monitoring of glucose, information on symptoms of hyperglycemia and hypoglycemia, and avoiding dehydration [11, 23, 25–30]. Studies also recommend collaboration of healthcare professionals for shared decision-making to resolve cultural perspective differences and unique cultural needs of patients [31]. A systematic review indicates empowering patients and healthcare professionals with the information of Ramadan fasting and disseminating the knowledge in major regional languages of the world to reach the information in underprivileged communities [32, 33]. Religious leaders and healthcare professionals play a pivotal role in providing information pertaining to safer fasting to the patients [7]. The onus of safe fasting lies with the people who intend to fast by visiting their physician for pre-Ramadan counselling and adhering to the recommendations of lifestyle advice on diet, medication regimen, cessation of smoking, and light physical exercise or sports [34]. One of the randomized controlled trials done on Ramadan-specific education coupled with telemonitoring compared with usual care found a decrease in the reported symptoms of hypoglycemia in the telemonitoring group, with only two participants out of forty-five reporting symptomatic hypoglycemia in comparison to eight participants out of

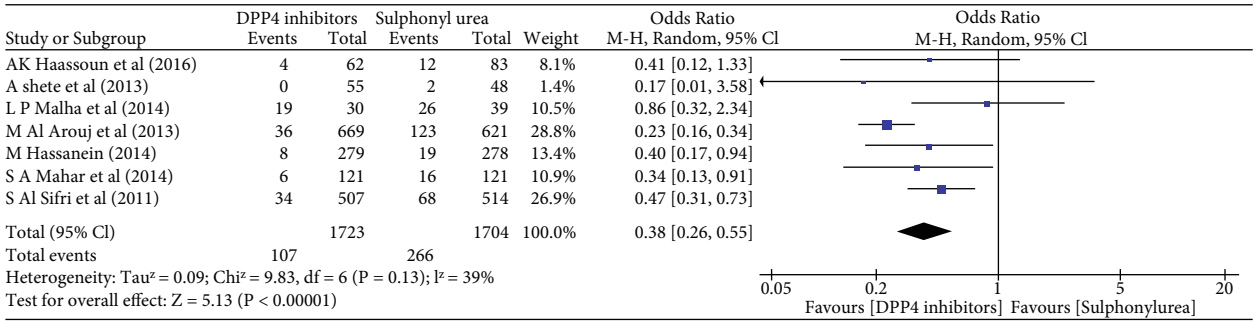

Figure 2: Forest plot for hypoglycemic event comparing DPP-4 inhibitors and sulphonylurea.

eighty in the usual care group at the end of the Ramadan period ($p = 0.04$). Significant reduction in HbA1c was achieved in the telemonitoring group as compared to the usual care group at the end of the study ($p < 0.01$) [35]. McEwen et al. conducted a study to determine if individualized education before Ramadan resulted in a safe fast for people with T2DM as compared to usual care. The study indicated a lower incidence of reporting of severe hypoglycemia that required medical assistance, glucagon injection, or intravenous infusion of glucose in patients receiving individualized education as compared to patients receiving usual care (23% vs. 34%, $p = 0.0017$). The proportion of patients who were hospitalized after getting individualized education was much lower than the proportion of patients who received usual treatment ($p = 0.0071$). HbA1c improved by $0.7 \pm 1.1\%$ in the individualized education group versus $0.1 \pm 1.3\%$ ($p < 0.0001$) in the usual care group. The BMI values also corresponded to a decrease of $1.1 \pm 2.4\,kg/m^2$ vs. $0.2 \pm 1.7\,kg/m^2$ in the intervention and control groups ($p < 0.0001$) [23]. Further larger studies are required to increase the knowledge on dietary and treatment changes for proper advice to the T2DM patients who fast during Ramadan [36]. The review of literature on structured education thus indicated that T2DM patients can undergo safe fasting during the month of Ramadan if provided with appropriate pre-Ramadan advice on nutrition, physical activity, and management of glucose-lowering drugs.

3.2. Effects of Glucose-Lowering Drugs during Ramadan. Managing T2DM becomes a challenge during Ramadan especially as the dietary pattern and circadian rhythm changes drastically. Desired blood glucose level can be achieved through a proper attention to diet, physical activity, and possible modification of pharmacologic treatment. As Ramadan fasting may involve refraining oneself from food and water for even twelve hours or more from dawn to dusk, it becomes an essential need that guidance concerning the use and modification of treatment during this period should be individualized [37, 38].

Of the available glucose-lowering agents, the choice of treatment to an individual should be customized considering the pathogenesis of T2DM which is heterogenic in nature [39]. Glucose-lowering drugs available today can be classified into the following major classes: biguanides, sulphonylureas (SUs), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 receptor (GLP-1) agonists, thiazolidinediones (TZDs), meglitinides, α-glucosidase inhibitors (AGIs), and insulin [40].

3.3. Biguanides. Metformin is widely preferred as the first-line glucose-lowering drug for the management of T2DM [40]. It is an insulin-sensitizing drug which exerts its effects by blocking liver gluconeogenesis thereby increasing the skeletal muscle uptake of glucose [41] but has also other potential modes of action. A pilot, open-label, observational study, conducted by Bonakdaran and Khajeh-Dalouie to determine the effects of fasting during Ramadan on glycemic excursions in patients with T2DM, reported that a remarkable difference was observed in the rate of hypoglycemic events (HEs) among the two groups of patients (patients administered metformin only versus patients administered SU). The percentage of HEs during Ramadan was found to be $0.11 \pm 0.3\%$ in the metformin group compared with $3.3 \pm 3.8\%$ in the SU group. The inclusion of SU in the treatment regimen increased the hypoglycemic risk of the study participants. The authors thereby concluded that in well-managed T2DM patients who are on metformin, the risk of hypoglycemia is minimal and patients may observe the Ramadan fast safely [27]. Generally, dosage modification is also not required with metformin. However, patients who take their usual dose at lunch may skip the dose during daytime fasting. A larger dose could be taken after breaking the fast while maintaining the morning dose as usual to prevent hyperglycemia. The dose can also be split into two: one-third can be taken at predawn while the rest at sunset [42, 43]. Though metformin once considered the first-line treatment for T2DM, many patients do not achieve glycemic control with this drug alone. A second drug is often needed to achieve glycemic control [41].

3.4. Sulphonylureas. SUs act by stimulating the insulin secretion by pancreatic β-cells and by decreasing the hepatic clearance of insulin. They are classified into two generations: first-generation SUs such as tolbutamide and chlorpropamide. Gliclazide, glipizide, glibenclamide, and glimepiride fall under the second generation of SUs [44]. As secretion of insulin is non-glucose-mediated, the traditional SUs result in a higher risk of hypoglycemia [45]. Zargar et al. evaluated the maintenance of glucose control with the intake of an

evening dose of a long-acting SU in male T2DM patients fasting during Ramadan in Bangladesh, Pakistan, and India. These patients exhibited glycemic control with gliclazide-modified release (MR) 60 mg monotherapy and were switched to an evening dose only of the same drug during Ramadan and resumed their usual morning dose thereafter. The study results indicated an improvement in glycated hemoglobin (HbA1c) and blood lipids compared with levels prior fasting. None of the patients withdrew due to hypoglycemia. Side effects were observed in a few patients and medication compliance with once daily dosage was good. The authors thus concluded that the frequency of hypoglycemia and weight gain were negligible for male patients with gliclazide MR 60 mg monotherapy being switched to an evening dose schedule which enabled them to safely observe the Ramadan fast [46]. VIRTUE, a multicenter, prospective study, was conducted to assess the effect of vildagliptin relative to SUs in Muslim patients with T2DM observing the Ramadan fast. The study patients received treatment with vildagliptin or SU monotherapy or as an add-on to metformin. There was a significant and clinically relevant ~3.5-fold lower incidence of HEs with vildagliptin in comparison with SU treatment. Also, fewer patients experienced ≥1 HE with vildagliptin compared with SUs. Among the individual SU drug types, glipizide was the preferred drug of choice due to its lower incidence of HE (12.5%) followed by glimepiride (17.9%), gliclazide (19.2%), and glibenclamide (31.85%). A significant reduction in HbA1c was observed in the vildagliptin group as compared with the SU group. Minor reductions from prefasting baseline levels in body weight were noted in both cohorts. [47]. A similar study was conducted by Al Sifri et al. to evaluate the incidence of symptomatic hypoglycemia in Muslim T2DM patients fasting during Ramadan who were on SU before the study or switched to sitagliptin (with or without metformin). The percentage of patients recorded with either symptomatic or asymptomatic HEs was lower in the sitagliptin group (8.5%) as compared with the SU group (17.9%). Among the patients in the SU group, 6.6% under the gliclazide subgroup reported symptomatic HEs followed by glimepiride (12.4%) and glibenclamide (19.7%) [48]. Anwar et al. concluded that there is no statistically significant difference in the reported incidence of hypoglycemia between repaglinide and short acting SU glimepiride in T2DM patients during Ramadan [49]. The Glimepiride in Ramadan (GLIRA) study group concluded that the efficacy and safety of glimepiride in T2DM patients remained unchanged during the month-long daylight fast of Ramadan when the administration schedule of glimepiride was changed from morning to evening [18]. Cesur et al. conducted another multicenter study with glimepiride, repaglinide, and insulin glargine to compare their glycemic effects in T2DM patients during Ramadan fasting. Patients were divided into fasting and nonfasting groups, and metformin was administered to all patients. Hypoglycemia was reported in 14.3% of patients in the glimepiride group followed by 11.1% in the repaglinide group and 10% in the insulin glargine group, but these differences among the drug groups were not significant. The levels of HbA1c did not show any remarkable change in either the fasting or non-fasting group. The fructosamine levels presented a notable increase at 1-month post-Ramadan compared with pre-Ramadan and post-Ramadan in both the fasting and nonfasting groups with no significant difference between the three drug groups. No changes were reported in the fasting group in body mass index (BMI) and plasma lipids [50]. Another multiregional, double-blind study randomized 557 patients with T2DM who were previously on metformin and any SU to receive either vildagliptin or gliclazide plus metformin. The percentage of hypoglycemia was not different between the vildagliptin group (6.0%) and the gliclazide group (8.7%) [51]. Newer generation SUs, especially gliclazide, seem to be safer in comparison to the older generation of SUs, owing mainly to their lower risk of HEs [44].

3.5. Dipeptidyl Peptidase 4 Inhibitors. Hypoglycemia and hyperglycemia remain the two major concerns associated with Ramadan fasting, and therefore, the treatment options should be aimed at reducing these risks in patients who intend to fast [52]. The DPP-4 inhibitors have a glucose-dependent mechanism of action and inhibit the disintegration of GLP-1 leading to an increase in its systemic concentration and secretion of endogenous insulin. This process in result reduces the secretion of glucagon [53]. Sitagliptin, the first drug from this class, was approved more than a decade ago by the United States Government's Food and Drug administration. This was followed by several other DPP-4 inhibitors such as vildagliptin, saxagliptin, linagliptin, and alogliptin which are currently available. Vildagliptin and sitagliptin are the most frequently studied DPP-4 inhibitors to assess the safety and efficacy in T2DM patients during Ramadan [54, 55]. The use of DPP-4 inhibitors has increased in the recent times due to its inherent potential to decrease the levels of blood glucose, along with an added advantage of good tolerability and reduced hypoglycemic risk [9, 53]. A study conducted by Hassoun et al. assessed the impact of vildagliptin in relation to SU on hypoglycemic occasions and concluded that the vildagliptin cohort reported a larger reduction in HbA1c and body weight from baseline to the end of the study as compared with the SU cohort during the Ramadan fasting period [56]. A 4-week, open-label, observational study comparing vildagliptin and SU with or without metformin did not exhibit any significant differences in HEs among the two groups. However, significant reductions in HbA1c level and body weight were observed in the vildagliptin cohort. Neither group exhibited drug-related serious adverse event or discontinuation of treatment due to adverse events [57]. Malha et al. conducted a randomized open-label clinical trial to determine the glycemic effects of vildagliptin in patients with T2DM before, during, and after Ramadan fasting. The SU group showed a numerically higher incidence of hypoglycemia during Ramadan compared with the vildagliptin group (26 versus 19, $p = 0.334$). The BMI value was higher at baseline in the vildagliptin group compared to post-Ramadan (29.5 Kg/m^2 versus 28.9 Kg/m^2) whereas contrasting values were noted in the SU group (28.9 Kg/m^2 baseline versus 29.8 Kg/m^2 post- Ramadan) [58]. A study by Halimi et al. assessed the rate of hypoglycemia during Ramadan in patients with

T2DM treated with their usual dual therapy of metformin-vildagliptin or metformin SU/insulin secretagogue (IS). A minimum of single episode of symptomatic hypoglycemia was seen in 37.2% of patients in the IS cohort versus 34.2% in the vildagliptin cohort. Severe hypoglycemia was also more evident in the IS group (10.4%) as compared to the vildagliptin group (2.6%). Glycemic and weight control were not different in these cohorts [59]. Based on the few studies available thus far, DPP-4 inhibitors appear to be effective in improving glycemic control with lower rates of hypoglycemia during fasting, making them a convenient treatment option during Ramadan.

3.6. Sodium-Glucose Cotransporter 2 Inhibitors (SGLT2is). SGLT2is act by inhibiting the absorption of glucose from the proximal convoluted tubule of the kidney thereby promoting the excretion of glucose in urine [60]. Commonly available drugs under this class include dapagliflozin, canagliflozin, ipragliflozin, empagliflozin, and ertugliflozin [54, 61]. Two studies assessed the safety and efficacy of SGLT2i and reported very few patients experiencing hypoglycemia in the SGLT2i group as compared with those treated with SUs. Though treatment with SGLT2i was generally well tolerated, patients were more prone to develop volume depletion symptoms such as dehydration in this group when compared to the SU group [62, 63]. More studies are therefore required to assess the safety and efficacy of SGLT2i especially for their use in Ramadan.

3.7. Glucagon-Like Peptide-1 (GLP-1 Agonists). GLP-1 agonists that are incretin mimetics are increasingly used in the treatment of T2DM in recent years. They act by binding to the GLP-1 receptor, thereby reducing the glucagon concentration with improved insulin sensitivity. They also act by delaying gastric emptying, increasing satiety, and decreasing free fatty acid concentrations and body weight. Several drugs in this class have been developed [64]. The most commonly studied GLP-1 agonists for their safety and efficacy in T2DM patients during the month of Ramadan are exenatide and liraglutide. A study compared liraglutide and SU, both in combination with metformin, during Ramadan and reported 2% of patients in the liraglutide group experienced hypoglycemic episodes as compared to 11% of patients in the SU group. The trial also reported that body weight reduced more with the liraglutide group ($p = 0.0091$). The fructosamine levels were similar in both groups from beginning to the end of Ramadan (liraglutide $-12.8\,\mu$mol/L vs. SU $-16.4\,\mu$mol/L). However, there was a significant reduction in fructosamine levels with liraglutide versus SU from baseline to the beginning of Ramadan ($p = 0.0024$) [65].

A triple-blind, placebo-controlled study was performed by Buse et al. to evaluate the glycemic control of exenatide in T2DM patients treated with SU. Subjects were randomized to $5\,\mu$g or $10\,\mu$g subcutaneous exenatide or placebo twice daily in two different groups. The subjects followed their usual SU therapy for a period of at least three months before screening. The study concluded that long-term use of exenatide at fixed subcutaneous doses of $5\,\mu$g and $10\,\mu$g twice daily appears to be an effective treatment option for patients with T2DM who are not adequately managed with SUs [66]. Literature to evaluate the safety and efficacy of exenatide in T2DM patients who fast during Ramadan is scanty, and hence, more studies are welcome in this area.

3.8. Thiazolidinediones. TZDs act by improving the insulin sensitivity and increasing the insulin stimulated glucose with negligible effect on the hepatic glucose output. The signaling of insulin is stimulated in vitro with the assistance of muscle culture cells. Moreover, they are known to diminish circulating free fatty acid levels leading to insulin resistance [67]. Drugs under this class include troglitazone, rosiglitazone, and pioglitazone. Studies evaluating the effects of TZDs in T2DM patients are limited. A double-blind, randomized controlled trial was conducted by Vasan to evaluate the effects of pioglitazone in Muslim patients fasting during Ramadan reported remarkable improvement in glycemic control when pioglitazone was used as an add-on therapy with other oral glucose-lowering drugs (SUs, metformin, meglitinides and acarbose) without any increase in HE. However, significant weight gain was observed with the pioglitazone group. Unlike other classes of drugs, dose adjustment is not required with pioglitazone [68]. Although this class of drugs are safe to use in T2DM patients during Ramadan due to their lower risk of hypoglycemia, not many studies are available to confirm the same. The availability of pioglitazone is also restricted in few countries, which eventually has led to fewer studies being conducted.

3.9. Meglitinides. This class of drugs belongs to insulin secretagogues which increase the secretion of insulin similar to the action of SUs but has a shorter half-life [69]. Repaglinide and nateglinide are the commonly studied drugs under this class. A study to compare the treatment efficacy of repaglinide versus glimepiride was conducted in T2DM patients fasting during Ramadan, by Anwar et al. The study inferred that glycemic control was better controlled in the glimepiride group as compared to the repaglinide group. However, there was no statistically significant difference with respect to the incidence of hypoglycemia between the two groups. In comparison to repaglinide, the study concluded that glimepiride may be a better choice of drug during Ramadan due to its longer duration of action [49]. Another study was carried out by Bakiner et al. to evaluate the efficacy of repaglinide three times a day along with a single dose of insulin glargine. The participants were grouped under the fasting and nonfasting groups. The results showed that glycemic control did not change during this period. Also, none of the participants from the fasting group reported a HE. Thus, combining repaglinide with insulin glargine was proved to be safer in T2DM patients who fast in the month of Ramadan [70]. More studies are required to confirm the safety of this class of drugs.

3.10. α-Glucosidase Inhibitors. AGIs act by inhibiting the enzymes that are responsible for the conversion of complex nonabsorbable carbohydrates into simple absorbable carbohydrates. This process delays the digestion rate, thereby leading to a reduction in the postprandial glucose (PPG)

and levels of insulin. Acarbose, miglitol, and voglibose are the drugs that fall under this class [71]. The review of the existing literature indicates a lower risk of hypoglycemia with this class of drugs and can be used without any dosage adjustment. However, gastrointestinal side effects remain the cause of concern [8, 9, 42, 43, 53]. Currently, there are no randomized control trials available that have investigated the use of AGIs in T2DM patients fasting during the Ramadan period. They can be used in Ramadan as monotherapy mainly due to their little chances of causing hypoglycemia; however, the treatment should be individualized.

3.11. Insulin and Its Analogs. Insulin is generally indicated in patients who are intolerant to one or more oral glucose-lowering drugs, who do not exhibit adequate glycemic control with oral monotherapy, and who personally prefer insulin over other oral glucose-lowering agents. An open-label randomized clinical trial by Hassanein et al. to compare the safety and efficacy of insulin degludec/insulin aspart (IDegAsp) and biphasic insulin aspart 30 (BIAsp 30) was conducted in T2DM patients fasting before, during, and after Ramadan. The treatment initiation period was fixed to a minimum of 8 weeks before the commencement of Ramadan. The dose of insulin was decreased at suhoor by 30–50% on the first day of Ramadan and readjusted back to pre-Ramadan levels at the end of the month. The patients were observed for another 4 weeks (post-Ramadan period). The treatment period witnessed similar glycemic efficacy in both arms. There was no significant difference observed between the two groups in terms of HbA1c reduction and change of fructosamine levels from baseline to end of Ramadan or end of 4 weeks post-Ramadan. Throughout the treatment period, the overall rate of hypoglycemia was significantly lower in the IDegAsp arm in comparison with the BIAsp 30 arm ($p < .0001$) [72]. The Ramadan study group compared Insulin lispro Mix25™ with human insulin 30/70 to determine their impact on morning and evening PPG control and on average daily blood glucose in T2DM patients fasting during Ramadan. All patients were administered human insulin 30/70 during the lead-in period. At visit 2, the patients were administered with Insulin lispro Mix25 (Humalog R Mix25™) for the initial two weeks of Ramadan, followed by therapy with human insulin 30/70 for the remaining two weeks or vice versa. Though the numbers of hypoglycemic episodes were reported to be similar in both the groups, the treatment with lispro Mix25 was associated with a lower average daily glycemia as compared with human insulin 30/70. There was no significant change in body weight reported in either group [73].

3.12. Meta-Analysis. The main objective of this meta-analysis was to compare the safety profile of DPP-4 inhibitors versus SU in T2DM patients fasting during Ramadan. The primary outcome analyzed for this purpose was the number of confirmed symptomatic HEs that occurred during the fasting period. The statistical analysis was carried out using Review Manager version 5.3. Odds ratio was calculated to compare the degree of fasting risk in T2DM patients with 95% Confidence Interval (CI) using the Mantel-Haenszel method. A random effects model was applied to determine the effect size.

Seven papers, with a total of 1723 participants, were included in this meta-analysis [47, 48, 51, 57, 58, 74, 75]. The sample size ranged between 30 and 669. Patients with T2DM age 18 or older were included. One study used sitagliptin [48] while the other six used vildagliptin [47, 51, 57, 58, 74, 75]. The seven studies compared DPP-4 with SU (glimepiride, gliclazide, glibenclamide, or glipizide). All the seven studies assessed HEs during the fasting period. Six studies assessed changes in HbA1c and body weight apart from HE [47, 51, 57, 58, 74, 75].

The number of hypoglycemic episodes with DPP-4 inhibitors was considerably lower compared to with SUs (107 vs. 266) (Figure 2). The meta-analysis indicates DPP-4 inhibitors would significantly reduce the risk of hypoglycemia as compared to sulphonylurea (odds ratio = 0.38, 95% CI: 0.26 to 0.55, p 0.00001). Among the DPP-4 inhibitors, vildagliptin was found to be an effective, safe, and well-tolerated regimen with a low incidence of hypoglycemia accompanied by good glycemic control. Physicians also recommend the use of vildagliptin over SU for use in high-risk populations such as the elderly. One of the studies reported that the use of a sitagliptin-based regimen decreased the risk of hypoglycemia compared with a SU-based regimen. Among the SUs, gliclazide was found to be much safer with a lower incidence of hypoglycemia as compared to other listed drugs in this category [48]. This meta-analysis concluded that DPP-4 inhibitors remain the preferred drug class among T2DM patients fasting when compared with SUs. One previous meta-analysis conducted by Gray et al. also reached at the same conclusion [76].

4. Limitations

The main limitation is that meta-analysis included all types of study design. Differences in the reporting methods of hypoglycemia whether it is hypoglycemia incidence, hypoglycemia event, or symptoms in the studies are included. This can lead to publication bias as symptoms are subjective to an individual. Further, our study evaluated only patients with T2DM who wish to fast during Ramadan, and thus, the conclusion cannot be generalized to T1DM patients. We performed the extraction of literature for review from only one search engine (PubMed). Publication bias could not be established as the number of articles included for meta-analysis is less than 10. However, visual inspection of the funnel plot indicates a slight asymmetry which might be subjective.

5. Conclusions

Our review indicates that structured education and counselling play an important role in preventing the complications associated with Ramadan fasting in T2DM patients. Structured education and diabetes treatment program during Ramadan in patients with T2DM who wish to fast, empowered patients to self-manage diabetes, lose weight, improve glycemic control, and avoid other complications of fasting.

Ramadan-specific structured education along with telemonitoring also decreased the symptomatic hypoglycemia in T2DM patients, thus achieving safer fast during Ramadan. Early and effective counselling at least one to two months prior to Ramadan with a particular emphasis on blood glucose measurement, information about signs and symptoms of hyperglycemia and hypoglycemia, changes in meal pattern, physical activity, and medications is recommended according to the risk stratification of the T2DM patients willing to fast to avoid complications during Ramadan. A patient-focused educational program that encourages optimal health care during the fasting period is the need of the hour. Both the healthcare providers and T2DM patients are recommended to have customized education preferably in their regional language for a safe Ramadan fasting. Regarding the choice of glucose-lowering drugs during Ramadan, DPP-4 inhibitors are the preferred class because of their safety and efficacy. Our meta-analysis corroborates this finding. However, we recommend assessing the safety and efficacy profile of other classes of drugs such as SGLT2is, TZDs, meglitinides, and AGIs through randomized clinical trials as the literature on the use of drugs in these categories during Ramadan is scarce.

Abbreviations

AGIs: α-Glucosidase inhibitors
BMI: Body mass index
DPP-4: Dipeptidyl peptidase 4
GDP: Gross Domestic Product
GLP-1: Glucagon-like peptide-1
HbA1c: Glycated hemoglobin
HE: Hypoglycemic event
IDF-DAR: International Diabetes Federation-Diabetes and Ramadan (DAR) International Alliance
MENA: Middle East and North Africa
MESH: Medical Subject Headings
PICOS: Population Intervention Comparison Outcome Study Design
PPG: Postprandial glucose
PRISMA: Preferred reporting items for systematic reviews and meta-analysis
SU: Sulphonylurea
SGLT2i: Sodium-glucose cotransporter 2 inhibitor
T1DM: Type 1 Diabetes Mellitus
T2DM: Type 2 Diabetes Mellitus
TZDs: Thiazolidinediones.

Data Availability

The data supporting this systematic review and meta-analysis are from previously reported studies and datasets, which have been cited.

Conflicts of Interest

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

Authors' Contributions

RS conceived the idea; designed, analyzed, and interpreted data; and revised the draft manuscript. AA contributed to extraction of data from the database and writing manuscript. ST contributed to extraction of the data from the database and reviewing the manuscript. AB was involved in study design and critically reviewed and revised the manuscript. JT and MA were involved in critical review and revision of the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Supplementary 1. Table 1: characteristics of included studies.

Supplementary 2. Table 2: characteristics of classes of drugs and studies.

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Review Article

An Insight into Potential Pharmacotherapeutic Agents for Painful Diabetic Neuropathy

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Diabetes is the 4th most common disease affecting the world's population. It is accompanied by many complications that deteriorate the quality of life. Painful diabetic neuropathy (PDN) is one of the debilitating consequences of diabetes that effects one-third of diabetic patients. Unfortunately, there is no internationally recommended drug that directly hinders the pathological mechanisms that result in painful diabetic neuropathy. Clinical studies have shown that anticonvulsant and antidepressant therapies have proven fruitful in management of pain associated with PDN. Currently, the FDA approved medications for painful diabetic neuropathies include duloxetine, pregabalin, tapentadol extended release, and capsaicin (for foot PDN only). The FDA has also approved the use of spinal cord stimulation system for the treatment of diabetic neuropathy pain. The drugs recommended by other regulatory bodies include gabapentin, amitriptyline, dextromethorphan, tramadol, venlafaxine, sodium valproate, and 5 % lidocaine patch. These drugs are only partially effective and have adverse effects associated with their use. Treating painful symptoms in diabetic patient can be frustrating not only for the patients but also for health care workers, so additional clinical trials for novel and conventional treatments are required to devise more effective treatment for PDN with minimal side effects. This review gives an insight on the pathways involved in the pathogenesis of PDN and the potential pharmacotherapeutic agents. This will be followed by an overview on the FDA-approved drugs for PDN and commercially available topical analgesic and their effects on painful diabetic neuropathies.

1. Introduction

A number of reviews on peripheral neuropathy have been published in general and on diabetic neuropathic pain, in particular [1]. Most of these reviews gave us an insight about the classification and mechanism of painful diabetic neuropathy (PDN) which can help us in developing correct diagnosis and successful treatment against it. An ideal treatment for PDN can be defined as those compounds that prevent the progressive loss of nerve function and improve the symptoms with minimal side effects. Several combinations of drugs have been approved by the Food and Drug Authority (FDA), American Academy of Neurology (AAN), American Association of Clinical Endocrinologists (AACE), American Diabetes Association (ADA), European Federation of Neurological Sciences (EFNS), and National Institute of Clinical Examination (NICE) which have been proven partially fruitful in managing symptoms of PDN. Many other drugs are under trials, and some drugs have been withdrawn from the market as they pose serious health risks in long-term usage [2]. Management of PDN is still challenging because of its complex and underassessed pathophysiology.

Neuropathic pain is defined by International Association for the Study of Pain (IASP) as "pain caused by a lesion or disease of the somatosensory nervous system" [3]. Diabetes mellitus (DM) is a complex metabolic disorder which is characterized by high blood glucose levels, due to inadequate insulin secretion by the pancreas or inability of target cells to reuptake glucose from the blood [4]. Diabetic peripheral neuropathy (DPN) is one of the consequences of diabetes which is accompanied by other risks of getting cardiovascular, peripheral, and cerebrovascular diseases [2]. Globally,

diabetes was the 7th leading cause of death in 2016 [5], and according to "The Lancet Commission on Diabetes: using data to transform Diabetes Care and Patient Lives" report published by International Diabetes Care, the number of people affected by DM is 463 million which is 5.9% of total world population, and it is expected that by 2045, 629 million (6.3%) people are to be affected by DM [6]. In general, peripheral diabetic neuropathy and more specifically painful peripheral diabetic neuropathy are the main causes that deter the quality of life and lead to high morbidity rate. A study conducted by Baxi et al. on larger population shows that 28.85% of the diabetic population suffered from DPN and out of which 88% of the population showed pain symptoms [7].

In this paper, the metabolic pathways involved in the pathogenesis of PDN and the potential targets for its treatment will be discussed. Followed by which, a brief overview of the topical agents available in the market and the FDA-approved drugs will be provided. In the end, the nonpharmacological treatment modalities including the recently FDA-approved spinal cord stimulation system will also be discussed.

2. Methodology

A comprehensive literature review was undertaken, incorporating articles from electronic databases (Google Scholar and PubMed) by using keywords like "diabetes, painful diabetic neuropathies, pathological mechanisms, anticonvulsants drugs, FDA/EU approved drugs etc." and other relevant lists of articles with author name. Explanatory data from those articles was taken and incorporated in this review in a descriptive manner. All the preclinical and clinical studies mentioned in this review solely focus on painful diabetic neuropathy and not on any other form of neuropathies.

3. Classification

Diabetic neuropathy can be classified into two broad categories: diffuse and focal neuropathies. Diffuse neuropathies branch into diabetic peripheral neuropathy (DPN) and diabetic autonomic neuropathy (DAN). Peripheral neuropathies usually affect the nerves present in the extremities. Both small and large nerve fibers are affected by DPN. Damage to large nerve fibers interferes with the body movement and body position whereas demyelination of smaller nerve fibers in the peripheral region causes dysesthesias and paresthesia linked with neuropathic pain [8]. There are numerous types of peripheral diabetic neuropathy, the most common being the distal symmetry diabetic sensorimotor polyneuropathy (DSPN). DSPN accounts for 75-90% of all the diabetic neuropathy cases and can present itself as either painful (pDSPN) or nonpainful. The clinical appearance of the most prevalent forms of diabetic neuropathy, as well as the progressive sensory loss that occurs throughout the course of DSPN, are depicted in Figure 1. In pDSPN, burning, stabbing, numbing, or deep ache pains are felt in the periphery where multiple neurons are affected [9]. Oxidative stress caused by diabetes due to underlying pathogenic events results in the sensory sensitization and demyelination of neurons in the periphery. A study conducted on rats showed that metabolic flux is the primary cause of demyelination and progression of peripheral neuropathy [10]. Diffused autonomic neuropathy is associated with poor lifestyle and progresses slowly. It affects the physiological systems which are controlled by autonomic nervous system, i.e., cardiovascular, gastrointestinal, and genitourinary [1].

Mononeuropathies affect the medial, ulnar, and lateral popliteal nerves [11]. Radiculoplexopathy affects the motor neurons in the lumbosacral region [12]. Sensory losses are difficult to detect and may remain hidden for a longer period. Both the above-mentioned neuropathies come under focal and multifocal neuropathies which are less common as compared to peripheral neuropathies.

Painful distal symmetrical diabetic sensorimotor polyneuropathy (pDSPN) is mostly referred to when painful diabetic neuropathy (PDN) is stated, as it is the most common subtype of PDN. The other subtypes of PDN include mononeuritis multiplex, mononeuropathy, small fiber neuropathy, diabetic lumbosacral radiculoplexus neuropathy, and treatment-induced neuropathy. pDSPN or PDN is defined as "pain caused by a lesion of the somatosensory system attributed to diabetes" [13]. This article will focus on painful diabetic neuropathy, and hereinafter, PDN will indicate pDSPN throughout this text.

4. Systemic/Localized/Topical Treatments

Systemic therapies affect the entire body whereas localized therapies target the specific injured areas. Multiple drugs are used in systemic treatment and management, but there are a few drugs for the local management of PDN. The anticonvulsants, antidepressants, and opioids recommended for PDN are given as systemic therapies [14, 15]. These drugs are administered orally which provides temporary relief from pain but exhibits adverse side effects, e.g., dizziness, dry mouth, and muscle weakness [16]. Topical treatment options are available in the form of 5% lidocaine patch, capsaicin, and nitric oxide spray to manage pain symptoms. Recently, topical treatments have been in the spotlight as they pose lesser health risk compared to orally administered treatment options, but they are still not efficacious enough [17].

5. Metabolic Pathways of Painful Diabetic Neuropathy (PDN) and Potential Therapeutic Agents

The potential therapeutic agents for PDN include the inhibitors of signaling molecules or activators of suppressor signals indicated in the pathophysiology of painful diabetic neuropathy (Figure 2).

5.1. Polyol Pathway. Polyol pathway comprises two main enzymes named as aldose reductase (AR) and sorbitol dehydrogenase which are responsible for the metabolism of excessive glucose [18]. AR converts glucose in the presence of NADPH cofactor into sorbitol, which is in turn converted into fructose in the presence of NAD^+. A small amount of

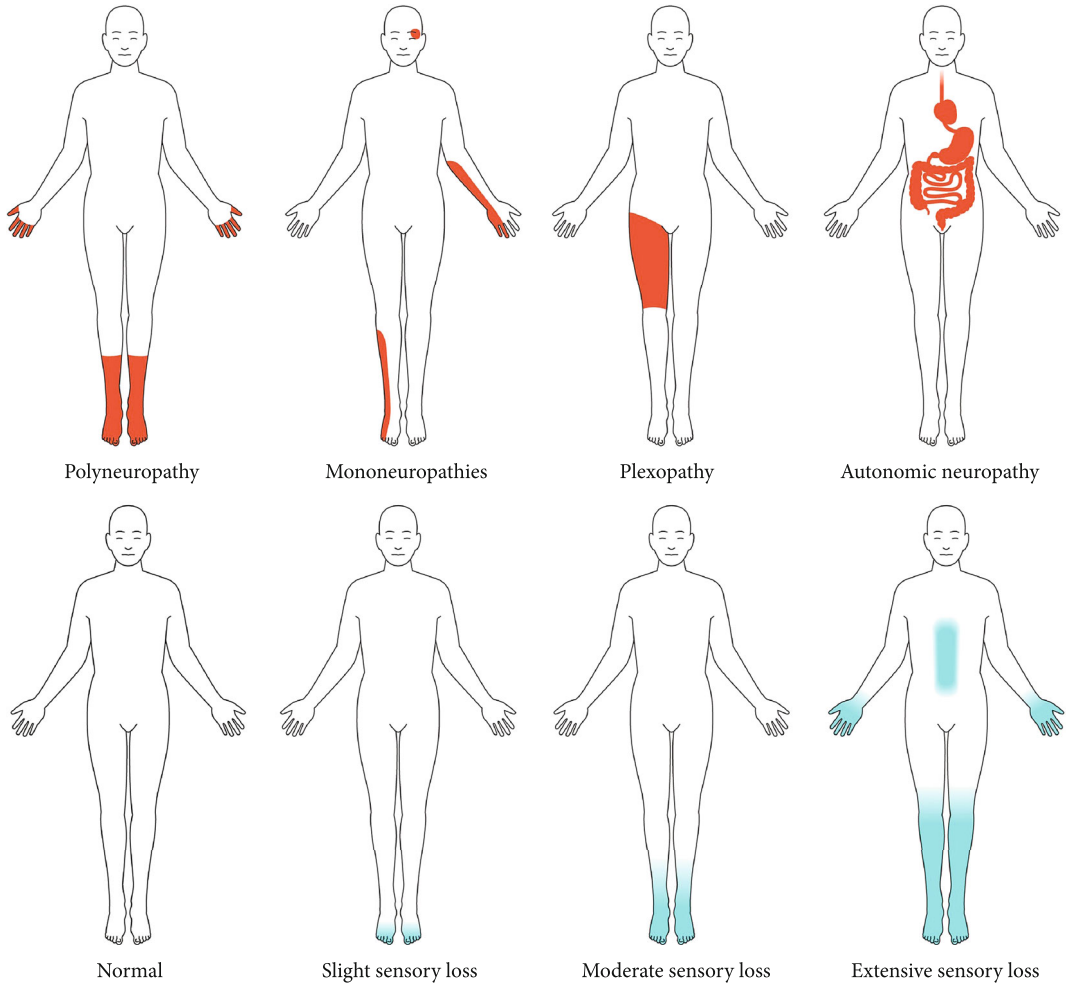

Figure 1: Clinical appearance of the most prevalent type of diabetic neuropathy and progression of sensory loss over the course of DSPN. Adapted from [13] (CC BY 4.0)

glucose concentration gets metabolized through this pathway as AR exhibits lower affinity for glucose. In hyperglycemic state, the excess glucose is consumed using this pathway resulting in an increase in NADPH level and reductive stress [19]. This stress along with the mitochondrial dysfunction impairs the Schwann cell function causing compromised myelination, abnormal neurotrophic support to the axon, and therefore a loss of axon function [20]. Because of increased sorbitol and fructose concentration, events like reduced efflux of myoinositol, inhibition of ATP synthesis, and a resultant compromised Na^+ and K^+ ATPase activity are observed. Additionally, axon-glia dysfunction and reduced nerve conduction velocity because of structural degeneration of nerves are also examined. It also causes the downregulation of glutathione reduction pathway which causes the accumulation of free radical and peroxides, thus aggravating the nerve damage and resulting in NO-mediated vasodilation [21].

Inhibition of Aldose reductase pathway is one of the primary targets for many therapeutic agents. Epalrestat is reversible aldose reductase inhibitor which has been proven effective against peripheral diabetic neuropathy. Clinical studies demonstrated the alleviation of spontaneous pain in the lower limbs of 48.6% diabetic patients [22]. Epalrestat, a carboxylic derivative, has been found beneficial in inhibiting polyol pathway and shielding against nerve damage with no side effects until recently a study conducted by Le et al. highlighted the induction of liver fibrogenesis due to increased oxidative stress [23, 24]. Studies conducted on tolrestat, zenarestat, and sorbinil suggested their withdrawal from human use because of their adverse effects, i.e., liver dysfunction, increased creatinine levels, and serious hypersensitivity, respectively [25]. Other aldose reductase inhibitors (ARIs) (fidarestat, ponalrestat, zopolrestat, and lidorestat) have been used for the management of diabetic complication, but due to their adverse effects, they are not capable of producing desired outcomes (Singh [26]). Ranirestat is one of the ARI that has advanced to human trials, because it demonstrated positive results in improving the nerve conduction velocity, sensory perception, and nerve fiber density in patient suffering from diabetic polyneuropathy, but the effects on PDN are yet to be investigated [27, 28].

5.2. Hexosamine Pathway. One of the glycolysis product fructose-6-phosphate is converted into glucosamine-6-phophate in the presence of glutamine fructose-6-

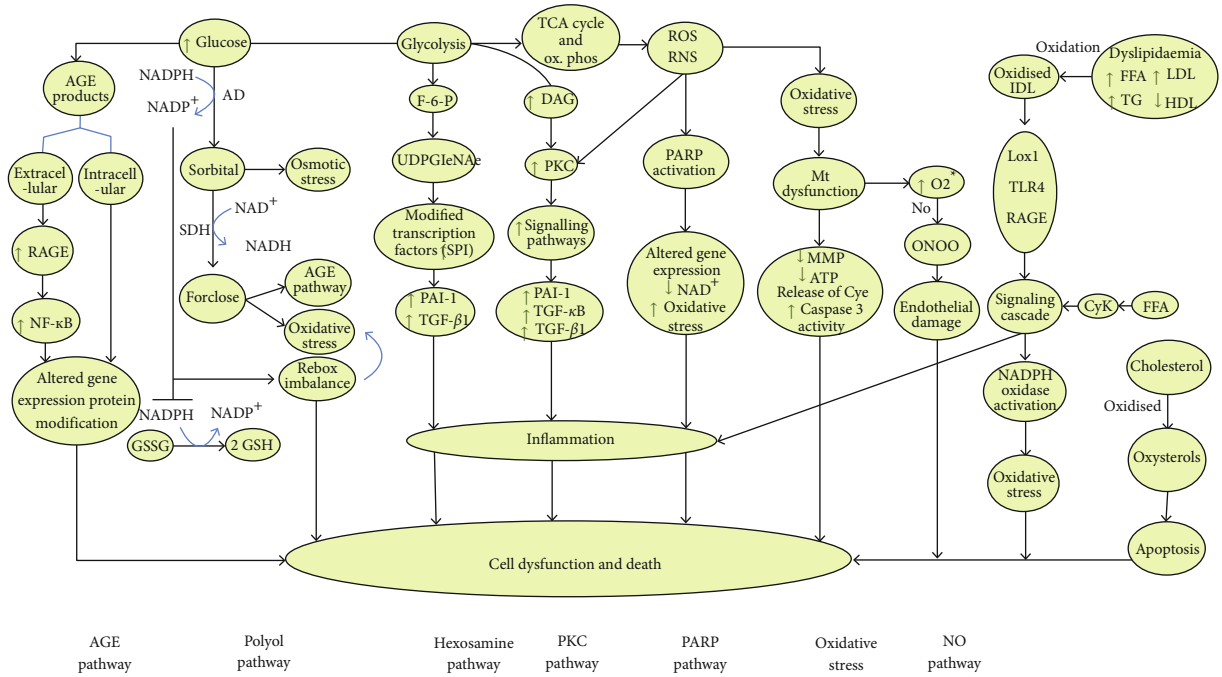

Figure 2: Important pathophysiological pathways involved in diabetic neuropathy [47]

phosphate amidotransferase (GFAT) which is in turn converted into uridine-5-diphosphate-N-acetyl glucosamine [29]. In type 2 diabetes, glutamine fructose-6-phosphate amidotransferase (GFAT) is involved in insulin resistance and hyperinsulinemia, while the end product of this pathway, uridine-5-diphosphate-N-acetyl glucosamine, causes gene transcription factor specificity protein 1 (Sp1) to increase which then activates the transforming growth factor beta (TGF-β) and plasminogen activator inhibitor-1 (PAI-1), responsible for damaging endothelial cells and prompting smooth muscle cell division. This further leads to microvascular complications, especially diabetic neuropathy which damages the vessels supplying blood to the nerves [19, 30].

Benfotiamine reduces the production of advanced glycosylation end products by impeding glucose metabolism via the hexosamine pathway. In 2008, a study was conducted to evaluate the safety and efficacy of benfotiamine in diabetic polyneuropathy patients. It was observed that the drug ameliorated pain to a greater extent, but the results were not statistically significant [31]. Recently, the effect of coadministration of benfotiamine and alpha lipoic acid (free radical scavenging drug) was assessed in a total of 120 patients diagnosed with PDN. The drugs were orally administered with the coadministration test group given a dose of 300 mg/day and 600 mg/day respectively. It was found that the coadministration of these drugs exhibited a more pronounced efficacy than the monotherapy of the individual drugs [32].

5.3. Protein Kinase C Pathway. Multiple studies have confirmed the involvement of protein kinase C (PKC) in diabetic neuropathy [33]. Protein kinase C consists of serine/threonine protein kinase family which are responsible for many cellular processes and affect signaling transduction cascade associated with apoptosis, differentiation, and proliferation. In general, PKC isoforms in humans are divided into three subtypes; classical, atypical, and novel, depending on the secondary messenger they require for activation. Upregulation of diacylglycerol and Ca^{+2} activate conventional PKC isoforms (α, βI, βII, and γ); on the other hand, only diacylglycerol (DAG) is responsible for the activity of novel isoforms of PKC (δ, ε, η, and θ). Atypical isoforms get activated in the absence of diacyl glycerol (DAG) and Ca^{+2} and consist of protein kinase Mζ and ι/λ isoform. According to Borghini et al., protein kinase isoforms α, βI, βII, γ, ε, and δ are present in the nerves [34].

In the diabetic condition, PKC pathway originates from the glyceraldehyde-3-phosphate of the glycolysis pathway. As part of PKC pathway, the glyceraldehyde-3-phosphate is converted to dihydroxyacetone which is then converted into glycerol-3-phosphate and ultimately into DAG. DAG and/or advanced glycation end products (AGEs) activates the PKC which then upregulates a number of signaling cascades by protein phosphorylation. PKC is involved in the activation of vascular endothelial growth factor (VEGF), plasminogen activator ihibittor-1 (PAI-1), transforming growth factor beta-1 (TGF-β), and nuclear factor kappa-B (NF-κB) which leads to microvascular complications. PKC also down regulates Na^+/K^+ ATPase causing normalization of sciatic nerve conduction velocity and nerve blood flow (Simran et al. 2019). It has been evidenced that the inflammatory reaction induced by hyperglycemia activates the PKC pathway which in turn phosphorylates the transient receptor potential vanilloid (TRPV1), present in the plasma membrane of Aδ and C fibers (nerve fibers for relaying pain). The phosphorylation of TRPV1 results in hypersensitivity of nociceptors and causes pain in diabetic state [35, 36].

By inhibiting PKC in diabetic rats, reduction in hyperexcitability of C-fiber and hyperalgesia was observed. This may be because of the upregulation of the P2X3 receptor in the dorsal root ganglia by PKC in response to the nerve injury. P2X3 is an ionotropic receptor for ATP expressed in the DRG nociceptors [37]. Berberine, a plant alkaloid, was found to relieve PDN in rats by inhibiting TNF-alpha (a proinflammatory cytokine) and modulating PKCε and TRPV1 [36].

5.4. AGEs (Advanced Glycation End Products). Accumulation of advanced glycation end products (AGEs) and their receptor (receptor for advanced glycation end products RAGE) occurs when glucose and other saccharides undergo nonenzymatic reaction which modifies the structure and function of lipids and proteins [25]. Several studies on AGEs have shown that AGEs along with methylglyoxal cause vascular damage. Elevated levels of AGE-RAGE and reduced levels of glyoxalase-1 (which is responsible for the detoxification of methylglyoxal) were reported in type 1 diabetic patients [38]. AGE-RAGE presence has been confirmed in endothelial and Schwann cells. High levels of AGEs cause diabetic neuropathy by elevation of p65 subunit of NF-kB which triggers inflammation and injury in myelinated neuron. AGEs are also associated with induction of apoptosis of Schwann cells [39]. Oxidative stress because of NADPH oxidase activity happens due to neuronal AGE-RAGE interaction [40, 41].

Many recent studies have targeted AGEs in an attempt to ameliorate diabetic neuropathy and painful diabetic neuropathy. Xu et al. reported that the coadministration of AGEs and 1,25-$(OH)_2D_3$ to Schwann cells resulted in suppression of apoptosis (induced by AGEs) through PKA-NF-κB pathway, and it was concluded that vitamin D may be investigated further for diabetic neuropathy [39]. This study complies with the findings by Basit et al. who demonstrated that the intramuscular injection of vitamin D relieves PDN symptoms in the Pakistani population [42]. Therefore, the administration of vitamin D may potentially promote the neuroprotective effects of Schwann cells by neutralizing the catastrophic effects of AGEs. Similarly, interleukin 10 (IL-10, an anti-inflammatory cytokine) was also observed to protect Schwann cells from the effects of AGE via NF-κB pathway [43]. Another research disseminated the partial alleviation of PDN followed by the administration of pyridoxamine in diabetic rats. Pyridoxamine exhibited this activity by inhibiting the effects of RAGE-NF-κB/ERK signaling pathway [44]. Recently, the neuroprotective activity of a medicinal herbal formulation Compound XiongShao Capsule was also published. It was observed that the formulation suppressed thermal and mechanical hyperalgesia significantly by decreasing serum advanced glycation end products, superoxide dismutase, and nitric oxide synthase levels and by inducing apoptosis [45].

5.5. Oxidative Stress. The oxidative stress is created intracellularly when there are more free radicals produced than they are being eliminated or utilized by the pathways or enzymes. Oxidative stress plays a key role in diabetic neuropathy. It is mainly caused by free oxygen and nitrogen reactive species like hydroxyl group, hydrogen peroxide, and superoxide. Nitrosative stress is caused due to reactive agents like peroxynitrite and nitrotyrosine which can cause diabetes-induced pain [46]. These free-radicals are generated as a result of shunting of excess glucose to polyol pathway, hexosamine pathway, PKC pathway, and AGE-RAGE interaction [47] which further result in the buildup of cytotoxic metabolites and NADPH overconsumption. These factors combine to enhance intracellular redox stress and aberrant protein, lipid, and DNA changes, resulting in mitochondrial damage and ROS overproduction. The loss of Schwann cells, myelinated axons, and sensory neurons in the dorsal root ganglia brings harm to the peripheral nervous system. Furthermore, inadequate mitochondrial energy generation impairs the ability to transport information down the axons and aggravates axonal damage in diabetic neuropathy. Collectively, oxidative stress in hyperglycemic patient is caused due to increase in lipid peroxidation, GSSH/GSH ratio, 4-hydroxynonenal protein adduct, taurine, and quinine reductase activity in diabetic patients and results in hypoxia, nerve conduction velocity, apoptosis of Schwann cells and neurons, loss of neurotrophic system, and mitochondrial dysfunction. Nrf2 is a transcription factor triggered by the redox status in the milieu and functions to regulate the antioxidant system while NF-κB—another transcription factor—is implicated in the production of inflammatory response. In healthy cells, the regulation of both these factors is coordinated to maintain the redox balance, but in diabetic neuropathy, this balance is disturbed (Ganesh [48]).

A number of agents targeting the oxidative-nitrosative stress have been assessed in the past few decades in an attempt to ameliorate the diabetic neuropathic pain. Berberine has been evidenced to partially bring down the blood glucose level (BGL) and body weight and suppress thermal and mechanical hyperalgesia in diabetic rats. It was suggested that the subject compound ameliorates diabetes and PDN by controlling the elevated oxidative stress and inflammation in the neurons [49]. Likewise, tocotrienol in combination with insulin was observed to reverse PDN in diabetic rats by controlling oxidative-nitrosative stress, caspase 3, and proinflammatory cytokines [46]. Nerunjil (*Tribulus terrestris*) was demonstrated to ameliorate pain threshold in PDN by regulating the oxidative stress and inflammatory response [50]. On the other hand, fisetin was reported to relieve thermal and mechanical pain in diabetic neuropathic rats by normalizing the regulation of Nrf2 and NF-κB [51]. Additionally, *Rosmarinus officinalis L.* has an antinociceptive and neuroprotective effect in diabetic rats, due to its ability to decrease caspase and Bax-Bcl-2 ratio which are the key signaling molecules in causing apoptosis. The neuroprotective activity of the plant is also because of its antioxidant and radical-scavenging activity. Signification effect was seen in the form of reduced thermal hyperalgesia in STZ-induced diabetic rats [52]. Partial reduction in pain has been reported in an animal model of hyperglycemia when treated with kaempferol extracted from *Eruca sativa* [53].

Alpha lipoic acid, a widely tested drug for diabetic neuropathy, appears to slow down or cure it by exhibiting multiple antioxidant activities. Administration of alpha lipoic acid results in an increase in reduced glutathione, which is a vital endogenous antioxidant. A dose of 600 mg alpha lipoic acid was observed to ameliorate neuropathic defects hyperalgesia, numbness, and paresthesia in clinical trials [54–56].

Acetyl L-carnitine has been evidenced to relieve PDN symptoms [57, 58] by a number of mechanisms including antioxidant [59], cytoprotective, and antiapoptotic activity. Its analgesic activity is mediated by bringing down glutamate level in the synapse, contributing majorly via the epigenetic mechanism in which a transcription factor, p65/Rela, of the NF-κB family is acetylated. The acetylated transcription factor then facilitates the upregulation of type-2 metabotropic glutamate (mGlu2) receptors in the DRG and dorsal horn, resulting in a decline in glutamate release from the nociceptors (Di [60, 61]).

5.6. PARPs (Poly ADP-Ribose Polymerase). Under normal circumstances, poly ADP-ribose polymerase (PARP) is involved in repairing DNA and inducing apoptosis. Excessive PARP leads to tissue damage in diabetes mellitus. Hyperglycemic conditions lead to the formation of reactive nitrogen and oxygen species in which single DNA breaks are severed (single-strand DNA break). The consequent upregulation of PARP causes depletion of NAD^+ in the cell (thus decelerating glucose metabolism and energy generation) and ribosylation of ADP for the production of glyceraldehyde-3-phosphate dehydrogenase (GADPH) because of which vessels supplying the blood to nerves get damage [62]. PARP activation is also associated with nerve conduction deficit in sensory and motor nerves, dysfunction of neurovascular system, gene expression, alteration of transcriptional regulation, and energy failure processes in diabetic animals [63].

In the rodent model of PDN, 1,5-isoquinolinediol (which is a PARP inhibitor) was demonstrated to ameliorate the thermal hyperalgesia, tactile allodynia, and mechanical hyperalgesia [64]. Additionally, 10-(4-methylpiperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-one which is another PARP inhibitor when administered orally in the rodent model of PDN resulted in partial alleviation of PDN symptoms along with reduction of intraepidermal nerve fiber degeneration [65].

5.7. MAPKs (Mitogen-Activated Protein Kinases). Mitogen-activated kinases are subdivided into extracellular signal-related kinase (ERK), p38, and c-Jun N-terminal kinase (JNK); all are involved in signal transduction. ERK domains 1 and 2 are associated with neural survival, while JNK and p38 facilitate the neural apoptosis. Upregulation of these three leads to neuropathic pain. JNK upregulation causes phosphorylation of neurofilaments, and downregulation causes neuronal regeneration in diabetic rats [63]. Furthermore, research suggests that the role of long noncoding RNAs in PDN is because of the activation of ERK1/2 and p38 MAPK, and their inhibition may heighten the threshold of thermal and mechanical pain sensitivity, thus relieving PDN [66]. The long noncoding RNAs will be discussed in detail later.

It has been reported that p38 MAPK inhibitors: SB203580 and SD-282, JNK inhibitor: SP600125, and MAPK inhibitor: U0126, have a role in fixing mechanical allodynia and hyperalgesia in diabetic rats [67–69]. It was also reported that berberine exhibits its neuroprotective efficacy in diabetic neuropathy by regulating the MAPK pathway [70] in addition to modulating PKC and inhibiting TNF-alpha in PDN [36].

5.8. NF-κB (Nuclear Factor Kappa Light Chain Enhancer of Activated B Cells). Immune responses and apoptosis are regulated by transcription factors like NF-κB and are triggered by inflammatory stimuli. Studies revealed that the activated NF-κB is found in sciatic, sural nerve and DRG of diabetic animals. NF-κB activity was more prominent in Schwann cells cultivated in higher glucose concentration as compared to cell grown in low glucose medium. Overexpression of p65 subunit of NF-κB causes inflammatory demyelination [71, 72] and oxidative-nitrosative stress which induce insults in the nerve fibers and the vessels supplying blood to these tissues resulting in impaired blood supply and elevated release of inflammatory mediators: prostaglandins and bradykinins. This sequence of events increases the sensitivity to noxious stimulus resulting in neuropathic pain (Ganesh [48]).

Bioactive extracts from Annona reticulata Bark (or) Zizyphus jujuba Root bark have shown positive results in PDN by decreasing the oxidative stress and inhibiting the cascade of NF-κB [73]. Alpha lipoic acid is also found to relieve PDN in diabetic rats by modulating NF-κB cascade and TRPV1 expression [74]. Fisetin has been shown to reduce heat and mechanical stimulus-related hyperalgesia in diabetic animals by restoring Nrf2 and NF-κB regulation [51].

5.9. Hh (Hedgehog). Hh family consists of proteins that usually express in peripheral nervous system and play role in cell growth, cell fate, and survival. The insult to the peripheral nerve initiates a process of degeneration and regeneration during which Hh pathway plays a leading role. The effectors of these pathways Sonic Hedgehog and Desert Hedgehog are involved in the nerve regeneration. Sonic Hedgehog initiates the neovascularization in the vicinity of the injured nerve to facilitate regeneration in diabetic rats [75]. A recent study demonstrated that the upregulation of microRNAs, miR-9 and miR-29a, leads to diabetic neuropathy and the painful symptoms. This was evidenced to be instigated via insulin gene enhancer binding protein-1- (ISL1-) mediated activation of the sonic hedgehog signaling pathway [76]. Decreased Hh levels were seen in diabetic animals causing downregulation of motor neuron conduction velocity, sensory nerve conduction velocity, and reduced pain perception to heat, nerve growth factor and nerve blood flow. But blocking of Hh pathway showed decreased pain perception and neuropathic pain in diabetic rodents, possibly witnessed due to increased endothelial cell permeability, and decreased claudian5 expression [77].

5.10. Inflammatory Cytokines. Greater than 30 isoforms of interleukin exist which are classified into anti-inflammatory ILs (IL-4 and IL-10) and proinflammatory (IL-1 beta, IL-6, and IL-8). TNF-alpha is another proinflammatory cytokine that is activated by different immune cells like lymphocytes, natural killer cells, macrophages, and mast cells which confirms that their upregulation produces an immune response. Proinflammatory cytokines are mostly involved in pathogenic signal transduction in diabetic neuropathy. Yu-Wen et al. revealed the substantially elevated levels of IL-6 and TNF alpha in the peripheral nerves and spinal cord of sedentary STZ-induced diabetic rats and the role of these cytokines in PDN [78]. It is well recognized that TNF alpha is also elevated in the human subjects of PDN and the level of TNF alpha is directly related to the severity of pain. The TNF alpha and iNOS immunoreactivity is also prominent and related to pain in PDN patients [79]. The increased level of IL-10 was also noticed in PDN patients, and it is believed that this increment may be the result of the activation of the compensatory mechanism [80, 81].

Minocycline relieves the diabetic neuropathic pain in STZ-treated rats and potentiates the analgesic effects of morphine by upregulating the production of IL-10, IL-2, IL-1 alpha, and sTNF RII. Furthermore, it is believed that minocycline can inhibit PARP and pancreatic beta cell necrosis [82]. It was also observed that neural mobilization (discussed later in detail) in the STZ-induced diabetic rats reduced the mechanical allodynia by cutting the levels of TNF-alpha and IL-1beta [83]. Curcumin derivative J147 is another neuroprotective and powerful neurogenic drug candidate which promotes AMP kinase pathway and inhibits TNF-α and other neuroinflammatory markers that cause neurodegeneration, thereby reversing the touch triggered allodynia [84].

5.11. COX (Cyclooxygenase). Two forms of cyclooxygenase enzymes (COX) are reported COX-1, involved in cellular hemostasis and COX-2 which remains silent under normal circumstances and are activated under high glucose level, oxidative stress, PKC activation, and inflammatory cytokines [85]. Studies suggested that COX-deficient rodents are resistant to diabetes related complications like decreased nerve conduction velocity, reduced blood flow around myelin sheath, and diminished nerve fiber density [86].

The combinatorial administration of nimesulide (COX-2 inhibitor) and Rutin (targeting Nrf-2/HO-1) was corroborated to raise the pain thresholds in the diabetic rats [87]. Likewise, celecoxib which is a selective COX-2 antagonist is recognized for countering allodynia and hyperalgesia in diabetic rats. The suggested mechanism of action is the modulation of opioid receptor or voltage-gated sodium and potassium ion channels [88]. The synergistic administration of proglumide (nonselective cholecystokinin (CCK) inhibitor receptor) and celecoxib resulted in a significant reduction of painful symptoms in diabetic rats [88]. Meloxicam, another COX-2 antagonist, is also suggested to relieve allodynia in diabetic rodents [89]. COX-2 inhibitor (SC-58125 and NS-398) when administered intrathecally produced a pronounced antihyperalgesic effect in diabetic animals [90].

Nonsteroidal anti-inflammatory drugs which include ibuprofen, acetaminophen, and aspirin are COX inhibitors and are widely prescribed for relieving pain, but their efficacy is not proven for PDN in humans [91]. The efficacy and safety of the combined administration of Tramadol (an Opioid) and Acetaminophen (an NSAID) were evaluated for PDN. The combination ameliorated the PDN symptoms: pain, sleep quality, mood, anxiety, and quality of life, but the study was discontinued ahead of time because of the adverse outcomes [92]. In another study, it was witnessed that the coadministration of selective serotonin reuptake inhibitors and aspirin increased the risk of gastrointestinal (GI) bleeding [93]. Further, it is suggested that administration of these drugs may also impair the renal function in addition to causing GI bleeding in diabetic patients [94, 95].

5.12. NGF (Nerve Growth Factors). Nerve growth factor as the name suggest is related to nerve propagation and development. Abnormal increase and decrease in NGF concentration can cause serious neuronal damage as it affects many pathways of survival. Some other factors like glia cell-derived neurotrophic factor, brain derived neurotrophic factor, and neurotrophic (NT-3, NT-4, and NT-5), and insulin growth factor I and II are also involved in propagation, angiogenesis, sensitization, and cell growth. NGF is responsible for the propagation of small nerve fiber and sympathetic neurons. Retrograde axonal transportation and NGF-dependent sensory neurons with diminished expression of neuropeptides substance P and calcitonin gene-related peptide (SP, CGRP) are affected by the absence of TRKA (tropomyosin receptor kinase A) in hyperglycemia (Tomlinson, Fernyhough, and Diemel 1997). In diabetic rats, reduced skin fiber through SP was found in association with decreased level of NGF which can act as an evidence for developing polyneuropathy other than vascular and metabolic changes. NGF also affects the developmental and regulatory pathways of cardiac nervous system.

In addition, in PDN, the cutaneous neurotrophin nerve growth factor (NGF) level rise. NGF gives rise to mechanical allodynia in mouse models [96], and NGF/p38 signaling enhances intraepidermal nerve fiber density (IENFD) in PDN [96, 97]. NGF stimulates cutaneous nociceptors in humans and is thought to be the source of hypersensitivity and hyperalgesia in PDN [98]. It has a two-edged effect. NGF levels in the dorsal root ganglion (DRG) and dorsal horn in rat models, when explored, were discovered to drop in the DRG 1 week after diabetes induction and in the dorsal horn 2 weeks after diabetes induction. Hyperalgesia is caused by decreased NGF expression in the DRG, while allodynia is caused by decreased NGF expression in the dorsal horn of the spinal cord. Exogenous NGF has been shown to alleviate diabetic neuropathic pain [99]. But it is already known that the administration of endogenous NGF in PDN patients resulted in pain relief in phase II trial but failed to perform better than placebo in the phase III trial [100–102].

5.13. Autophagy. Autophagy is a metabolic pathway triggered by oxidative stress in which cytoplasmic materials are sent to the lysosome for breakdown and reuse of the by-products. It comprises molecules which are key players of recycling pathways and maintain cellular homeostasis. Autophagosomes, a double membrane bounded vesicle, are formed when a phagophore internalizes a damaged component from the cytoplasm. Degradation machinery is a combination of autophagosomes and lysosomes [103]. The downregulation of autophagy in PDN is mediated by P13K/AKT/mTOR. It was found that autophagy downregulation in spinal cord could play a role in the etiology of PDN while the maintenance of PDN is somewhat aided by increased autophagy in the spinal cord. It was revealed that rapamycin injection reduced the mechanical pain threshold in diabetic rats. The expression of LC3-II (biomarker of autophagy) and Beclin1 protein (well known to trigger autophagy) was considerably higher in the spinal cords of rapamycin-treated diabetic rats than in nonsupplemented diabetic rats [104, 105].

In diabetic rats, inhibiting the PI3K/AKT/mTOR pathway increases autophagy and alleviates hyperalgesia [104]. *Lycium barbarum* polysaccharide heightened the nociceptive thresholds in diabetic rats by inhibiting of mTOR/p70S6K, thereby augmenting autophagy [106].

5.14. GSK3 (Glycogen Synthase Kinase 3). Glycogen synthase kinase 3 (GSK3) facilitates the addition of phosphate on serine and threonine amino acid. Diverse genes encode for GSK3-alpha and GSK3-beta. Pathways that it controls involve migration, apoptosis, cellular proliferation, and glucose regulation. Neuronal anterograde axon transportation has been also found to be lined with GSK3. Peripheral and central inflammatory responses are governed by GSK3-beta (King et al. 2015). In diabetic rats, the mRNA of GSK3-beta is upregulated and it was found that the nonpharmacological endurance training of the rats having PDN can regulate this increment [107].

5.15. Pyruvate Dehydrogenase Kinases (PDKs). In normal metabolism, there is a balance between nutrient intake and consumption. There are two distinct routes for metabolism of fatty acids and glucose that converge just before the TCA cycle. Briefly, fatty acid converts to Acyl-CoA in the cytosol which then moves inside the mitochondria for further metabolism. Fatty acid oxidation in the mitochondria results in the conversion into acetyl-CoA. On the other hand, glucose metabolizes to pyruvate in glycolysis which then moves into the mitochondria and experiences oxidative decarboxylation to form acetyl CoA under the action of mitochondrial gatekeeping enzyme pyruvate dehydrogenase complex (PDC). Acetyl CoA in both cases (fatty acid and glucose metabolism) is, subsequently, used in TCA cycle. Pyruvate dehydrogenase kinases (PDKs) may phosphorylate the PDC and inhibit its action. Upon inhibition of PDC, the excess pyruvate is covered to lactic acid [108].

PDK is believed to be abnormally upregulated in dorsal root ganglion (DRG) cells in PDN; this may be due to the hypoxia in the DRG. The cells in the DRG include the neuronal cell bodies, satellite glial cells, and the infiltrating macrophages. The surge in lactic acid as a result of the induction of PDKs contributes to the pathogenesis of PDN by triggering reactive gliosis, macrophage infiltration, acidic microenvironment, proinflammation, and sensitization of the peripheral neurons which ultimately results in central sensitization and pain hypersensitivity [109]. In a study published in 2016, it was witnessed that the genetic ablation of PDK2 and PDK4 mitigated PDN in the streptozotocin-induced diabetic rats and it was concluded that the glucose-PDK2/4-PDC-lactate axis in the DRG may serve as a potential pharmacotherapeutic target for PDN [109].

5.16. Satellite Glial Cells (SGCs). Satellite glial cells are present in the sensory, parasympathetic and sympathetic ganglia, and functions to envelop the neuronal cell bodies. The activation of SGCs in sensory ganglia may contribute to PDN via a number of ways: altered enzymatic activity in SGCs (aldose reductase, PDK2 and PDK4) [109, 110], upregulation of P2X4R and P2X7R [111, 112], and excitation of nociceptors by SGC-derived cytokines [113, 114].

5.17. Long Nonprotein Coding RNA. The long nonprotein coding RNA NONRATT021972 is upregulated in pathogenesis of diseases of nervous system, and it was evidenced to be upregulated in PDN as well. In DRG SGCs of diabetic rats, BzATP-activated currents are substantially higher than in control rats. When the effect of small interfering RNA (siRNA) for NONRATT021972 was assessed, it was found that the intravenous injection of NONRATT021972 siRNA resulted in downregulation of P2X7, TNF-alpha, and glial fibrillary acidic protein (GFAP). Further, the ATP-activated currents and resultant diabetic neuropathy pain symptoms were reduced by NONRATT021972 siRNA treatment [111]. Similarly, uc.48+ siRNA and BC168687 siRNA alleviate the PDN symptoms by downregulating the levels of proinflammatory cytokines [66].

6. Topical Agents

Topical analgesic agents for painful diabetic neuropathy include lidocaine, capsaicin, and nitric oxide sprays [17]. The effects of these drugs are discussed in detail in this section.

6.1. Lidocaine. Lidocaine is a topical agent imparting the antagonistic effect on the sodium-gated voltage channels, i.e., Na_v 1.7 and Na_v 1.8. It is recommended by the American Association of Clinical Endocrinologists (AACE) and American Academy of Neurology (AAN) for the management of pain in PDN patients [115]. It helps in stabilizing the membrane potential of the small nerve fibers by retaining hyperexcitability causing reduced release of neuronal active substances. Studies regarding lidocaine are being conducted; for example, the application of lidocaine in healthy subjects resulted in a substantial change in the thresholds of the pain stimulus relayed by small fibers. Small nerve fibers are associated with the pain as a result of thermal and mechanical stimulus. It was observed that this effect was due to the

partial blockage of these nerve fibers [116]. In another study, the safety and efficacy of the 5% lidocaine patches were assessed and it was confirmed that the application of about four patches in a duration of 18 hours per day was effective in alleviating pain and improving life quality and was well tolerated in PDN patients [117]. Pharmacodynamics and pharmacokinetic studies conducted on rat models with diabetic neuropathy showed that as compared to local anesthesia, lidocaine patch showed lower inhibitory concentration for blocking of sodium ion channels in the nerve fibers associated with pain conduction to CNS (Ten [118]). Meta-analysis and systematic review studies have shown that 5% lidocaine patch has the similar pain reduction capacity when compared with pregabalin but has a better safety profile [119].

6.2. Nitrates. Topical nitrates are not recommended in any of the guidelines for treating PDN, but they are used off-label [115]. A randomized, placebo-controlled, double-blind study showed promising results in reducing overall neuropathic symptoms ($p = 0.02$) along with the burning sensation (0.006) with the usage of isosorbide dinitrate spray [120, 121]. Study involving the administration of L-arginine to the rat model of PDN showed a decrease in thermal and tactile allodynia and mechanical hyperalgesia by regulating plasma level of nitric oxide [122]. A study by Quattrini et al. highlighted a reduced sympathetically mediated vasoconstriction in the foot of PDN patients. It was anticipated that the local sympathetic dysfunction may induce a heightened cutaneous shunting and compromised dermal nutritional blood flow, resulting in hypoxia which may trigger PDN symptoms ultimately. Correction of this condition could be the possible mechanism of action by which pain relief is imparted by local vasodilators, isosorbide dinitrate patches, and glyceryl trinitrate spray [123].

7. FDA-Approved Drugs

The FDA-approved drugs for painful diabetic neuropathy include pregabalin, duloxetine, tapentadol, and capsaicin (for foot pain only). These are discussed below in detail.

7.1. Duloxetine. Duloxetine inhibits the reuptake of neurotransmitters, i.e., norepinephrine and serotonin, and shows less affinity towards dopamine transporters. Having lesser to no affinity for glutamate, dopaminergic, opioid, adrenergic, GABA, cholinergic receptors, and no inhibitory action on monoamine oxidase, duloxetine is simply classified as SNRI (selective norepinephrine-serotonin reuptake inhibitor) [124]. Therefore, the mechanism through which duloxetine lowers diabetic neuropathy pain is through inhibition of reuptake of serotonin-norepinephrine. As SNRIs affect the neurotransmitter balance in the brain, this indicates that their central pain inhibitory action may be due to the inhibition of noradrenergic and serotonergic neuronal activity. Reduction in these inhibitory signaling may lead to persistent pain perception in the brain. Both neurotransmitters (serotonin and norepinephrine) play a pivotal role in relaying pain signals in spinal cord and brainstem. Synergistic inhibition of these neurotransmitters causes less transmission of pain signals from periphery to CNS. A number of preclinical and clinical trials have shown increased tolerance to pain with reduced pain symptoms in diabetic-induced painful neuropathy [125–127]. Pathogenic mechanism involving NF-κB can cause spinal glial cells to secrete active substances and cytokines that can cause a sustained neuropathic pain. Duloxetine (SNRI) reportedly inhibits secretion of NF-κB and TLR4 in dorsal root ganglion of rat [128]. Inactivation of microglial secretion has shown a neuroprotective and restoring effect on peripheral nerve injury with increased levels of nerve growth factors especially in sciatic nerve of diabetic rats [126]. Comparative double blinded study was conducted with gabapentin and duloxetine which suggested that the former shows more side effects whereas the later shows more medication compliance [129].

7.2. Gama Aminobutyric Acid (Pregabalin). Health experts prescribe gabapentinoid drugs: pregabalin and gabapentin for the management of peripheral and central neuropathies. Gabapentinoids are considered the first line of treatment in mitigating these complications. These are analogues of GABA and are known to bind with auxiliary subunits of calcium channels ($\alpha_2\delta$-1 and $\alpha_2\delta$-2). Pregabalin does not exhibit any interaction with GABA A and B receptors or triggers GABA uptake [130]. The potency ratio of pregabalin is higher than gabapentin, as the former has more affinity towards $\alpha_2\delta$-1 subunit [131]. For the management of painful diabetic neuropathy, pregabalin is a first line of treatment according to international guidelines. Dose-dependent studies have been conducted on pregabalin drug to quantify the pain intensity in diabetes-induced neuropathies [125, 132, 133]. Pain reduction was less than 50% in 3 : 10 patients taking 300/600 mg pregabalin daily as compared to 2 : 10 with placebo. With 600 mg daily dose, patients reported somnolence 15% and dizziness 22% [134]. In another research, a comparative analysis between venlafaxine, carbamazepine, and pregabalin was done, showing pregabalin more potent in curing painful diabetic neuropathy [135]. Researchers have documented that 600 mg/d pregabalin is well tolerated, reduces pain significantly, and does not affect the nerve conduction velocity [136].

On the other hand, gabapentin has been reported in treating neuropathies in animal models, but it is not approved by the FDA for the treatment of diabetic neuropathic pain. It is widely used and recommended in other guidelines for PDN [137]. It synergistically interacts between elevated GABA production, non-NMDA receptor inhibition, and strong affinity for alpha-2-delta subunit of voltage-gated calcium channel [138]. Gabapentinoids interacts with the highest-affinity binding sites present in the brain membrane. In the *in vitro* studies, it has been found that these gabapentinoids modulate the activity of GABA synthase enzyme, glutamic acid decarboxylase (GAD), and glutamate synthase, branched amino acid chain transaminase [139]. A study was conducted by Andrew Moore *et al.* to check the effect of gabapentin on 5914 patients. According to the study, gabapentin (1200 mg/day) has been proven efficacious in treating painful neuropathies—38% patients

reported reduction in pain by 50% vs placebo [140]. A novel study involving coadministration of gabapentin and tramadol showed a synergic effect on neuropathic pain reduction by IL-1β proinflammatory suppression in the mouse model. The effect was not tested on the PDN model, but this study indicated that gabapentin can be combined with other drugs to increase its effectiveness in managing painful symptom [141]. Carbamazepine, another anticonvulsant, has exhibited effectiveness against different neuropathic syndromes and especially PDN [142]. But its efficacy is comparatively lower than that of pregabalin [135], and it is not recommended for PDN in international guidelines.

The effectiveness of pregabalin against diabetic neuropathic pain may be because of its antidepressant nature. PDN is a major determining factor of depression and is significantly associated with it [143–145]; noradrenergic antidepressants and gabapentinoid anxiolytics are antineuropathic medications that have been licensed and/or recommended for PDN [146]. Pregabalin is approved to treat neuropathic pain and PDN, and it may also be used to treat concomitant anxiety and sleep disturbances [147]. It may have an antinociceptive effect on its own, but this is still subject to debate [148].

7.3. Opioids (Tapentadol Extended Release). Tapentadol is the first representative of a class of drugs referred to as mu-opioid receptor agonist/noradrenaline reuptake inhibitor (MOR-NRI) drugs, i.e., centrally acting analgesic drugs. It is FDA approved, is taken orally, and has analgesic and noradrenergic properties making it effective towards managing pain symptoms [149]. Clinical trials have shown positive result of tapentadol in managing diabetic peripheral neuropathy and chronic lower back pain. Tapentadol has dual mechanism of action, i.e., u-opioid receptor agonism and norepinephrine reuptake inhibition contributing to its antineuropathic potential in the substantia gelatinosa of spinal cord [150]. Tapentadol extended release has found to have some adverse effects: nausea, vomiting, headache, somnolence dizziness, and constipation. Long-term opioid administration greatly increases the risk of addiction [151]. Based on their poor tolerance by the body and safety concerns associated with their use, opioids are considered the second line of treatment in neuropathic pain management [152].

Lastly, one of the characteristic properties of tapentadol is its minimal serotoninergic activity which is beneficial for pain management in patients. Studies related to tapentadol-extended release are being done to check its neuroprotective effect. In a study, patients were titrated with dose of tapentadol ER 100-250 mg twice a day, following which the changes in pain intensity were reported in placebo 1.28 (2.41) and tapentadol ER 0.08 (1.87) on 11-point numerical rating scale. Adverse effects associated with placebo were 56.0% and with tapentadol ER 74.7%. Results obtained from this pooled analysis exhibited analgesic efficacy of tapentadol ER in managing painful diabetic neuropathy. An important point to consider is that the results were consistent among different PDN subcategories in terms of treatment effectiveness [153]. Large-scale, phase 3 studies showed that tapentadol ER was well tolerated among the patients suffering from chronic osteoarthritis, lower back pain, and painful diabetic peripheral neuropathy [154].

7.4. Capsaicin. Topical capsaicin is approved by the FDA only for relieving the foot pain in PDN [155]. It is a TRPV1 agonist which triggers depolarization in the nociceptor by facilitating the influx of Na^+ and Ca^{++} and by triggering the release substance P. Recurrent exposure of TRPV1 to capsaicin results in the scarcity of substance P and renders desensitization and inactivity of TRPV1 [17]. The 8% capsaicin patch has been tailored to deliver high levels of capsaicin rapidly, which facilitates the inhibition of the hyperexcited nociceptor, thus tampering the ectopic release of nerve impulse [156]. It has been proven that the application of 8% patch for a period of 30 minutes relieves pain and ameliorated the life quality in PDN population [156]. However, the lower concentration of capsaicin gel 0.025% showed insignificant results in managing painful symptoms in patients with PDN but was safe and well tolerated as compared to higher concentration of capsaicin [157]

8. Nonpharmacological Treatment Modalities for PDN

8.1. Exercise. It was observed that physical activity in diabetic rats alleviated the PDN symptoms at least temporarily by increasing the levels of heat shock protein 72 (Hsp72) [78]. Neurodynamics or neural mobilization is a treatment modality that mobilizes the nervous system and/or the structures encompassing it through exercise or manual methods. The aim in this intervention is to reinstate the homeostasis of the nervous system and its associated structures. A number of preclinical and clinical studies have demonstrated the effectiveness of this intervention in restoring the fluid dispersion within the neuron and immune reaction and in healing the intraneural edema and thermal and mechanical hyperalgesia [158]. It STZ-induced diabetic rats, it was revealed that the neural mobilization relieved the mechanical allodynia by cutting the levels of TNF-alpha and IL-1beta [83].

8.2. Spinal Cord Stimulation (SCS). SCS is an invasive modality for treating chronic algesia that involves activation of the dorsal columns of the spinal cord using a low-voltage electric current. The mechanism of action remains elusive; however, this intervention is thought to affect both spinal and supraspinal regions. In most cases, the SCS device is implanted in two stages. The electrode lead is first inserted percutaneously in the epidural space and attached to a temporary pulse generator external to the body (the trial phase). The external pulse generator is only replaced by an implanted pulse generator if the treatment results in significant pain reduction; otherwise, the lead is removed and no SCS therapy is offered [159, 160].

SCS was shown to improve the pain symptoms and life quality of 60 PDN patients for a period of 6 months [160]. Another recent study revealed the success of this intervention for 86% of PDN patients after 1 year of initiation of

Table 1: List of therapeutic drugs involved in management of PDN. (-) shows decrease in the level of that component, and (+) shows increase in the level of that component.

Sr	Drugs	Target	Observation	Clinical or Preclinical	References
1	Epalrestat	Polyol pathway	Spontaneous pain (-), MNCV (+), SNCV (+), vibration perception threshold (+), F-wave latency (-)	Clinical and preclinical	[22, 162]
2	Sorbinil	Polyol pathway	Polyol pathway (-), Na pump defect (-), defective axonal transport (-), NCV (+), myelinated fiber repair (+)	Preclinical and clinical	[163, 164]
3	Fidarestat	Polyol pathway	Sorbitol accumulation (-), spontaneous pain (-), median nerve FCV (-), minimal latency (-), NCV (+)	Clinical	[14, 165]
4	Zenarestat	Polyol pathway	Nerve conduction velocity (NCV) (+), sorbitol in sciatic nerve (-)	Clinical and preclinical	[165, 166]
5	Tolrestat	Polyol pathway	MNCV (+), polyol influx in nerve (+), neuropathic pain (-)	Clinical	[167]
6	Benfotiamine	Hexosamine pathway	Pain (-) when coadministered with alpha lipoic acid	Clinical	[32]
7	Berberine	PKC pathway, MAPK pathway, TNF-alpha, oxidative stress, TRPV1	Thermal hyperalgesia (-), mechanical hyperalgesia (-)	Preclinical	[36]
8	Vitamin D	AGE	Pain (-) neuroprotective effect on Schwann cells (+),	Clinical and preclinical	[39, 42]
9	Pyridoxamine	RAGE/NF-kB/ERK	Mechanical allodynia (-)	Preclinical	[74]
10	Compound XiongShao Capsule	AGEs	Thermal hyperalgesia (-), mechanical hyperalgesia (-),	Preclinical	[45]
11	Tocotrienol	ROS	Reversed PDN when administered in combination with insulin	Preclinical	[46]
12	Tribulus terrestris extract	ROS, inflammatory mediators	Pain threshold (+)	Preclinical	[50]
13	Fisetin	ROS, NF-κB,	Thermal and mechanical pain (-)	Preclinical	[51]
14	*Rosmarinus officinalis L.*	ROS	Antinociceptive (+), anti-neuropathic (+)	Preclinical	[52]
15	Kaempferol extracted from Eruca sativa	ROS	Partial pain reduction	Preclinical	[53]
16	Alpha lipoic acid	NF-κB, ROS, TRPV1	Hyperalgesia (-), reduced glutathione (+)	Clinical	[54–56]
17	Acetyl L-carnitine	ROS	Mechanical allodynia (-), synaptic glutamate level (-), NCV (+), nerve regeneration (+)	Clinical	[58, 60, 61]
18	1,5-Isoquinolinediol	PARP inhibitor	Thermal hyperalgesia (-), tactile allodynia (-), mechanical hyperalgesia (-)	Preclinical	[64]
19	10-(4-Methylpiperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-one	PARP inhibitor	Intraepidermal nerve fiber degeneration (-), partial reduction of pain (+)	Preclinical	[65]
20	SB203580	p38a MAPK inhibitors	Mechanical allodynia (-), hyperalgesia (-)	Preclinical	[67–69]
21	SD-282				
22	SP600125	JNK inhibitor			
23	U0126	MAPK inhibitor			
24	Ziziphus jujuba Root bark	NF-κB, ROS	Thermal hyperalgesia (-), mechanical hyperalgesia (-), cold allodynia (-)	Preclinical	[73]
25	Desert Hedgehog deficiency	Hedgehog pathway	Thermal hyperalgesia (-)	Preclinical	[77]
26	Minocycline	Cytokines, PARP	Neuropathic pain (-) in combination with morphine	Preclinical	[82]

Table 1: Continued.

Sr	Drugs	Target	Observation	Clinical or Preclinical	References
27	Curcumin derivative J147	AMP kinase pathway, TNF-α	Touch triggered allodynia (-)	Preclinical	[84]
28	Nimesulide	COX-2	Pain threshold (+) when administered in combination with rutin	Preclinical	[87]
29	Celecoxib	COX-2	Allodynia (-), hyperalgesia (-)	Preclinical	[88]
30	Meloxicam	COX-2	Allodynia (-)	Preclinical	[89]
31	SC-58125 and NS-398	COX-2	Hyperalgesia (-)	Preclinical	[90]
32	Endogenous NGF	NGF	Pain relief in phase II, but no statistically significant pain relief in phase III trials	Clinical	[100–102]
33	Exogenous NGF	NGF	Mechanical pain threshold (+)	Preclinical	[99]
34	Lycium barbarum polysaccharide	Autophagy, mTOR/ p70S6K,	Pain thresholds (+)	Preclinical	[106]
35	NONRATT021972 siRNA	Long nonprotein coding	ATP activated currents (-), spontaneous pain (-), P2X7 (-), TNF-alpha (-), GFAP (-)	Preclinical	[111]
36	uc.48+ siRNA	Long nonprotein coding	Spontaneous pain (-), proinflammatory cytokines (-)	Preclinical	[66, 168]
37	BC168687 siRNA	Long nonprotein coding		Preclinical	[169]
FDA/EU-approved drugs					
1	Pregabalin	α_2-δ ligand	Neuropathic pain (-)	Clinical	[136]
2	Duloxetine	SNRI inhibitor	Neuropathic pain (-)	Clinical	[124]
3	Tapentadol ER	Mu-opioid receptor agonist and norepinephrine reuptake inhibitor.	Pain reduction (+)	Clinical	[153]
4	Capsaicin	TRPV1 agonist	Pain sensitivity (-)	Clinical	[156]
Topical drugs					
1	Lidocaine	Blockers of voltage-gated Na^+ channels	Na^+ ion influx (-), pain transduction pathway (-)	Clinical	[117]
2	Nitrate	NO donor	NO (+), vasodilation (+)	Clinical	[121]

MNCV: motor nerve conduction velocity; NCV: nerve conduction velocity.

SCS treatment and in 55% of PDN patients after 5 years [161]. Recently, FDA accorded premarket approval for senza spinal cord stimulation system for the treatment of diabetic neuropathy pain.

9. Discussion

Diabetic neuropathy is a complex and grave nerve degenerative disorder, which affects 40-80% of people suffering from diabetes globally. Signaling pathways are essentially responsible for the initiation and pathogenesis of this disorder. Whereas comprehensive studies are required on transcriptional and translational levels to understand the exact development and regulation of these pathological mechanism causing difficulty in developing exact treatment for diabetic neuropathy, slowing down this pathological mechanism is the main aim for the symptomatic based management of painful diabetic neuropathy. For the attenuation of painful symptoms, several tricyclic antidepressants, anticonvulsant, and opioids are prescribed to diabetic patients. Curative effect of medicine is dependent on the way they cause the suppression/activation of neuropathic signaling/inhibiting molecules. Evidence from clinical and preclinical studies shows that neuropathic suppression of signaling molecules via aldose reductase, MAPKs, PKC isoforms, and oxidative stress has proven effective in dealing with painful symptoms of diabetes. These have been proven beneficial in enervating nerve damage. Other than the above-mentioned pathway initiation of neuropathic pathology inhibition through PARPs, siRNAs, and inhibitors of hedgehog pathway need to be investigated further. Currently, the management of painful diabetic neuropathy is achieved locally as well as systemically. The commercially available drugs for relieving diabetic neuropathic pain involving localized treatment are capsaicin, 5% lidocaine patch, and nitrates spray whereas drugs for systemic suppression of diabetic neuropathic pain are gabapentinoids (pregabalin and gabapentin) duloxetine and opioids. The recent FDA approval of the spinal cord stimulation system also seems promising in relieving this agonizing disorder called PDN. Furthermore, large-scale clinical trials with comparative analysis of existing and novel pharmacotherapeutic agents are required in order to develop a more localized and potent therapeutic alternative with fewer side effects. In this regard, building understanding regarding the mechanisms involved in painful diabetic neuropathy, molecular targets, and devising a novel drug

(inhibitors or suppressors)/drug delivery system should be the researcher's approach in managing diabetes induced painful neuropathy in the future. Table 1 summarizes the mechanism of action of the pharmacotherapeutic agents that have been investigated preclinically or clinically so far. It also includes the FDA-approved drugs for PDN, and the topical agents available in the market that are used for PDN.

10. Conclusion

Painful diabetic neuropathy has high prevalence, is under diagnosed, requires expensive treatments, and lacks effective therapy. Lack of understanding regarding the pathogenesis of painful diabetic neuropathy is the main reason behind the shortfall of its treatment whereas management of this disease is being done by symptomatic pain management, glycemic control, risk factor management, and pathogenic mechanism-based management. Currently available therapies which significantly reduce diabetes-induced painful neuropathies mainly include agents that work on ion channels; i.e., pregabalin antagonizes calcium channels. Recent ongoing research is targeting mechanisms, e.g., polyol pathway, hexamine pathway, PKC, oxidative stress, PARP, MAPK, AGE, NF-KB, Hh, COX, IL, TNF-alpha, NGF, autophagy, and GSK3 which either inhibit or activate molecules responsible for either signaling or suppressing these pathological mechanisms. Therefore, mechanism-based approaches should be the way forward in tackling diabetes induced painful neuropathies. However, current agents (duloxetine, opioids, r-aminobutyric acid, etc.) have limited efficacy and possess intolerable side effects in the long run. Adjuvant therapies are also efficacious but are under moderate clinical use. Furthermore, clinical trials on large scale with comparative analysis among already existing drugs and novel drugs need to be done to develop a localized and more potent treatment option with minimal side effects.

Abbreviations

12(S)HETE:	12-Hydroxyeicosatetraenoic Acid
15(S)HETE:	15-Hydroxyeicosatetraenoic Acid
ACE:	Angiotensin-converting enzyme
AGE:	Advanced glycation end products
AGE-RAGE:	Advanced glycation end product-receptor for advanced glycation end products
AMP:	Adenosine monophosphate
AR:	Aldose reductase
ATP:	Adenosine triphosphate
Ca^{+2}:	Calcium ion
CGRP:	Calcitonin gene-related peptide
COX:	Cyclooxygenase
DAG:	Diacylglycerol kinase
DNA:	Deoxyribose-adenine-dinucleotide
DRG:	Dorsal root ganglion
ERK:	Extracellular signal-regulated kinases
FDA:	Food Drug Authority
GABA:	Gamma-aminobutyric acid
GAD:	Glutamic acid decarboxylase
GADPH:	Glyceraldehyde 3-phosphate dehydrogenase
GSK3:	Glycogen synthase kinase 3
GSSH/GSH:	Glutathione oxidized state/glutathione-reduced state
Hh:	Hedgehog pathway
HMG- CoA:	3-Hydroxy-3-methylglutaryl-CoA
IL:	Interleukin
JNK:	c-Jun N terminal kinase
LOX:	Lipoxygenase
MAPK:	Mitogen-activated protein kinases
MOR-NRI:	Mu-opioid receptor agonist/noradrenaline reuptake inhibition
Na^{+}:	Sodium ion
NAD^{+}:	Nicotinamide adenine dinucleotide
NADPH:	Nicotinamide adenine dinucleotide phosphate
NF-κB-Kappa:	Light-chain-enhancer of activated B cell
NF-κB-P65-Kappa:	Light-chain-enhancer of activated B cell-transcription factor p65
NGF:	Nerve growth factors
NO:	Nitric oxide
NT:	Neurotransmitter
PAI-1:	Plasminogen activator inhibitor type 1
PARP:	Poly-ADP ribose polymerase
PKC:	Protein kinase C
SP:	Subunit protein
STZ:	Streptozotocin
SIRT2:	NAD^{+}-dependent protein deacetylase sirtin-2
TNF-α:	Tumor necrosis factor alpha
TRKA:	Tropomyosin receptor kinase A
VEGF:	Vascular endothelial growth factor.

Conflicts of Interest

The authors have no conflict of interest to declare.

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Research Article

Predictive Ability of Visit-to-Visit Variability of HbA1c Measurements for the Development of Diabetic Kidney Disease: A Retrospective Longitudinal Observational Study

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Aims. This study is aimed at clarifying the relationship between visit-to-visit variability of glycated hemoglobin (HbA1c) and the risk of diabetic kidney disease (DKD) and to identifying the most useful index of visit-to-visit variability of HbA1c. *Methods*. This clinic-based retrospective longitudinal study included 699 Japanese type 2 diabetes mellitus patients. Visit-to-visit variability of HbA1c was calculated as the internal standard deviation of HbA1c (HbA1c-SD), the coefficient of variation of HbA1c (HbA1c-CV), the HbA1c change score (HbA1c-HVS), and the area under the HbA1c curve (HbA1c-AUC) with 3-year serial HbA1c measurement data, and the associations between these indices and the development/progression of DKD were examined. *Results*. Cox proportional hazards models showed that the HbA1c-SD and HbA1c-AUC were associated with the incidence of microalbuminuria, independently of the HbA1c level. These results were verified and replicated in propensity score (PS) matching and bootstrap analyses. Moreover, the HbA1c-SD and HbA1c-AUC were also associated with oxidized human serum albumin (HSA), an oxidative stress marker. *Conclusions*. Visit-to-visit variability of HbA1c was an independent risk factor of microalbuminuria in association with oxidative stress among type 2 diabetes mellitus patients. HbA1c-AUC, a novel index of HbA1c variability, may be a potent prognostic indicator in predicting the risk of microalbuminuria.

1. Introduction

Diabetic kidney disease (DKD) is a microvascular complication of diabetes and is considered to be a renal symptom caused by long-term exposure to hyperglycemia [1]. Patients with DKD are not only at increased risk of developing end-stage kidney disease but are also at increased risk of cardiovascular morbidity and mortality [1, 2]. Therefore, optimizing glycemic control is most important for preventing the development and progression of DKD [2, 3]. Glycated hemoglobin (HbA1c) is traditionally used as an average blood glucose measurement to monitor blood glucose control in the treatment of type 2 diabetes [2, 3]. This rationale is based on clinical and observational evidence that lowering

HbA1c reduces the risk of micro- and macrovascular complications of diabetes [2, 3]. However, a decreased HbA1c value alone has been reported to be insufficient to ensure optimal clinical outcomes in patients with type 2 diabetes [4]. The Action to Control Cardiovascular Disease in Diabetes (ACCORD) trial showed that the intensive reduction of blood glucose concentration only delayed the onset of albuminuria and was not associated with a decreased risk of other measures of renal dysfunction [5]. Therefore, other approaches—in addition to lowering HbA1c—are required to prevent the onset and progression of DKD.

Visit-to-visit variability of HbA1c, another indicator of glycemic control, has recently received attention for its relationship to the incidence of diabetic complications [6–16], although the importance of visit-to-visit variability in HbA1c to the risk of DKD is still under debate because of inconclusive evidence [16]. Since visit-to-visit variability of HbA1c is related to various factors (i.e., HbA1c), analyses to investigate the effect of visit-to-visit variability in HbA1c on the risk of DKD need to be adjusted for confounding factors [16]. Meanwhile, in many previous studies, visit-to-visit variability of HbA1c has been measured by the internal standard deviation of HbA1c (HbA1c-SD) and/or the coefficient of variation of HbA1c (HbA1c-CV) [6–11, 13]. HbA1c-SD and HbA1c-CV are influenced by the number of measurements and measurement intervals; thus, accurate quantitative evaluation of visit-to-visit variability of HbA1c has not been achieved. Recently, the HbA1c change score (HbA1c-HVS), which is calculated by dividing the number of times HbA1c changed by >0.5% (5.5 mmol/mol) by the total number of HbA1c measurements has been used as an index of visit-to-visit variability of HbA1c [14, 15]. However, it is unclear which index of visit-to-visit variability of HbA1c is most useful for predicting the risk of DKD, and there may be other useful indices. In order to clarify the causal relationship between visit-to-visit variability of HbA1c and the risk of DKD, it is necessary to conduct a comparative study using various indices.

In addition to clarifying the impact of visit-to-visit variability of HbA1c on the risk of DKD, it is also necessary to elucidate the mechanisms underlying the association between visit-to-visit variability of HbA1c and the development/progression of DKD. Oxidative stress is considered to be a key pathogenic factor in the association between visit-to-visit variability of HbA1c and diabetic complications [8–10, 17, 18]; however, the details are not clear, especially in DKD. We have recently reported that high levels of oxidized human serum albumin (HSA), a marker of oxidative stress, predict the development and progression of DKD [19]. Therefore, examining the association between oxidized HSA and visit-to-visit variability of HbA1c may help elucidate mechanisms in the relationship between visit-to-visit variability of HbA1c and the development/progression of DKD.

In the present study, in addition to three indices that have been used in previous studies (i.e., HbA1c-SD, HbA1c-CV, and HbA1c-HVS), the area under the HbA1c curve above or below the individual mean HbA1c (HbA1c-AUC) was also used as an index of visit-to-visit variability of HbA1c. We then investigated which index expressing visit-to-visit variability of HbA1c is the most associated with the development/progression of DKD, while also considering potential confounders. Moreover, we further investigated whether oxidized HSA could affect the visit-to-visit variability of HbA1c.

2. Materials and Methods

2.1. Subjects. In this study, the records of 728 consecutive type 2 diabetes mellitus patients who visited the Jinnouchi Clinic, Diabetes Care Center in Kumamoto, Japan, between July 1999 and October 2019 were reviewed. Among them, patients whose HbA1c level was measured ≥5 times within a 3-year period following the start of observation, and whose renal function was followed for ≥1 year, were included. Consequently, 699 subjects (477 males and 222 females) were included in the retrospective longitudinal study. For the diagnosis of type 2 diabetes, since all subjects enrolled in this retrospective study were Japanese patients, we applied the criteria of the Japan Diabetes Society (JDS), which are optimized for the Japanese population [20]. The diagnostic criteria are based on values of plasma glucose, HbA1c, typical symptoms of chronic hyperglycemia, and/or a prior diagnosis of diabetes, and the diagnostic cut-off values of plasma glucose and HbA1c for diabetes were a fasting plasma glucose level ≥ 126 mg/dl (≥7.0 mmol/l), 2 h oral glucose tolerance test value ≥ 200 mg/dl (≥11.1 mmol/l), casual plasma glucose level ≥ 200 mg/dl (≥11.1 mmol/l), and HbA1c ≥ 6.5 % [20].

2.2. Study Design. The retrospective longitudinal study regarding the association between visit-to-visit variability of HbA1c and DKD was conducted as shown in Supplementary Figure 1. Considering possible intense changes of HbA1c in patients who started hypoglycemic treatment in primary care or replaced hypoglycemic medicines in use after transfer from another hospital, clinical data from 6 months after the first visit was used. In a 3-year period following the start of observation, HbA1c data were used to calculate the indices of visit-to-visit variability of HbA1c. After the 3-year period for calculating visit-to-visit variability of HbA1c, survival analyses with the baseline at the end of the 3-year period were performed to investigate the associations of visit-to-visit variability of HbA1c with the incidence of microalbuminuria and progression of the estimated glomerular filtration rate (eGFR) categories according to Kidney Disease: Improving Global Outcomes (KDIGO) GFR categories [21]. The length of follow-up for survival analyses was up to 10 years (median, 9.9 years; 2002-2019). In the analysis regarding the association between oxidized HSA and visit-to-visit variability of HbA1c, 194 subjects who underwent one time blood collection and the measurement of HbA1c more than 5 times in a 2.5-year period after blood collection were included. In the analysis, we calculated the visit-to-visit variability of HbA1c using HbA1c measurement data obtained for 2.5 years after oxidized HSA measurement and examined the association between oxidized HSA and

visit-to-visit variability of HbA1c. The study protocol was approved by the institutional ethics committee of the Faculty of Life Sciences, Kumamoto University (Approval No.169), and the study was performed in accordance with the Declaration of Helsinki.

2.3. Indices of Visit-to-Visit Variability of HbA1c. In this study, the SD, CV, AUC, and HVS of HbA1c were employed as the index of visit-to-visit variability of HbA1c, denoted as HbA1c-SD, HbA1c-CV, HbA1c-AUC, and HbA1c-HVS, respectively. The 4 indices of visit-to-visit variability of HbA1c were calculated for each subject. HbA1c-HVS was calculated as the number of times HbA1c changed by >0.5% (5.5 mmol/mol) divided by the total number of HbA1c measurements [15]. For example, if a patient had 5 successive measurements of HbA1c with values of 6.7%, 7.0%, 7.8%, 7.4%, and 8.0%, then the HbA1c-HVS was calculated as 40% (i.e., $2/5 \times 100\%$) [15]. HbA1c-AUC was calculated as the area of under the HbA1c curve above or below the individual mean HbA1c as shown in Supplementary Figure 2. Quartiles for each index were calculated and represented by Q1, Q2, Q3, and Q4. The lower quartile was denoted as Q1 (low HbA1c variability), and the upper quartile was denoted as Q4 (high HbA1c variability). In this study, HbA1c was measured using an ADAMS A1c HA-8181 (Arkray Inc., Tokyo, Japan), a glycohemoglobin analyzer that uses ion-exchange high-performance liquid chromatography assay to measure HbA1c and that has been certified by the Diabetes Control and Complications Trial and the National Glycohemoglobin Standardization Program (NGSP) reference assay (http://www.ngsp.org/docs/methods.pdf).

2.4. Endpoint Definitions. The development of DKD was evaluated by two assessments: the incidence of microalbuminuria and eGFR stage progression according to the KDIGO GFR category [21]. Survival analyses regarding the incidence of microalbuminuria were conducted among 533 subjects with normoalbuminuria at baseline. Normoalbuminuria was defined as a urine albumin- (ACR-) to-creatinine (Cr) ratio (Alb/Cr) of <30 mg/gCr or a negative urine dipstick test result, while microalbuminuria was defined as an Alb/Cr ≥ 30 mg/gCr or a positive urine dipstick test result followed by a subsequent Alb/Cr ≥ 30 mg/gCr. Survival analyses regarding eGFR stage progression were performed among subjects stratified according to the eGFR stage at baseline. The eGFR stage was determined according to the KDIGO GFR categories, as follows [21]: G1: normal, eGFR ≥ 90 ml/min/1.73 m^2; G2: mild chronic kidney disease (CKD), eGFR: 60-89 ml/min/1.73 m^2; G3a: mild-moderate CKD, eGFR: 45-59 ml/min/1.73 m^2; G3b: moderate-severe CKD, eGFR: 30-44 ml/min/1.73 m^2; G4: severe CKD, eGFR: 15-30 ml/min/1.73 m^2; G5: end-stage kidney disease, eGFR < 15 ml/min/1.73 m^2. In this study, G3a and G3b were combined as G3, since few subjects in the two groups experienced progression. A ≥25% decrease in eGFR was defined as progression of the eGFR stage. For example, if a patient had a baseline eGFR of 92 ml/min/1.73 m^2 and a follow-up eGFR value equal to or lower than 69 ml/min/1.73 m^2, this patient would be considered to have experienced progression from G1 (i.e., $(92 - 69)/92 \times 100\% = 25\%$). The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation: $\text{eGFR}\,(\text{ml/min/1.73 m}^2) = 0.808 \times 175 \times \text{Cr}^{-1.154} \times \text{Age}^{-0.203} \times 0.742$ (if female) [22].

2.5. Clinical Information. The clinical information that was used was obtained from medical records. This included HbA1c values, sex, age, duration of diabetes, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, Alb, Cr, Alb/Cr, and eGFR. Type 2 diabetes mellitus was diagnosed according to the criteria of the JDS [20].

2.6. Determination of the Degree of Oxidized HSA. Among all subjects, we measured oxidized HSA in 194 subjects who underwent one time blood collection and the measurement of HbA1c > 5 times in a 2.5-year period after blood collection. HSA was collected by solid phase extraction and measured using time-of-flight mass spectrometry. The degree of oxidized HSA was calculated as follows: degree of oxidized HSA = [oxidized form of HSA/(oxidized form of HSA + unoxidized form of HSA)] × 100 [23].

2.7. Statistical Analysis. Continuous variables were compared by Student's *t*-test or a one-way ANOVA; categorical variables were compared by Fisher's exact test. The longitudinal associations of visit-to-visit variability of HbA1c with the incidence of microalbuminuria and eGFR stage progression were analyzed using Kaplan-Meier survival curves and Cox proportional hazards models. A comparison of the cumulative incidence between the groups was carried out using Kaplan-Meier survival curves with a log rank test. Multivariable adjusted hazard ratios (HRs) and 95% confidence intervals (CI) was calculated using a Cox proportional hazards model adjusted for age, sex, mean HbA1c over the 3-year period, duration of diabetes, SBP, LDL-C, eGFR, presence of ischemic heart disease and heart failure at baseline. Bootstrap analyses were performed to validate the associations of visit-to-visit variability of HbA1c with the incidence of microalbuminuria and eGFR stage progression [24]. One thousand replicated datasets were generated by random sampling with replacement [24]. Additionally, in order to reduce confounding bias, propensity score (PS) matching was performed to verify the results of the longitudinal analyses [24]. The PS was constructed using a logistic regression model. The following variables were included in the PS matching model: the mean HbA1c over the 3-year period, sex, age, HbA1c level, duration of diabetes, SBP, and BMI at baseline. Subjects were matched at a ratio of 1 : 1, using the nearest neighbor matching algorithm without replacement on the logit of the PS, with a caliper of width equal to 0.25 SD of the logit of the PS [24]. Besides, the longitudinal associations of continuous variables of the index of visit-to-visit variability of HbA1c with the incidence of microalbuminuria and eGFR stage progression were also analyzed using a Cox proportional hazards models adjusted

for the same cofounders in the analysis containing category variables of variability of HbA1c. The association between oxidized HSA and visit-to-visit variability of HbA1c was analyzed using a multiple linear regression analysis with calculation of unstandardized partial regression coefficient (B), partial correlation coefficients (β), and standardized error (SE) adjusted by sex, mean HbA1c over the 2.5-year period, duration of diabetes, SBP, eGFR, and BMI. p values of <0.05 were considered to be statistically significant. PS matching was performed with a contributed R package ("Matching") using the R software program (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria). Other statistical analyses were performed using the SPSS software package for Windows (Version 23.0, IBM Japan Ltd., Tokyo, Japan).

3. Results

A total of 699 type 2 diabetes mellitus patients (477 males and 222 females) were included in this study. Clinical characteristics of all subjects are shown in Table 1. At the start of the observation, the mean duration of diabetes was 9.0 ± 7.8 years, and the mean HbA1c was 7.7 ± 1.5%. The mean number of HbA1c measurements over the 3-year period was 28 ± 10 times (at least 5 measurements per subject), and the mean HbA1c over the 3-year period was 7.7 ± 1.1% (Table 1). The mean and quartile values of the 4 indices of visit-to-visit variability of HbA1c (HbA1c-SD, HbA1c-CV, HbA1c-AUC, and HbA1c-HVS) are shown in Supplementary Table 1. To observe the characteristics of subjects with low and high visit-to-visit variability of HbA1c, the overall subjects were divided by quartiles of the 4 indices, respectively, and results are shown in Supplementary Table 2-5. We found that whichever index was employed to measure the visit-to-visit variability of HbA1c, subjects with high HbA1c variability were characterized by significant younger age and higher mean HbA1c, BMI, SBP, triglycerides, and LDL-C values in comparison to those with low HbA1c variability (Supplementary Table 2-5).

Microalbuminuria is an early manifestation of the progression of DKD. In the present study, we performed survival analyses to investigate the association of visit-to-visit variability of HbA1c with the incidence of microalbuminuria among 533 subjects who had normoalbuminuria at the baseline of the survival analyses. The subjects were divided into 4 groups based on the quartiles of the 4 indices (HbA1c-SD, HbA1c-CV, HbA1c-AUC, and HbA1c-HVS). The incidence of microalbuminuria at endpoint was 52% (incidence rate: 10.8 cases per 1000 person-year). For all indices, Kaplan-Meier curves showed that the incidence of microalbuminuria differed among the 4 groups (Figure 1), and multivariable Cox proportional hazards models showed that the incidence of microalbuminuria in the Q4 groups of HbA1c-SD, HbA1c-CV, and HbA1c-AUC was higher in comparison to their respective Q1 groups (Table 2). Additionally, the risk was verified by a bootstrap analysis using 1,000 replicated datasets.

eGFR decline is another manifestation of the progression of DKD; thus, we also performed survival analyses to investigate the association of visit-to-visit variability of HbA1c with eGFR stage progression. First, we investigated the association of visit-to-visit variability of HbA1c with eGFR stage progression from stage G1 among 280 subjects who were in G1 at the baseline of the survival analyses. Subjects of stage G1 were divided into 4 groups based on the quartiles of the 4 indices (HbA1c-SD, HbA1c-CV, HbA1c-AUC, and HbA1c-HVS), respectively. The incidence of eGFR stage progression from stage G1 was 75% (incidence rate: 16.1 cases per 1000 person-year). Kaplan-Meier curves showed the incidence of eGFR stage progression from G1 among each group of the 4 indices (Supplementary Figure 3), and the multivariable Cox proportional hazards models showed that the eGFR stage progression from G1 was higher in the Q4 groups of HbA1c-SD and HbA1c-CV in comparison to their respective Q1 groups (Supplementary Table 6). The bootstrap analysis verified the associations of HbA1c-SD and HbA1c-CV with the incidence of eGFR stage progression from G1. Next, we investigated the association between visit-to-visit variability of HbA1c and eGFR stage progression from G2 or G3. However, there was no association between visit-to-visit variability of HbA1c and eGFR stage progression from G2 or from G3 (Supplementary Figure 4-5, Supplementary Table 7).

Subjects with high visit-to-visit variability of HbA1c were characterized by a higher HbA1c level, younger age, and undesirable clinical parameters, such as higher SBP (Supplementary Table 2-5), which may affect the development of DKD. Considering the impact of confounding bias on the result, we performed PS-matching among subjects in the Q1 (the lowest variability group) and Q4 (the highest variability group) groups of each index to further confirm the association of visit-to-visit variability of HbA1c with the incidence of microalbuminuria as well as eGFR stage progression from G1. The information of the PS-matched

Table 1: Clinical characteristics of all subjects.

	All subjects ($n = 699$)
Male/female	477/222
Start of the observation	
Age (years)	56.1 ± 10.4
Duration of diabetes (years)	9.0 ± 7.8
HbA1c (%)	7.7 ± 1.5
BMI (kg/m^2)	24.3 ± 4.0
SBP (mmHg)	135.8 ± 18.1
DBP (mmHg)	82.1 ± 11.4
HDL-C (mmol/l)	1.4 ± 0.4
LDL-C (mmol/l)	3.2 ± 0.9
Triglycerides (mmol/l)	1.6 ± 1.4
3-year period following the start of observation	
Mean HbA1c (%)	7.7 ± 1.1
Number of HbA1c measurements (times)	28 ± 10

Data are shown as the number or the mean ± SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SD: standard deviation.

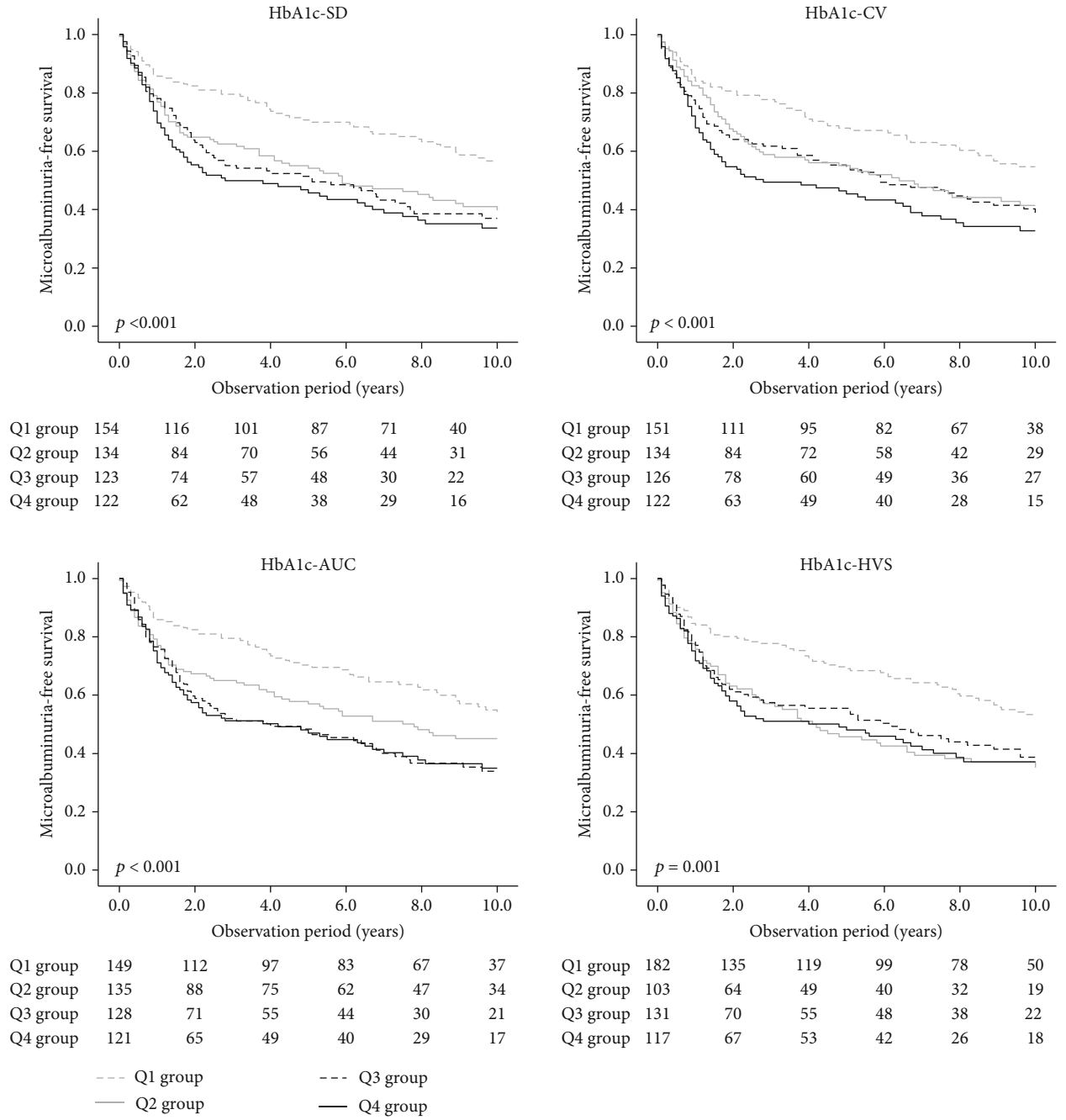

Figure 1: Kaplan-Meier curves for microalbuminuria-free survival among subjects divided by quartiles of the 4 indices of visit-to-visit variability of HbA1c. The quartiles of each index are represented by Q1, Q2, Q3, and Q4. The lower quartile is denoted as Q1, and the upper quartile is denoted as Q4. The comparison of the cumulative incidence among groups was carried out using a log rank test. HbA1c-SD: internal standard deviation of HbA1c; HbA1c-CV: coefficient of variation of HbA1c; HbA1c-AUC: area under the HbA1c curve; HbA1c-HVS: HbA1c change score.

subjects can be found in Supplementary Table 8-9, which showed there was no significant difference in the variables used in PS matching between matched subjects. Survival analyses were conducted to compare the risk of microalbuminuria and eGFR stage progression from G1 between the matched subjects with the highest and lowest visit-to-visit variability of HbA1c. In the analyses for HbA1c-SD, HbA1c-AUC, and HbA1c-HVS, the incidence of microalbuminuria was higher in the subjects with the highest HbA1c variability in comparison to those with the lowest HbA1c variability (Figure 2, Table 2). However, regarding eGFR stage progression, no significant difference was found in the risk of eGFR stage progression from G1 between the highest and lowest HbA1c variability groups; this was found with all indices (Supplementary Figure 6, Supplementary Table 6).

Table 2: Association of the risk of microalbuminuria with visit-to-visit variability of HbA1c, measured as 4 indices using a Cox proportional hazards model.

Index	HR (95% CI)[a]	p value[b]	p value[c]	PS-matched subjects HR (95% CI)	PS-matched subjects p value[b]
HbA1c-SD					
Q1	1	—	—	1	—
Q2	1.60 (1.11-2.30)	0.011	0.012		
Q3	1.52 (1.00-2.30)	0.050	0.076		
Q4	**1.77 (1.12-2.79)**	**0.014**	**0.015**	**3.18 (1.06-9.58)**	**0.040**
HbA1c-CV					
Q1	1	—	—	1	—
Q2	1.51 (1.06-2.17)	0.024	0.022		
Q3	1.19 (0.79-1.77)	0.404	0.407		
Q4	1.61 (1.06-2.44)	0.026	0.025	1.54 (0.57-4.17)	0.391
HbA1c-AUC					
Q1	1	—	—	1	—
Q2	1.28 (0.89-1.85)	0.183	0.158		
Q3	1.51 (1.01-2.26)	0.047	0.027		
Q4	**1.60 (1.02-2.49)**	**0.039**	**0.032**	**3.29 (1.13-9.61)**	**0.027**
HbA1c-HVS					
Q1	1	—	—	1	—
Q2	1.52 (1.07-2.17)	0.019	0.018		
Q3	1.19 (0.82-1.72)	0.365	0.371		
Q4	1.33 (0.83-2.12)	0.235	0.258	4.23 (1.51-11.85)	0.006

The quartiles of each index are represented by Q1, Q2, Q3, and Q4. The lower quartile is denoted as Q1, and the upper quartile is denoted as Q4. [a]Adjusted by age, sex, mean HbA1c over the 3-year period, duration of diabetes, SBP, LDL-C, and presence of ischemic heart disease and heart failure at baseline. [b]Analyzed using a Cox proportional hazards model. [c]Analyzed using a bootstrap analysis based on 1,000 replicated datasets. HR: hazard ratio; CI: confidence interval; PS: propensity score; HbA1c-SD: internal standard deviation of HbA1c; HbA1c-CV: coefficient of variation of HbA1c; HbA1c-AUC: area under the HbA1c curve; HbA1c-HVS: HbA1c change score; SBP: systolic blood pressure; LDL-C: low-density lipoprotein cholesterol.

Besides of analyzing the associations between category variables of variability of HbA1c, which were represented by the quartile groups, with incidence of microalbuminuria and eGFR stage progression in the Cox proportional hazards models, we also analyzed the continuous variables of variability of HbA1c in the Cox proportional hazards models. Increases in HbA1c-SD, HbA1c-CV, and HbA1c-AUC values were associated with an increase in the risk of microalbuminuria onset as well as the eGFR stage progression from G1 (Table 3).

Additionally, we investigated the association between visit-to-visit variability of HbA1c and oxidative stress. The degree of oxidized HSA was determined in 194 subjects, and the subjects were divided into 4 groups based on the quartiles of HbA1c-AUC and HbA1c-SD over a 2.5-year period. Multiple linear regression models showed significant associations between HbA1c-AUC and HbA1c-SD with the degree of oxidized HSA, and oxidized HSA was higher in the subjects with the highest HbA1c variability in comparison to those with the lowest HbA1c variability (Table 4).

4. Discussion

The present longitudinal study showed that the visit-to-visit variability of HbA1c was associated with the incidence of microalbuminuria in type 2 diabetes mellitus patients, independently of HbA1c. In addition, this is the first study to show that the HbA1c-AUC was associated with the incidence of microalbuminuria among the indices of visit-to-visit variability of HbA1c. Furthermore, oxidized HSA was associated with the HbA1c-AUC and HbA1c-SD, independently of the mean HbA1c. These results emphasize the importance of visit-to-visit variability of HbA1c in the early stage of DKD in relation to oxidative stress and indicate the potential of HbA1c-AUC as a novel predictive marker of the incidence of microalbuminuria.

A novel feature of our study was our use of four indices (HbA1c-SD, HbA1c-CV, HbA1c-AUC, and HbA1c-HVS) of visit-to-visit variability of HbA1c and the comparison of their predictive ability for the development of DKD. Dozens of studies have reported the association between HbA1c-SD and/or HbA1c-CV and the appearance of albuminuria and/or eGFR decline [8–11, 13]; however, studies on HbA1c-AUC and HbA1c-HVS are rare [14, 25]. Our study reveals that HbA1c-AUC quartiles were significantly associated with the appearance of microalbuminuria, the same as HbA1c-SD. Results from the bootstrap analysis and PS-matching analysis supported this association. Of note, the HRs of the quartiles of visit-to-visit variability of HbA1c for microalbuminuria showed an increasing trend from the lower quartile to the upper quartile in the results of HbA1c-AUC, which was not observed in the results of the other indices (Table 2). The

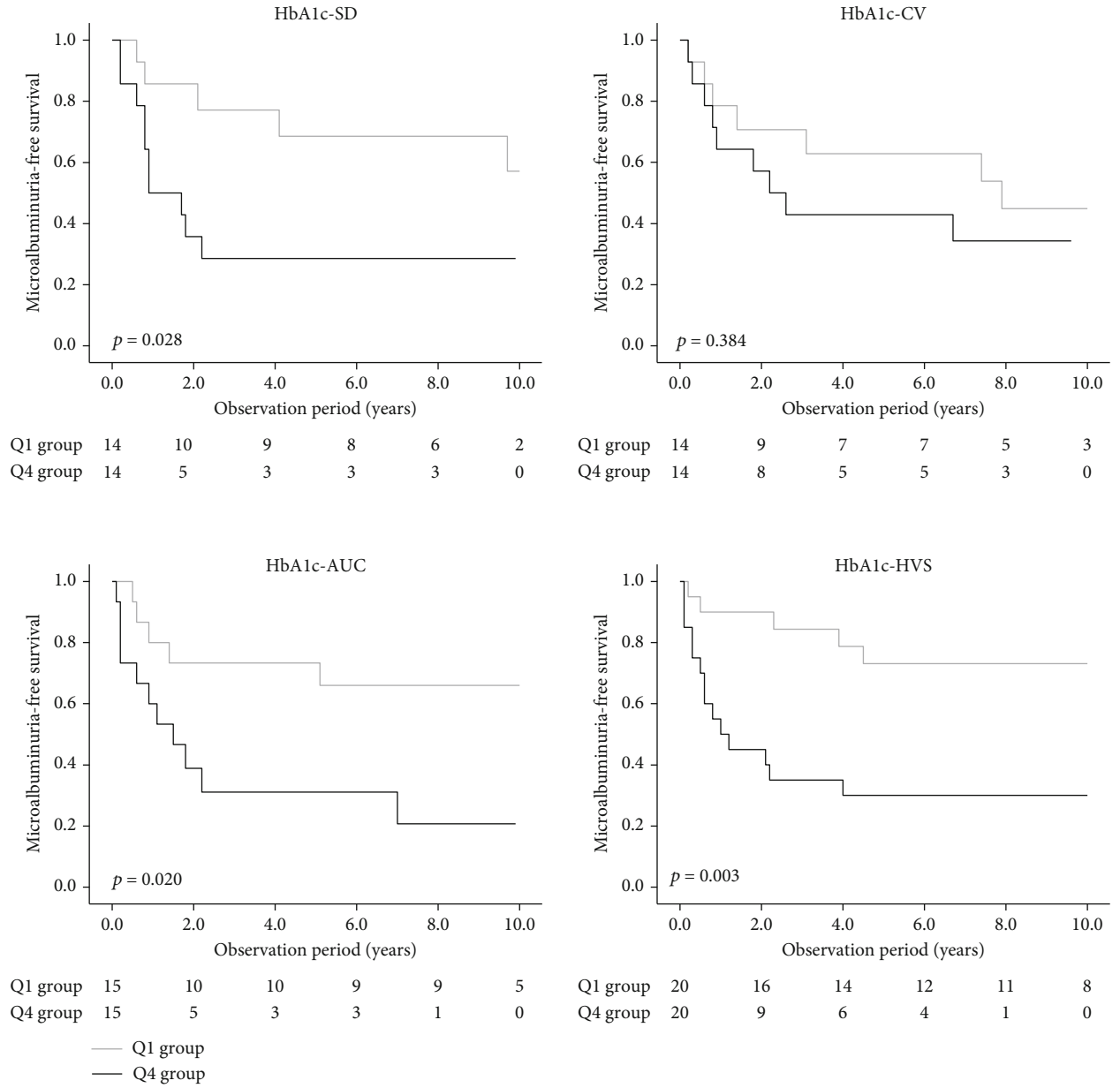

Figure 2: Kaplan-Meier curves for the microalbuminuria-free survival among PS-matched subjects from Q1 and Q4 group of each index of visit-to-visit variability of HbA1c. The lower quartile is denoted as Q1, and the upper quartile is denoted as Q4. The comparison of the cumulative incidence among groups was carried out using a log rank test. HbA1c-SD: internal standard deviation of HbA1c; HbA1c-CV: coefficient of variation of HbA1c; HbA1c-AUC: area under the HbA1c curve; HbA1c-HVS: HbA1c change score.

two conventional indices (i.e., HbA1c-SD and HbA1c-CV) are calculated with a discrete HbA1c value and are thought to be affected by the number of HbA1c measurements and the measurement intervals. In contrast, HbA1c-AUC, is calculated nearly as the definite integral of a curve that describes the variation of HbA1c, which takes the continuous change between two discrete HbA1c value into account, thus considered to be less influenced by the frequency of measurement. This may be why the tendency for the incidence of microalbuminuria to increase from the lower quartile to the upper quartile was only observed for HbA1c-AUC in the present study. Taken together, HbA1c-AUC may be a candidate predictor of microalbuminuria risk.

The mechanisms underlying the association between visit-to-visit variability of HbA1c and diabetic complications are not fully understood; however, oxidative stress and subsequent endothelial dysfunction due to the overproduction of the superoxide radical by glycemic variability are commonly considered to be key pathogenic factors [8–10, 17, 18, 26]. Conversely, oxidative stress may also affect HbA1c variability because it is associated with impaired insulin secretion from beta cells and impaired glucose uptake and utilization by hepatocytes and skeletal muscle cells [27]. Some oxidative stress markers, such as 8-iso-prostaglandin F2α, thiobarbituric acid-reactive substance, and 8-hydroxydeoxyguanosine were reported to be positively correlated with visit-to-visit variability

Table 3: Association of the risk of microalbuminuria and eGFR stage progression with continuous variables of visit-to-visit variability of HbA1c, measured as 4 indices using a Cox proportional hazards model.

Index	Incidence of microalbuminuria		eGFR stage progression from G1		eGFR stage progression from G2		eGFR stage progression from G3	
	HR (95% CI)[a]	p value	HR (95% CI)[a]	p value	HR (95% CI)[a]	p value	HR (95% CI)[a]	p value
HbA1c-SD	**1.18 (1.01-1.37)**	**0.033**	**1.23 (1.06-1.43)**	**0.008**	1.09 (0.86-1.37)	0.483	1.09 (0.73-1.61)	0.675
HbA1c-CV	**1.17 (1.03-1.34)**	**0.019**	**1.21 (1.05-1.39)**	**0.008**	1.09 (0.89-1.33)	0.400	1.09 (0.77-1.53)	0.634
HbA1c-AUC	**1.20 (1.03-1.40)**	**0.019**	**1.22 (1.05-1.42)**	**0.010**	1.09 (0.84-1.43)	0.509	1.04 (0.67-1.62)	0.848
HbA1c-HVS	1.00 (0.85-1.17)	0.952	1.03 (0.88-1.21)	0.735	1.18 (0.96-1.46)	0.105	0.99 (0.57-1.69)	0.958

[a]Adjusted by age, sex, mean HbA1c over the 3-year period, duration of diabetes, SBP, LDL-C, eGFR (in the analysis of eGFR stage progression), and presence of ischemic heart disease and heart failure at baseline. eGFR: estimated glomerular filtration rate; HR: hazard ratio; CI: confidence interval; HbA1c-SD: internal standard deviation of HbA1c; HbA1c-CV: coefficient of variation of HbA1c; HbA1c-AUC: area under the HbA1c curve; HbA1c-HVS: HbA1c change score; SBP: systolic blood pressure; LDL-C: low-density lipoprotein cholesterol.

Table 4: Association between degree of oxidized HSA and visit-to-visit variability of HbA1c, as assessed by a multiple linear regression model.

Index	B[a]	β	SE	p value
HbA1c-SD				
Q1	—	—	—	—
Q2	1.75	0.15	0.91	0.056
Q3	1.84	0.15	0.97	0.059
Q4	**2.35**	**0.19**	**1.18**	**0.047**
HbA1c-AUC				
Q1	—		—	—
Q2	**1.87**	**0.16**	**0.92**	**0.044**
Q3	**2.03**	**0.17**	**0.99**	**0.041**
Q4	**2.56**	**0.21**	**1.16**	**0.029**

The quartiles of each index are represented by Q1, Q2, Q3, and Q4. The lower quartile is denoted as Q1, and the upper quartile is denoted as Q4. [a]Adjusted by sex, mean HbA1c over the 2.5-year period, duration of diabetes, SBP, eGFR, and BMI. HSA: human serum albumin; HbA1c-SD: internal standard deviation of HbA1c; HbA1c-AUC: area under the HbA1c curve; B: unstandardized partial regression coefficient; β: partial correlation coefficients; SE: standardized error SBP: systolic blood pressure; eGFR: estimated glomerular filtration rate; BMI: body mass index.

of HbA1c [28]. In this study, we found that the degree of oxidized HSA, a marker of oxidative stress [19, 23, 29], affects HbA1c-AUC and HbA1c-SD, independently of the mean HbA1c value. We recently reported that oxidized HSA could be an early predictive marker of a declining renal function in patients with type 2 diabetes [19]. Therefore, visit-to-visit variability of HbA1c may influence the decline in the renal function of patients with type 2 diabetes in close association with the oxidative stress status. Moreover, since multiple measurements of HbA1c (i.e., multiple visits to the hospital and blood sampling) are required to calculate the visit-to-visit variability of HbA1c, we suggest that the single measurement of oxidized HSA, although not a completely predictive marker, may be useful for predicting subsequent visit-to-visit variability of HbA1c.

This study showed that the visit-to-visit variability of HbA1c is an independent risk factor for microalbuminuria among type 2 diabetes patients, emphasizing the importance of optimizing an unfluctuating HbA1c to prevent early development of DKD. Higher visit-to-visit variability of HbA1c may be the consequence of suboptimal diabetes management and less responsible behaviors, such as poor treatment adherence and unhealthy habits [30]. Preferred pharmacological strategies, such as new antidiabetic drugs combined with basal insulin or metformin as well as lifestyle modification, including exercise training (i.e., resistance exercise) and dietary intervention (i.e., low carbohydrate diet) may be potential strategies to alleviate glycemic variability [31–34]. Besides, seasonal changes contribute to HbA1c variability, with high and low HbA1c values generally observed in winter and summer, respectively [35]. In our study, we observed a similar influence of seasonal changes on the variation of HbA1c: the peak and trough HbA1c values appeared in March and August, respectively (Supplementary Figure 7). Yamada et al. reported that add-use of SGLT2 inhibitors attenuated the tendency for HbA1c to worsen towards the winter season [36], suggesting that a moderate adjustment in antiglycemic medication is a valuable strategy for treating seasonal variation in HbA1c.

HbA1c-HVS, weighted for clinically significant changes in HbA1c (>0.5%), was also measured in this study [15]. However, our data reveal that HbA1c-HVS was not associated with the development of DKD, which is likely caused by the distribution bias of the subjects; in our study, a high percentage of subjects (31%) had an HbA1c-HVS value of 0% and was classified into the Q1 group. Li et al. reported the role of HbA1c-HVS in the prediction of cardiovascular events and several microvascular complications among newly diagnosed type 2 diabetes patients [25], while HbA1c-HVS has not been widely used, the utility of HbA1c-HVS in representing visit-to-visit variability of HbA1c and predicting diabetic complications requires further exploration.

In this study, subjects with high visit-to-visit variability of HbA1c were characterized by younger age, higher BMI, blood pressure and triglyceride values, and lower HDL-C. Part of these characteristics were also observed in people at risk of developing CVD [37], which was reported to be associated with visit-to-visit variability in previous studies [8, 38–42]. Thus, subjects with high visit-to-visit variability of HbA1c may have a higher risk of developing CVD, along with clinical characteristics of CVD; however, whether there are biological mechanisms underlying the association between the clinical characteristics of CVD and high visit-to-visit variability of HbA1c is unclear.

The present study was associated with some limitations. In the present study, the effect of the HbA1c level on the association between visit-to-visit variability of HbA1c and DKD was minimized by adjusting for the mean HbA1c during the 3-year period. However, prescription patterns, such as intensive treatment, monotherapy or polytherapy, which can affect HbA1c levels, were not accounted for in the multivariable analysis, and this should be further investigated in a future study. The incidence of microalbuminuria in the subjects of this study was relatively high at 52% [43]. Some of the study subjects had been transferred from other hospitals and were under special management for diabetes at the diabetes center; these patients had a history of severe diabetes or suboptimal management, which may have contributed to the high incidence of microalbuminuria. In addition, the presence of an older age (48% of the subjects were over 60 years old), longer duration of diabetes (50% of the subjects had diabetes for more than 10 years), and more comorbidities such as hypertension (30%), dyslipidemia (54%), and macrovascular disease (25%) at baseline may also have been associated with the high incidence of microalbuminuria in this study. This study had a relatively small sample size, and subjects were limited to a Japanese population; accordingly, the results should be interpreted with some caution. Further investigations in a large-scale population involving subjects of different ethnicity or in different countries are necessary to confirm the findings.

The abovementioned limitations may partly explain why we did not find a significant association between visit-to-visit variability of HbA1c and eGFR stage progression. The effects of visit-to-visit variability of HbA1c were likely to have been masked by unadjusted confounding factors. Also, the small sample size in the analysis of eGFR stage progression may have been insufficient to reach statistical significance. On the other hand, another explanation is that the appearance of albuminuria and GFR decline occur independently of each other as risk factors in patients with diabetes [44]. Although visit-to-visit variability of HbA1c is associated with the appearance of microalbuminuria, it may not be associated with early eGFR stage progression, and two previous studies reported similar findings [9, 45].

The present study was also associated with some strengths. In addition to HbA1c-SD, HbA1c-CV and HbA1c-HVS and a new index, HbA1c-AUC, were employed and explored in this study. Since the subjects in our study had received monthly glycemic checkups in a diabetes clinic for a number of years, a large number of serial HbA1c measurements for individual patients were available in the analyses, which ensured the reliability of our findings. Additionally, a bootstrap analysis and sensitivity analysis were conducted to verify the robustness of the findings. Furthermore, we showed that oxidized HSA was associated with subsequent visit-to-visit variability of HbA1c.

5. Conclusions

In conclusion, our study showed that visit-to-visit variability of HbA1c is a novel predictor of microalbuminuria, independent of HbA1c. In particular, HbA1c-AUC could be an accurate and stable indicator of visit-to-visit variability of HbA1c. Moreover, we suggest that oxidized HSA may be a predictive marker of the visit-to-visit variability of HbA1c. This study provided evidence to support that visit-to-visit variability of HbA1c plays an important role in the development of DKD among type 2 diabetes patients and pointed out the importance of paying attention to the control of HbA1c variability in diabetes treatment.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

YY contributed to the conception, design, and statistical analysis of the study and the drafting of the manuscript. NK contributed to the conception, design, data collection, and statistical analysis of the study. KO contributed to the conception, design, data collection, and statistical analysis of the study and the drafting of the manuscript. HW, TI, YS, HM, TM, and HJ contributed to the data collection and offered critical comments. TS, A. Maruyama, HN, TK, A. Morita, and AY contributed to the data collection. JS reviewed the conception and design of the study, offered critical comments, and contributed to the writing of the manuscript. All authors read and approved the final version of the article. Yunyi Yan, Nozomi Kondo, and Kentaro Oniki contributed equally to this work.

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Supplementary Materials

Supplementary Figure 1: study design to investigate the association between visit-to-visit variability of HbA1c and DKD risk. Supplementary Figure 2: calculation method of HbA1c-AUC. Supplementary Figure 3: Kaplan-Meier curves for eGFR stage progression-free survival from G1 among subjects divided by quartiles of the 4 indices of visit-to-visit variability of HbA1c. Supplementary Figure 4: Kaplan-Meier curves for eGFR stage progression-free survival from G2 among subjects divided by quartiles of the 4 indices of visit-to-visit variability of HbA1c. Supplementary Figure 5: Kaplan-Meier curves for eGFR stage progression-free survival from G3 among subjects divided by quartiles of the 4 indices of visit-to-visit variability of HbA1c. Supplementary Figure 6: Kaplan-Meier curves for eGFR stage progression-free survival from G1 among PS-matched subjects from the Q1 and Q4 groups of each index of visit-to-visit

variability of HbA1c. Supplementary Figure 7: seasonal changes affected HbA1c. Supplementary Table 1: value of the mean and quartiles of the 4 indices of visit-to-visit variability of HbA1c. Supplementary Table 2: baseline characteristics of all subjects divided by the quartiles of HbA1c-SD. Supplementary Table 3: baseline characteristics of all subjects divided by the quartiles of HbA1c-CV. Supplementary Table 4: baseline characteristics of all subjects divided by the quartiles of HbA1c-AUC. Supplementary Table 5: baseline characteristics of all subjects divided by the quartiles of HbA1c-HVS. Supplementary Table 6: association of the risk in eGFR stage progression from G1 with visit-to-visit variability of HbA1c, measured as 4 indices. The results were analyzed using a Cox proportional hazards model. Supplementary Table 7: association of the risk in eGFR stage progression from G2 and from G3 with visit-to-visit variability of HbA1c, measured as 4 indices. The results were analyzed using a Cox proportional hazards model. Supplementary Table 8: information of PS-matched subjects in the longitudinal analyses for the risk of microalbuminuria. Supplementary Table 9: information of PS-matched subjects in the longitudinal analyses for the risk of eGFR stage progression from G1. *(Supplementary Materials)*

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Research Article

A Comparative Study of Health Efficacy Indicators in Subjects with T2DM Applying Power Cycling to 12 Weeks of Low-Volume High-Intensity Interval Training and Moderate-Intensity Continuous Training

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This study is aimed at comparing the effects of different exercise intensities, namely, high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), on body composition, heart and lung fitness, and blood glucose, and blood pressure indices in patients with type 2 diabetes mellitus (T2DM), using power cycling. A total of 96 T2DM volunteers who met the inclusion criteria were recruited from a hospital in Yangpu, Shanghai. Based on the blood index data of their medical examination results which comprised blood pressure, fasting blood glucose, hemoglobin A1c (HbA1c), and insulin, 37 volunteers were included in the study. Exercise prescription was determined based on T2DM exercise guidelines combined with medical diagnosis and exercise test results, and the patients were randomly assigned to three groups: HIIT group, MICT group, and control (CON) group. HIIT involved one-minute power cycling (80%–95% maximal oxygen uptake (VO2max)), one-minute passive or active rest (25%–30% VO2max), and two-minute rounds of eight groups. MICT required the use of a power bike for 30 minutes of continuous training (50%–70% VO2max) five times a week. The CON group was introduced to relevant medicine, exercise, and nutrition knowledge. The exercise interventions were completed under the supervision of an exercise instructor and hospital doctors. The same indicators were measured after 12 weeks of intervention, and the results of the two tests within and between groups were analyzed for comparison. The weight index of the MICT intervention showed statistically significant within-group differences (difference = 3.52, 95% CI = 2.11–4.92, $p = 0.001 < 0.01$); group differences for the MICT and CON groups were also statistically significant (difference = 3.52 ± 2.09, Cd1 = -0.39 ± 1.25, $p = 0.004 < 0.01$). Body mass index (BMI) analysis revealed that the overall means of BMI indicators were not statistically different between groups ($F = 0.369$, $p = 0.694 > 0.05$) and the before and after values of the MICT and CON (difference = -1.30 ± 0.79, Cd1 = -0.18 ± 0.45, $p = 0.001 < 0.01$). No statistically significant difference was observed in the overall mean VO_2max index between the groups after the 12-week intervention ($F = 2.51$, $p = 0.100 > 0.05$). A statistically significant difference was found in the overall means of the data between the two groups (difference = 0.32, 95% CI = 0.23–0.40, $p = 0.001 < 0.01$). Analysis of fasting blood glucose (FBG) indicators revealed statistically significant differences between the MICT and control groups ($p = 0.028 < 0.05$). Analysis of HbA1c and fasting insulin (FI) indicators revealed no statistically significant difference in the overall HbA1c index after the 12-week exercise intervention ($F = 0.523$, $p = 0.598 > 0.05$), and the overall difference before and after the experiment between the groups was statistically significant ($F = 6.13$, $p = 0.006 < 0.01$). No statistically significant difference was found in the FI index overall after the 12-week exercise intervention ($F = 2.50$, $p = 0.1 > 0.05$). Analysis of systolic blood pressure (SBP) revealed statistically significant difference before and after the HIIT and CON interventions (Hd7 = -1.10 ± 1.79, Cd7 = 1.2 ± 1.31, $p = 0.018 < 0.05$) and statistically significant difference before and after the MICT and CON interventions (Md7 = -0.99 ± 0.91, Cd7 = 1.40 ± 1.78, $p = 0.02 < 0.05$). The diastolic blood pressure (DBP) revealed no statistically significant within-group differences before and after. Exercise interventions applying both low-volume HIIT and MICT, with both intensity exercises designed for power cycling, improved health-related indicators in the participants; low-volume HIIT had more time advantage. The current experiment compared HIIT with MICT in a safe manner: 50% of the

exercise time produced similar benefits and advantages in the two indicators of VO_2max and FI. However, MICT was superior to HIIT in the two indicators of body weight (weight) and BMI. The effect of power cycling on FI has the advantages of both aerobic and resistance exercise, which may optimize the type, intensity, and time of exercise prescription according to the individual or the type of exercise program. Our results provide a reference for the personalization of exercise prescription for patients with T2DM.

1. Introduction

Diabetes is a major public health problem worldwide [1], with type 2 diabetes mellitus (T2DM) accounting for more than 90% of cases. Diabetes is accompanied by a range of risk factors, such as cardiovascular diseases, hypertension, and dyslipidemia. Even with supplemental medications, the economic and social problems associated with T2DM treatment are becoming increasingly serious. With the multifaceted nature of T2DM etiology, with modifiable factors such as being overweight/obese, physical inactivity, and sedentary lifestyle, reducing the burden of disease requires effective and accessible lifestyle interventions [2, 3].

Exercise interventions are an important tool in the prevention and management of T2DM [4, 5] and can improve a wide range of cardiovascular and metabolic outcomes [6–8]. However, the optimal exercise prescription to maintain or improve the health status of the T2DM population remains uncertain. Exercise guidelines generally recommend at least 150 minutes of continuous moderate intensity (40%–60% of maximal oxygen uptake (VO2max)) or 75 minutes of higher-intensity (60%–85% VO2max) exercise per week [9, 10], but existing strategies face significant challenges, from lack of adherence to limited motivation and time to follow these guidelines [11]. The primary goal of exercise interventions in T2DM is to improve glycemia and insulin levels, but given the presence of comorbidities and the variety of causative factors in a larger T2DM population, improving body composition, aerobic fitness, and blood pressure and lipid levels are also important goals [12]. The increase in the prevalence of T2DM calls for more effective and targeted exercise prescriptions, and the type and intensity of exercise training should be tailored to the patient. Although the range of exercise guidelines for the T2DM population is currently expanding, specific evidence on the most recommended frequency, intensity, duration, and type of exercise for different conditions is lacking [13]. Therefore, understanding the different variables associated with the beneficial effects of exercise in the T2DM population is of practical interest [14, 15]. High-intensity interval training (HIIT) appears to be a viable and effective alternative exercise regimen to traditional moderate-to-high-intensity continuous training. It consists of alternating repetitions of short periods of high-intensity exercise interspersed with less active or passive recovery periods. HIIT has a moderating effect on clinical measures of the T2DM population and has also demonstrated effectiveness on glycemia, insulin, body composition, blood pressure, and aerobic capacity levels [16]. HIIT and MICT have different characteristics and effects as two training modalities, but there is not enough published data to conclude which is more effective [17]. Recently, published meta-analyses of the effects of HIIT practice have only demonstrated that HIIT is superior to MICT in improving cardiorespiratory fitness in patients with T2DM [18]. Body composition, blood glucose, and blood pressure were not found to differ between the two exercise modalities. Moreover, the high risk of study bias and low quality of evidence require the use of more randomized controlled trials [19].

As HIIT has several contraindications and risks in the T2DM population [20], it should only be recommended if the benefit or motivation is at least similar to that of MICT [10, 20, 21], and the potential benefits of HIIT should be compared with conventional MICT to ensure the best possible health benefits. Thus, people with T2DM may choose a more optimal intensity exercise prescription based on their own motivation or on the goal of improving different health indicators. Gibala et al. built on the original study by examining the impact of a lighter intensity exercise, a more realistic and feasible HIIT protocol [22, 23], proposed a low-volume HIIT protocol consisting of ten 60-second work rounds at 90% of maximum heart rate with 60-second recovery intervals, which could provide a more suitable duration and a better level of motivation [24–26]. Many effective HIIT protocols involved high-intensity uphill treadmill walking or running, which may be difficult for people with T2DM who are at a higher risk of falls and have limited lower extremity mobility. Thus, it is difficult to compare studies on the modalities, timing, and environmental conditions of exercise prescriptions needed to optimize efficacy [27].

Power cycling is desirable and commonly ideal in settings such as physical fitness and rehabilitation centers. This exercise mobilizes large muscle groups without weight bearing and foot-to-ground friction during running. Especially for the T2DM population, it is necessary to determine the HIIT and MICT protocols before applying power cycling for safety and effectiveness purposes. The present study is aimed at implementing a low-volume HIIT and MICT exercise intervention using power bikes as exercise equipment to provide evidence for HIIT and MICT exercise management and optimal exercise prescription in the T2DM population. Specifically, it aimed to (1) apply power cycling to the T2DM population for HIIT and MICT experimental data collection to test for good tolerance and (2) compare the different effects of HIIT and MICT exercise interventions on body composition, aerobic fitness, blood glucose, insulin, and other important indicators of health in a T2DM population.

2. Materials and Methods

2.1. Participant Inclusion and Exclusion. Thirty-seven male volunteers were recruited from the diabetes clinic of a hospital in Yangpu District, Shanghai, according to the succeeding criteria. The following were screen for the physical examination: diagnosis of T2DM for at least one year, meeting the WHO diagnostic criteria for diabetes [24], no major

macroscopic or microscopic vascular complications of diabetes, aged 32 to 47 years, and with a body mass index (BMI) of <35 kg/m^2. Participants also must have no physical limitations to the exercise intervention to be performed, no limitations in gait or balance, and no major health problems. For the behavioral habits, the following were checked: no smoking in the past six months, no participation in a supervised exercise program, and maintenance of a diet for at least six months. All participants were asked to complete the Physical Activity Questionnaire, which includes eating behavior. The recruitment also included exercise testing. Prior to participation in the trial, all participants underwent a detailed medical assessment to screen for relative or absolute contraindications to high-intensity exercise, including the use of the exercise plate test (Bruce protocol) to confirm the absence of potential cardiac contraindications. Meanwhile, the exclusion criteria were as follows: participants receiving exogenous insulin therapy; smokers; those with unstable weight (5 kg/6 months); those with a condition that precluded physical activity, such as evidence of acute disease or renal, hepatic, or cardiovascular disease; failure to perform all experimental conditions; failure to complete changes in medications prescribed for the experiment period; changes in dietary patterns; and participation in other supervised exercises (see Figure 1).

The experimental procedures and potential risks were explained to the participants prior to the study. Written informed consent was obtained from all the participants. They received good treatment at baseline, and their medications were not changed during the study. The study protocol was approved by the local hospital ethics committee (Ethics Committee Protocol Number: LL-KY-009).

2.2. Study Methodology

2.2.1. Experimental Design and Preexercise Adaptation. For participant allocation during the 12-week parallel randomized controlled clinical trial, after baseline assessment, researchers external to the project used a computer-generated random number list with a 1 : 1 : 1 allocation ratio. The participants were given opaque sealed envelopes and were randomized into three groups: HIIT ($n = 13$), MICT ($n = 12$), and CON ($n = 12$). Both the HIIT and MICT groups used the Swedish Monark power bike as a device for the exercise intervention, whereas the CON group received standard counseling on conventional T2DM exercise guidelines and did not perform organized exercise. For ethical reasons, all participants were provided with standard counseling on topics such as nutrition or exercise to improve trial adherence. Throughout the study period, participants also received information on maintaining activities of daily living (daily diet habitual physical activity and medication). Counseling covered overcoming barriers to exercise, enhancing self-regulation, self-efficacy, planning, and increasing awareness of the physical and mental benefits of exercise, considering that fluctuating blood glucose levels may be more harmful than stable high blood glucose levels. The HIIT, MICT, and CON groups were instructed in the 14-day real-time monitoring of blood glucose dynamics with a blood glucose monitor to avoid excessive fluctuations in blood glucose levels. They were given a detailed explanation of the heart rate scale, scores, and meaning of the ratings of perceived exercise (RPE) scale before the intervention. The patients were also allowed to rate themselves during the preexperiment to familiarize themselves with the form. They were introduced to their assigned exercise modality, namely, HIIT or MICT, to help them maintain consistent exercise adherence. Two weeks prior to the start of the study, the participants were organized to visit the laboratory and try the power bikes to help them acclimatize. The HIIT intensity was not standardized but based on the individual cardiorespiratory adaptations of the exercisers, and the experimental procedure was explained in detail to all participants. In the days leading up to the experiment, the participants were asked to maintain a normal diet and avoid engaging in extrasport exercise or strenuous physical activity. Participants in both exercise groups received 15 minutes of behavioral coaching three times a week (45 minutes in total) with the aim of preparing them for the transition to the exercise prescriptions by power cycling. The load used to achieve the different intensities of exercise tested in the study (HIIT and MICT) was progressively increased via one-on-one coaching.

2.2.2. HIIT and MICT Exercise Protocol. Both groups exercised for five times per week, supervised by an exercise instructor, and monitored using a heart rate band (polarT-31, USA). The maximum oxygen uptake of patients with T2DM was tested using the Astrand test method with the Swedish Monark power bike LC7, and their heart rate was monitored using a polar meter. A self-fatigue scale was placed directly in front of the power bike. The exercise intervention protocol was standardized according to extant guidelines [28] and consisted of 30 minutes per session for the MICT, except for preparatory and finishing activities, and 15 minutes per session for the HIIT group. This is consistent with the recommendations of at least 150 minutes of moderate-intensity exercise or 75 minutes of vigorous exercise per week for adults. All participants completed a five-minute warm-up and five-minute finishing activities at similar intensities during each supervised exercise session [29]. In summary, the time (minutes) allocated to each exercise session was as follows: a five-minute warm-up (three minutes off the bike, two minutes on the bike), moderate-intensity exercise (gradually adjusting the power bike load to a heart rate maximal oxygen uptake in the 50%–70% and 80%–95% range for MCT and HIIT, respectively) for 30 minutes for MICT and 15 minutes for HIIT (including eight minutes of intensity in the 80%–95% range and seven minutes of intervals for active recovery at approximately 25% intensity [30]), and five minutes to complete the relaxation and finishing process (see Figure 2).

2.2.3. Supervision and Exercise Intervention Process. The participants were contacted to determine specific times for each exercise intervention, and the exercise intervention protocol was generally performed under the supervision of both an exercise instructor and a physician. The intervention had

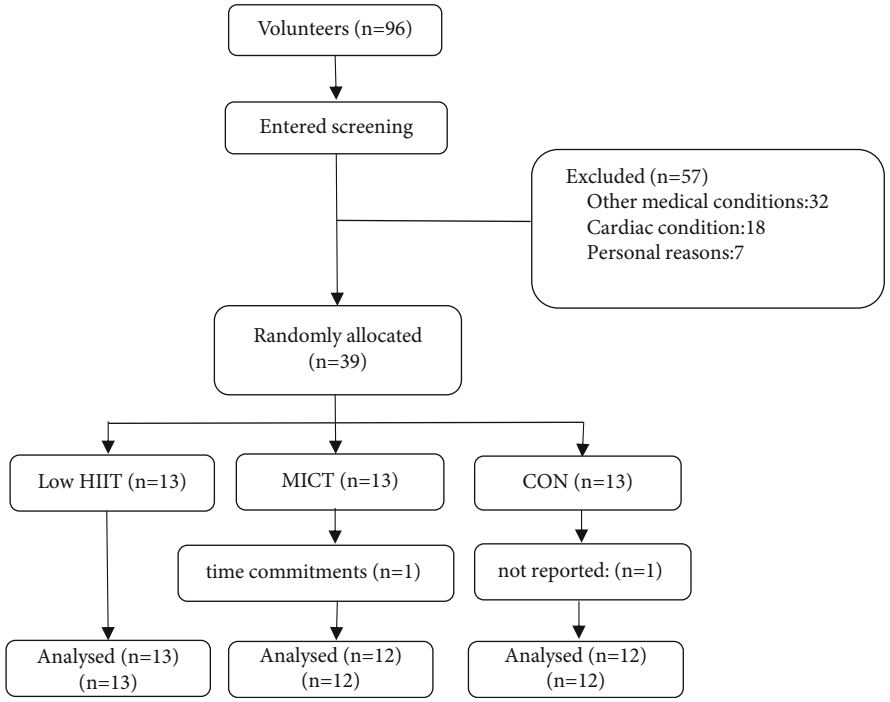

Figure 1: Flow chart of volunteer recruitment for the experiment.

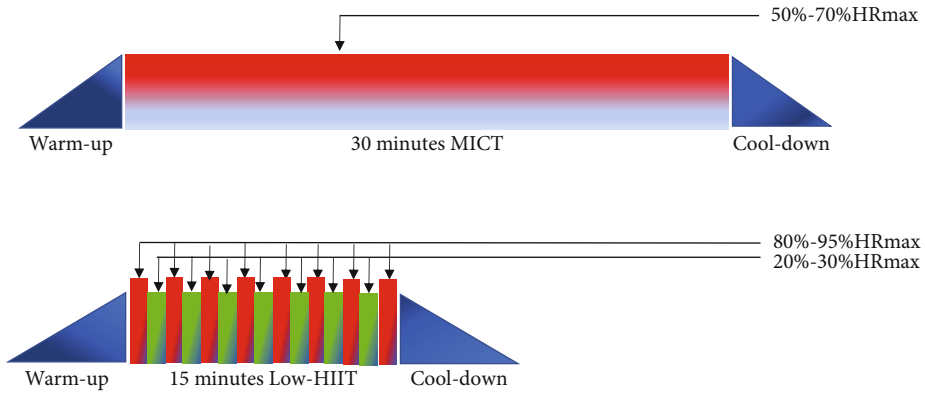

Figure 2: Schematic diagram of the two exercise schemes.

five scheduled exercise workouts per week with no more than two days of intervals between sessions, for a total of 60 scheduled workouts over the 12-week study. The HIIT and MICT protocols were maintained in the intensity range of one-two sessions over 12 weeks of training on an individual basis. Heart rate provided a basis for progression throughout the exercise intervention, with an increasing number of repetitions and duration of each repetition. Heart rate was continuously monitored during the supervised exercise intervention, and participants' heart rates were recorded using a downloadable Polar heart rate monitor (Polarft7, Finland) to ensure training at the intended intensity. During each session, the heart rate was recorded using a cycle tester. RPE was recorded using a subjective exertion rating scale (RPE 6-20) at the end of each week [12]. When a rapid rise in heart rate occurred during exercise, the researcher made inquiries and stopped the experiment if the participant reported discomfort. If chest tightness, heart pain, and head pain were reported, the experiment was stopped immediately. Participants were asked to engage in simple housework, walking, and other daily physical activities in addition to the HIIT and MICT exercise protocols during the exercise intervention.

2.2.4. Tests of Basic Physical Characteristics, Blood Biochemical Indicators. All participants were tested for height, weight, blood pressure, VO2max, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), fasting insulin (FI), and other blood biochemical indices at the experimental hospital before the exercise intervention was conducted. The participants' BMI was calculated, and the VO2max assessment test was performed using the Astrand test method available on the Swedish Monark power bicycle LC7. For glucose, the glucose oxidase method was used.

Table 1: Participant characteristics.

	HIIT	MICT	CON
Age (years)	38 ± 6	39 ± 5	40 ± 7
Height (cm)	166.9 ± 6.25	165.8 ± 5.56	166.7 ± 6.86
Time since diagnosis (years)	1.95 ± 0.55	1.79 ± 0.52	1.84 ± 0.49
Sex (m/f)	13	12	12
BMI	27.38 ± 5.53	26.75 ± 4.20	26.45 ± 4.97
Diet only	10	8	9
Comorbidities	3	4	3
Medication			
Metformin	6	6	7
Sulfonylureas	3	2	3
DPP-4 inhibitors	3	2	2
Alpha-glucosidase inhibitor	1		
Smokers	13/5	12/5	12/4

HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control.

For HbA1c, an affinity chromatography microcolumn assay was used. An enzyme-linked immunoassay using an automated biochemical analyzer was done to assess insulin level. The test indices were repeated after the 12-week experiment.

2.2.5. Statistical Analysis. Data analysis was performed using IBM SPSS Statistics for Windows, Version 22.0, and all data were expressed as mean ± standard deviation. To compare the data before and after the intervention in each group, normality test was performed. Paired sample t-test was used for results following normal distribution, and Wilcoxon test for two associated samples was used for those that do not. For comparison between the HIIT, MICT, and CON groups after the intervention, data following the normal curve were subjected to single-factor ANOVA. The Bonferroni post hoc test was used for two-way comparisons. Those that did not follow a normal distribution were subjected to the Kruskal–Wallis H-test. All pairwise comparisons of Kruskal–Wallis and one-way ANOVA multiple comparisons were used for two-by-two comparisons. When ANOVA results were not statistically significant, one-way ANOVAs or rank-sum tests were used for before and after differences for each group. In all tests, statistical significance was set at $p < 0.05$.

3. Results

3.1. Participants' General Conditions. Table 1 shows the characteristics of the participants in the three groups. The participants' baseline conditions were not statistically different and were comparable in terms of basic information, body morphology, cardiopulmonary function, and glucose, insulin, and lipid metabolism levels (Table 2). No adverse events were observed or reported by participants throughout the course of the exercise training protocol.

3.2. Comparison of Weight, BMI, and VO_2max Indicators between Groups after Exercise Intervention. The weight index values before and after MICT intervention were 73.12 ± 7.83 kg and 69.60 ± 5.91 kg, respectively. Statistical differences was observed within groups (difference = 3.52, 95% CI: 2.11–4.92, $p = 0.001 < 0.01$). The mean weight index did not differ significantly between the groups ($F = 0.953$, $p = 0.398 > 0.05$). The difference weight before and after the experiment was statistically different overall among the three groups ($F = 12.90$, $p = 0.002 < 0.01$), with differences observed before and after the experiment between HIIT and MICT (Hd = −0.51 ± 1.04, Md = 3.52 ± 2.09, $p = 0.011 < 0.05$), before and after the experiment between HIIT and CON (Hd = −0.72 ± 0.35, Cd = −0.39 ± 1.25, $p = 1.000 > 0.05$), and before and after the MICT and CON experiments (Md = 3.52 ± 2.09, Cd = −0.39 ± 1.25, $p = 0.004 < 0.01$).

BMI index analysis revealed a range of 26.75 ± 4.20 kg/m^2 before the MICT exercise intervention and a range of 25.45 ± 3.51 kg/m^2 12 weeks after the exercise intervention. The overall means of the data in the HIIT and MICT groups were statistically different (difference = 1.3, 95% CI: 0.77–1.83, $p = 0.001 < 0.01$). The overall means of BMI indicators were not statistically different between the groups ($F = 0.369$, $p = 0.694 > 0.05$). The overall pre- and postexperimental difference d2 of the groups was statistically different ($F = 13.02$, $p = 0.001 < 0.01$) between HIIT and MICT, (Hd = −0.21 ± 0.37, Md = −1.30 ± 0.79, $p = 0.001 < 0.05$), between HIIT and CON (Hd = −0.21 ± 0.37, Cd = −0.18 ± 0.45, $p = 1.000 > 0.05$), and between MICT and CON (Md = −1.30 ± 0.79, Cd = −0.18 ± 0.45, $p = 0.001 < 0.01$) (see Figures 3 and 4) (note: HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control).

The within-group MICT exercise VO_2max indicators before and after the intervention in each group were 3.46 ± 0.38 L/min and 3.77 ± 0.45 L/min, respectively, with a statistically significant difference in the overall means of the data between the two data sets (difference = 0.32, 95% CI: 0.23–0.40, $p = 0.001 < 0.01$). The HIIT exercise VO_2max index was 3.39 ± 0.44 L/min before and 3.92 ± 0.43 L/min

Table 2: Changes in participants' characteristic body composition, VO2max peak, blood glucose, and blood pressure control.

Groups	Pre	Post	*d*	*t*	*p*
Body mass (kg)					
HIIT	75 ± 9.98	74.49 ± 9.15	−0.51 ± 1.04		
MICT	73.12 ± 7.83	69.60 ± 5.91**	−3.52 ± 2.09§	5.56	0.001
CON	71.76 ± 9.72	71.37 ± 9.23	−0.39 ± 1.25		
F			12.90		
p			0.002		
BMI (kg/m^2)					
HIIT	27.38 ± 5.53	27.17 ± 5.23	−0.21 ± 0.37		
MICT	26.75 ± 4.20	25.45 ± 3.51**	−1.30 ± 0.79§	5.46	0.001
CON	26.45 ± 4.97	26.27 ± 4.88	−0.18 ± 0.45		
F			13.02		
p			0.001		
VO2max (L/min)					
HIIT	3.4 ± 0.4	3.9 ± 0.4**	0.52 ± 0.06§§	-27.29	0.001
MICT	3.5 ± 0.4	3.7 ± 0.5**	0.31 ± 0.13	-8.06	0.001
CON	3.5 ± 0.4	3.5 ± 0.5	−0.03 ± 0.10		
F			75.00		
p			0.001		
Fasting glucose (mmol/L)					
HIIT	7.80 ± 0.50	6.93 ± 0.33**#		9.70	0.001
MICT	7.60 ± 0.52	6.83 ± 0.44**		6.07	0.001
CON	7.47 ± 0.57	7.42 ± 0.62			
F		4.39			
p		0.022			
HbA1c (%)					
HIIT	7.18 ± 0.50	6.79 ± 0.41**	−0.20 ± 0.19	3.32	0.009
MICT	7.02 ± 0.44	6.88 ± 0.40**	−0.14 ± 0.14	3.26	0.009
CON	7.06 ± 0.38	7.09 ± 0.33	0.03 ± 0.12		
F			6.13		
p			0.006		
Fasting insulin (pmol/L)					
HIIT	27.04 ± 1.06	24.65 ± 1.38**	−2.39 ± 1.47§	5.14	0.001
MICT	26.35 ± 1.43	25.35 ± 1.49**	−0.99 ± 0.91	3.63	0.005
CON	26.88 ± 1.64	26.37 ± 2.21	−0.51 ± 1.26		
F			6.37		
p			0.005		
Systolic (mmHg)					
HIIT	140.2 ± 3.23	139.4 ± 2.88			
MICT	135.5 ± 7.23	134.7 ± 7.02			
CON	134.2 ± 8.44	135.4 ± 8.85*		-2.88	0.018
F					
p					

Table 2: Continued.

Groups	Pre	Post	d	t	p
Diastolic (mmHg)					
HIIT	75.40 ± 6.55	74.50 ± 5.19			
MICT	74.55 ± 6.92	74.45 ± 5.92			
CON	77.80 ± 6.29	78.10 ± 6.21			
F					
p					

Values are mean ± standard deviation; paired-sample t-test was used to compare data before and after the intervention in each group, $^{*}p < 0.05$, $^{**}p < 0.01$ for statistically significant differences; one-way ANOVA was used to compare postintervention differences between HIIT, MICT, and CON groups, and Bonferroni post hoc test was used for two-way comparisons. $\#p < 0.05$, $\#\#p < 0.01$ for statistically significant differences between groups; $\S p < 0.05$, $\S\S p < 0.01$ for statistically significant differences in HIIT vs. MICT differences. HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control.

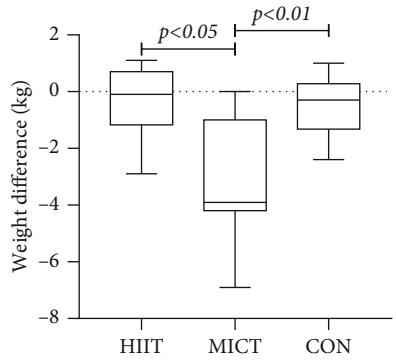

Figure 3: Comparison of weight difference between groups after the intervention.

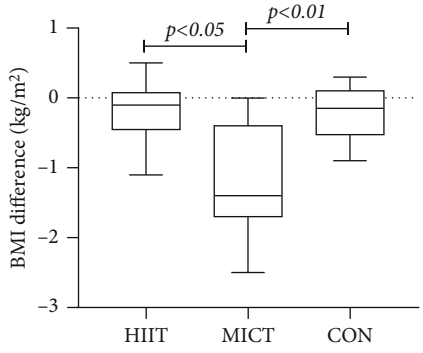

Figure 4: Comparison of BMI differences in each group after the intervention.

after the intervention, with a statistically significant difference in overall means between the two data sets (difference = 0.53, 95% CI: 0.48–0.57, $p = 0.001 < 0.01$). There was no statistically significant difference in the overall mean VO_2max index between the groups after the 12-week intervention ($F = 2.51$, $p = 0.100 > 0.05$). The pre- and post-experimental differences were statistically different overall ($F = 75$, $p = 0.001 < 0.01$), with pre- and postexperimental differences between HIIT and MICT (Hd = 0.52 ± 0.06, Md = 0.31 ± 0.13, $p = 0.001 < 0.01$), between HIIT and CON (Hd = 0.52 ± 0.06, Cd = −0.03 ± 0.10, $p = 0.001 < 0.01$), and before and after the MICT and CON (Md = 0.31 ± 0.13, Cd = −0.03 ± 0.10, $p = 0.001 < 0.01$) (see Figure 5) (note: HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control).

3.3. Comparison of Blood Glucose and Insulin Indices between Groups after Exercise Intervention. The within-group FBG indicators were 7.60 ± 0.52 mmol/L before and 6.83 ± 0.44 mmol/L after the exercise intervention for MICT, with a statistically significant difference in the overall means of the data between the two data sets (difference = 0.77, 95% CI: 0.49–1.05, $p = 0.001 < 0.01$). The HIIT FBG index was 7.80 ± 0.50 mmol/L before and 6.93 ± 0.33 mmol/L after the exercise intervention, with a statistically significant difference in overall means between the two data sets (difference = 0.87, 95% CI: 0.66–1.07, $p = 0.001 < 0.01$). The overall means of the FBG index were statistically different between groups after the 12-week intervention ($F = 4.399$, $p = 0.022 < 0.05$), with the MICT showing statistically different results from the control group ($p = 0.028 < 0.05$).

Analysis of HbA1c indicators revealed that the overall mean was statistically different between the HIIT and MICT groups (difference = 0.14, 95% CI: 0.04–0.23, $p = 0.009 < 0.01$) after the intervention. For the MICT group, the indicator was 7.02 ± 0.44 mmol/L before the intervention and 6.88 ± 0.40 mmol/L after. The HIIT HbA1c index was 7.18 ± 0.50 mmol/L before and 6.79 ± 0.41 mmol/L after, with a statistically significant difference in overall means between the two data sets (difference = 0.21, 95% CI: 0.07–0.34, $p = 0.009 < 0.01$). No statistically significant difference was found in the overall HbA1c index after the 12-week exercise intervention ($F = 0.523$, $p = 0.598 > 0.05$). The overall difference in before and after the experiment was statistically different between HIIT and MICT ($F = 6.13$, $p = 0.006 < 0.01$). The differences before and after the experiment were as follows: between HIIT and MICT (Hd = −0.20 ± 0.19, Md = −0.14 ± 0.14, $p = 0.972$), between HIIT and CON (Hd = −0.20 ± 0.19, Cd = 0.03 ± 0.12, $p = 0.006 < 0.01$), and between MICT and CON (Md = −0.14 ± 0.14, Cd = 0.03 ± 0.12, $p = 0.06 > 0.05$) (see Figures 6 and 7) (note: HIIT:

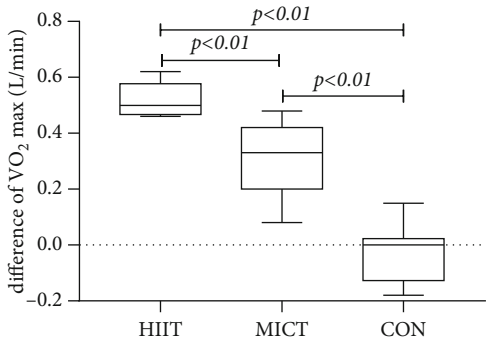

Figure 5: Comparison of difference of VO_2max in each group after intervention.

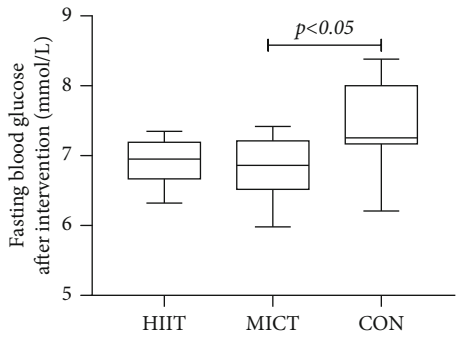

Figure 6: Fasting blood glucose values of each group after intervention.

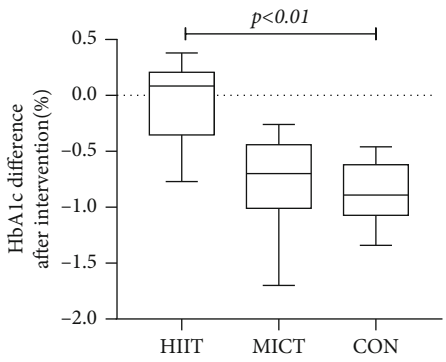

Figure 7: Difference of HbA1c in each group after intervention.

high-intensity interval training; MICT: moderate-intensity continuous training; CON: control).

The FI indicators were 26.35 ± 1.43 pmol/L before and 25.35 ± 1.49 pmol/L after the MICT exercise intervention, with a statistically significant difference in the overall means of the data between the two data sets (difference = 0.99, 95% CI: 0.38–1.60, $p = 0.005 < 0.01$). The FI index was 27.04 ± 1.06 pmol/L before and 24.65 ± 1.38 pmol/L after the HIIT exercise intervention, with a statistically significant difference between the overall means of the two data sets (difference = 0.51, 95% CI: -0.39–1.41, $p = 0.001 < 0.01$). There was no statistically significant difference noted in the FI index overall after the 12-week exercise intervention ($F = 2.50$, $p = 0.1 > 0.05$). The pre- and postexperimental differences were statistically different overall between the groups ($F = 6.37$, $p = 0.005 < 0.01$), between HIIT and MICT (Hd = −2.39 ± 1.47, Md = −0.99 ± 0.91, $p = 0.043 < 0.05$), between HIIT and CON (Hd = −2.39 ± 1.67, Cd = −0.51 ± 1.26, $p = 0.006 < 0.01$), and between MICT and CON (Md = −0.99 ± 0.91, Cd = −0.51 ± 1.26, $p = 1.000 > 0.05$) (see Figure 8) (note: HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control).

3.4. Comparison of Blood Pressure Indicators between Groups after Exercise Intervention. Analysis of systolic blood pressure indicators revealed ranges of 134.2 ± 8.4 mmHg before and 135.4 ± 8.9 mmHg after the CON exercise intervention, with a statistical difference in the overall mean of the data between the two (difference = 1.2, 95% CI: 0.26–2.14, $p = 0.018 < 0.05$). Twelve weeks of systolic blood pressure (SBP) showed no overall statistical difference after the exercise intervention ($F = 1.44$, $p = 0.25 > 0.05$). The overall pre- and postexperimental difference was statistically different between the three groups ($F = 5.766$, $p = 0.008 < 0.01$): between HIIT and MICT (Hd7 = −0.8 ± 1.69, Md7 = −0.73 ± 1.49, $p = 1.000 > 0.05$), between HIIT and CON (Hd7 = −1.10 ± 1.79, Cd7 = 1.2 ± 1.31, $p = 0.018 < 0.05$), and between MICT and CON (Md7 = −0.99 ± 0.91, Cd7 = 1.40 ± 1.78, $p = 0.02 < 0.05$). Analysis of diastolic blood pressure indices revealed no statistically significant differences within the group before and after, in DBP indexes after 12 weeks of exercise intervention ($F = 1.325$, $p = 0.282 > 0.05$), and overall in between the three experimental groups before and after exercise ($F = 1423$, $p = 0.258 > 0.05$) (see Figure 9) (note: HIIT: high-intensity interval training; MICT: moderate-intensity continuous training; CON: control).

4. Analysis and Discussion

HIIT has been proven effective or even has superior effects in the T2DM population in numerous studies. However, different exercise types, programs, intensities, and durations can have different effects, and the optimal exercise prescription still needs to be validated for different conditions. This study was designed to apply power cycling to compare the effects of low-volume HIIT and MICT aerobic exercise interventions on health-related indicators in a T2DM population. Participants were divided into HIIT, MICT, and CON groups by preexercise testing and were then randomly assigned to risk factors associated with T2DM and comorbidities, namely, basic body composition, cardiorespiratory fitness, glucose level, insulin level, and blood pressure. To investigate whether HIIT can be an effective alternative or an optimized protocol for MICT in the T2DM population with respect to different indicators, a lighter and more practical and feasible HIIT protocol was designed. This randomized controlled trial showed that under effective supervision, the application of low-volume HIIT or MICT on power bikes is safe, feasible, and well-tolerated in the T2DM population. Moreover, 12-week HIIT and MICT programs are

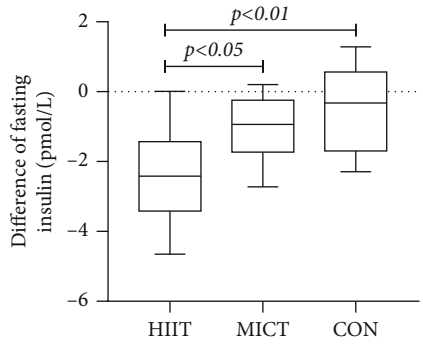

Figure 8: Difference in fasting insulin levels in each group after intervention.

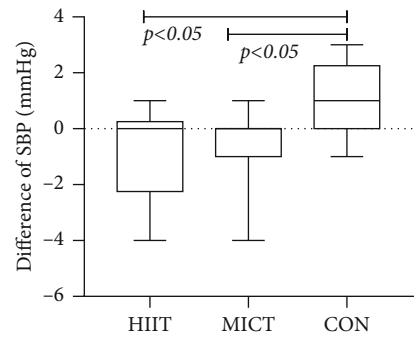

Figure 9: Blood pressure differences of each group after intervention.

not only effective but also ideal for targeting different health indicator.

4.1. Safe, Feasible, and Well Tolerated Exercise. The exercise protocol was performed under professional supervision. The application of power bikes considered not only the mobilization of large muscle groups and the absence of weight bearing on the legs and running friction on the ground. The HIIT intervention was also designed to be lower in volume and practically feasible. The total exercise time complied with the exercise guidelines of no less than 75 minutes per week at a high-intensity standard relative to the MICT. The training time was reduced by 50%. The complete experimental process included two late exclusions for personal objective reasons. The supervision records showed no adverse reactions or negative emotions during the experiment, and the interventions were well tolerated.

4.2. Response and Comparison of HIIT and MICT on Health-Related Indicators in the T2DM Population. Weight and BMI indicators are associated with an increased risk of cardiovascular complications in people with T2DM [31–33]. Weight loss mitigates the risk associated with diabetes [28, 34], and substantial weight loss can achieve long-term remission of diabetes [35, 36]. BMI is an indicator of general obesity and has been widely used to determine obesity and overweight in the population [37, 38]. Moreover, BMI has been linked to the time of diabetes diagnosis and the risk of death [39, 40]. Statistical differences in weight and BMI were found before and after the intervention in the MICT group, whereas only BMI was statistically different in the HIIT group. Weight loss from exercise training is mainly attributed to the energy expenditure accumulated during actual exercise [41]. The HIIT duration may have been too short in this study, resulting in limited energy expenditure. The effect of MICT exercise on weight loss in the participants was highlighted in the recorded weight loss, which also correlated with T2DM exercise guidelines (at least 150 minutes of moderate sustained and 75 minutes of high-intensity exercise are recommended). However, at least 200 minutes of moderate sustained training per week is recommended by the exercise guidelines for weight loss. Indeed, studies have shown that 150 minutes of moderate-intensity exercise per week is insufficient for weight loss [42]. Obesity is a key contributor to type 2 diabetes and may be the first factor to consider when encountering exercise prescription settings for such populations. However, this experiment was intended to compare relevant health indicators, not weight loss. These results could not adequately reflect the differential effects of body fat changes. First, although the cumulative weekly MICT exercise time was less than the minimum time standard recommended by the 200-minute weight loss exercise guidelines, the cumulative physical activity of preparation and finishing activities and daily living to achieve MICT may exceed 200 minutes. Moreover, the participants were encouraged to maintain good dietary and lifestyle habits during the experimental period. Second, participants in all groups were generally overweight, and exercise weight loss had a greater intervention effect on individuals with greater initial adiposity [43]. Considering factors such as the duration of obesity in the T2DM population and the fact that HIIT was conducted for half the time compared with the MICT group, the HIIT intervention could have shown similar effects on BMI indicators and a greater tendency to reduce BMI over a short period of time. Nonetheless, the insignificant change in HIIT weight was not unexpected, as one study found increased energy expenditure during the recovery phase of HIIT [44] and postexercise. Elevated plasma catecholamine levels in HIIT-driven lipolysis [45] are signs that HIIT affects appetite-regulating hormones, and that weight loss effects include a reduction in appetite after exercise in individuals. Similarly, studies [46] have stated that for patients with common metabolic disorders, such as T2DM, there is insufficient evidence that HIIT reduces weight compared with MICT, and that the role of HIIT in weight loss should not be exaggerated. The statistical differences in the pre- and postexperiment weight and BMI indicators between groups suggested that the current exercise duration and minimum energy expenditure standards for the HIIT training modality may be insufficient to have a significant weight loss effect. Future studies should clarify whether HIIT can provide an effective means of weight loss in patients with T2DM and elucidate the duration and intensity of HIIT training to be conducted.

VO2max is an important indicator for assessing cardiorespiratory endurance [19].

Low aerobic exercise capacity appears to be the strongest predictor of mortality among all known risk factors. Decreased cardiorespiratory fitness is common in patients with T2DM [47] and is strongly associated with mortality [48, 49]. Regular aerobic exercise, including the major muscle groups of the legs, arms, and trunk, is recommended to improve cardiorespiratory endurance capacity [50]. Maintaining or improving cardiorespiratory endurance in patients with T2DM is of great importance. In the present study, VO2max increased by 0.53 L/min in the HIIT group and by 0.32 L/min in the MICT group after the 12-week intervention. These results are clinically important because an increase of 0.35 L/min is associated with a 15% reduction in all-cause mortality and a 19% reduction in cardiovascular disease mortality [51]. In addition, higher aerobic capacity is associated with a higher quality of life in the T2DM population [52]. According to a recent meta-analysis comparing HIIT and MICT in the T2DM population, HIIT was significantly better than MICT relative to VO2max metrics. Nonetheless, evidence shows that in patients with cardiometabolic diseases, such as T2DM, vigorous or high-intensity exercise may lead to an increased risk of adverse effects (e.g., atrial tachycardia and myocardial infarction), at least temporarily [53]. The present study showed that both HIIT and MICT resulted in greatly improved VO2max in patients with T2DM. The effect of HIIT on VO2max was greater in the HIIT group than in the CON group, but the HIIT group did not show a significantly better increase in VO2max than the MICT group similar to other studies. In conventional studies, the maximum oxygen uptake of HIIT was significantly better than that of MICT. This inconsistency may be due to the following reasons. First, the power cycling participants may have had difficulty fully opening their chest with their upper body low on the handrail, creating a limitation on breathing and resulting in a lower expected increase in VO2max compared with running exercises. However, performing high-intensity intervals on a power bike reduced the risk of foot and ground friction and muscle or joint injury that tended to occur while running at high speed on the ground. Second, differences in type, program, and length of HIIT exercise could explain the uneven VO2max improvement and would require further in-depth study. Therefore, the results of this experiment suggest that the interventions were effective in improving VO2max in the T2DM population. The statistical difference between the pre- and postintervention VO2max indices indicates an advantage of HIIT, but it is not significant compared with other studies.

Both MICT and HIIT lowered fasting blood glucose in patients with T2DM and similar results were observed in a previous study. However, MICT required 45% more time than the HIIT protocol, and results of the current study emphasized the efficiency of HIIT in producing comparable effects on fasting blood glucose to MICT [54] in a more time-efficient manner. HIIT exercise on a cycle ergometer has been found to have no effect in terms of altering fasting blood glucose levels in subjects with T2DM [16]. However, most studies have shown that HIIT is an effective strategy for reducing fasting blood glucose concentrations in patients with T2DM. This study was not specifically designed to examine the effects of HIIT and MICT on glycemic control but rather to compare the inconsistent glycemic outcomes of MICT and HIIT on exercise interventions in the T2DM population. The main findings of this study were that (1) there was a statistical difference in fasting blood glucose for both MICT and HIIT before and after the intervention, with no statistical difference in fasting blood glucose between the two exercise intensities in a cross-sectional comparison after the intervention, and (2) there was a statistical difference with the control for MICT. Therefore, to achieve glycemic control in people with T2DM, easy-to-implement MICT can replace the more physically demanding HIIT.

HbA1c is not only the most widely used glycemic indicator but is also an important risk factor for cardiovascular disease in patients with T2DM. A 1% reduction in glycosylated hemoglobin levels is associated with a 37% reduction in the risk of microvascular complications and a 21% reduction in the risk of diabetes-related death [55, 56]. The lifespan of red blood cells is approximately four months, but many exercise training studies have been conducted for a shorter duration. HbA1c is a blood marker that quantifies the three-month average blood glucose concentration. Intuitively, an exercise program may take longer than 12 weeks to demonstrate an effect on HbA1c, yet many studies have a duration of 12 weeks or shorter. One study concluded that HbA1c decreases with each additional week of exercise compared with controls, and any reduction in HbA1c levels may reduce the risk of macrovascular and microvascular complications in patients with T2DM. Although this effect is small, it emphasizes the importance of sustained exercise interventions to improve health [16]. The present study found a statistical difference between the HIIT and CON groups only after the exercise intervention, probably because of (1) the duration of the exercise intervention and preparation time was approximately 13 weeks, and HIIT had an effect on HbA1c during the intervention period, and (2) the intensity difference. This experiment considered HIIT compared with MICT and found HIIT superior to MICT for HbA1c with consideration for intervention duration and effect.

Elevated FI has been suggested as a possible independent predictor of T2DM development [57]. Researchers have applied HIIT and MICT interventions to participants with T2DM after two randomized controlled trials: one experiment was superior in FI concentration, and the other was similar to MICT, which the authors attributed to the fact that both exercises improved insulin signaling in the skeletal muscle rather than to the effect of insulin secretion from pancreatic β-cells [10, 58]. Another meta-analysis comparing the effects of two different resistance intensities on FI in people with T2DM showed that resistance exercise leads to a significant reduction in FI only at high intensities, and that low to moderate intensities had no effect. The increase in GLUT4 protein levels in the skeletal muscle after strength training may be responsible for the enhanced insulin action in patients with T2DM [59]. Resistance exercise may improve insulin levels by increasing GLUT4 protein expression and insulin signaling without increasing muscle mass [15]. Based on the fact that different types of exercise lead

to a possible improvement in FI for skeletal muscle molecular mechanisms, HIIT resistance exercise may be a primary consideration for achieving FI reduction. The statistical difference between the pre- and postintervention FI in the three groups after 12 weeks in this study showed superior HIIT effects, possibly because power cycling requires exerting large muscle groups aerobically, with the improved leg muscle strength producing similar effects to resistance exercise.

Both HIIT and MICT modalities had similar effects on systolic and diastolic blood pressure in patients with prediabetes and T2DM. Elevated blood pressure is common in diabetic patients and is considered a strong risk factor for atherosclerotic cardiovascular disease, heart failure, and microvascular complications. The present experiment concluded that both exercise modalities had a blood pressure reduction effect. It is difficult to distinguish between specific advantages and disadvantages, but both can maintain blood pressure stability. Moreover, the control group showed some control over blood pressure increase.

5. Conclusions

Exercise interventions applying both low-volume HIIT and MICT designed for power cycling improved health-related indicators in subjects with T2DM. Notably, HIIT showed a temporal advantage. The current experiment compared HIIT with MICT. HIIT, which required 50% of the exercise time of MICT, produced similar benefits as MICT and advantages in the two indicators of VO2max and FI. However, MICT was superior to that of HIIT in terms of body weight and BMI. The effect of cycling on FI demonstrated the advantages of both aerobic and resistance exercise, which may optimize the type, intensity, and time of exercise prescription in the future according to the individual or the type of exercise program. These findings can provide a reference for the personalization of exercise prescriptions for patients with T2DM.

Abbreviations

T2DM: Type 2 diabetes mellitus
HIIT: High intensity interval training
MICT: Moderate intensity continuous training
CON: Control group
BW: Body weight
BMI: Body mass index
VO2max: Maximal oxygen uptake
FBG: Fasting blood glucose
HbA1c: Glycated hemoglobin type A1C
FI: Fasting insulin
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
HRmax: Maximal heart rate.

Data Availability

The data used to support the findings of this study are available from the request.

Conflicts of Interest

There is no conflict of interest regarding the publication of this paper.

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Review Article

Association of CDKAL1 RS10946398 Gene Polymorphism with Susceptibility to Diabetes Mellitus Type 2: A Meta-Analysis

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Background. Diabetes is one of the common chronic diseases in which susceptibility is determined by a combination of genetic and environmental factors, and more than 90% of diabetic patients are diabetes mellitus type 2 (T2DM). The existing studies on the association between CDKAL1 rs10946398 gene polymorphism and susceptibility to type 2 diabetes are inconsistent across populations. *Aim*. We aim to explore the association between CDKAL1 rs10946398 gene polymorphism and susceptibility to type 2 diabetes in different populations. *Methods*. We examined all studies before June 12, 2021, that associated CDKAL1 rs10946398 with T2DM. Heterogeneity was assessed by meta-analysis of allelic inheritance models (A vs. C), dominant inheritance models (AA vs. AC+CC), and recessive inheritance model (AA+AC vs. CC); I^2 was used to assess the heterogeneity (if $I^2 < 50\%$, the fixed-effects model was used; if $I^2 \geq 50\%$, the random-effects model was used for data consolidation); correlation was judged by a forest map; potential publication bias was tested by the Egger test ($p > 0.05$ indicates that there is no publication bias). *Results*. Fourteen data totaling 30288 subjects, including 19272 controls and 11016 patients with T2DM, met our inclusion criteria. In the Asian population, the differences were statistically significant ($p < 0.01$) for dominant genetic model (OR = 0.75, 95%CI = 0.64-0.88, $p = 0.0003$). But the allelic effect model (OR = 0.87, 95%CI = 0.75 -1.02, $p = 0.08$) and the recessive genetic model (OR = 0.85, 95%CI = 0.66-1.10, $p = 0.23$) were not statistically significant ($p > 0.01$). In the non-Asian population, the differences were statistically significant ($p < 0.01$) for the allelic effect model (OR = 0.83, 95%CI = 0.77-0.88, $p < 0.00001$), the dominant model (OR = 0.79, 95%CI = 0.72-0.87, $p < 0.00001$), and the recessive model (OR = 0.78, 95%CI = 0.70-0.87, $p < 0.0001$). *Conclusion*. In this study, CDKAL1 RS10946398 was positively associated with T2DM, but the association was different in Asian populations.

1. Introduction

According to the World Health Organization (WHO), approximately 3.4 million people died from developing diabetes in 2004, and it predicts that the number of diabetes deaths will double between 2005 and 2030. The International Diabetes Federation predicts that the global prevalence of diabetes will reach 642 million cases by 2040 (International Diabetes Federation, 2015), with type 2 diabetes accounting for more than 90% of diabetics [1].

Type 2 diabetes mellitus (T2DM), formerly known as non-insulin-dependent or adult-onset diabetes mellitus, is a type of diabetes mellitus. It is caused by poor insulin action which is the relative lack of insulin in patients, and its susceptibility is determined by both genetic and environmental factors [2]. In the context of increasing morbidity and

mortality of T2DM, it is of great significance to study the pathogenesis of T2DM.

Previous studies have shown that China [3–6] and other Populations of Asian countries' CDKAL1 RS10946398 locus mutation was significantly associated with T2DM [1, 7–10]. The United States [11, 12], Russia [13, 14], Mexico [15], and other non-Asian populations of CDKAL1 RS10946398 were also significantly associated with T2DM. It is noteworthy that a variant of the CDKAL1 RS10946398 locus in the population of the Asian country of the United Arab Emirates may not be directly associated with the development of T2DM [1]. These show that CDKAL1 rs10946398 locus variants play different roles in different study populations. Therefore, it is of great significance to study the relationship between CDKAL1 rs10946398 locus variation and T2DM susceptibility in different populations.

1.1. Retrieval Strategy. An advanced search of the literature search library was conducted by using "T2DM CDKAL1" and "CDKAL1 rs10946398" as key to search terms in the China National Knowledge Infrastructure (CNKI), PubMed, and WanFang digital databases, with the last search conducted on June 12, 2021.

1.2. Inclusion and Exclusion Criteria. The following studies were included [16, 17]:

(1) Case-control studies focus on the association between the CDKAL1 rs10946398 polymorphism and T2DM in adults

(2) Patients were randomly selected with no special restrictions on gender, family history, etc.

(3) Studies provide accurate control and case group data sources

(4) The data provided in the study report were statistically significant. The study results had specific OR values, 95% CI

(5) Studies met the diagnostic criteria of T2DM published by the World Health Organization (WHO) in 2019, and the control group all met the law of H-W genetic balance

The following studies were excluded:

(1) There were only case groups or a lack of sufficient controls

(2) Statistical data are erroneous or there are significant differences in the statistics of the same study in different literatures

(3) The overall sample size is insufficient

(4) Literature reviews and case reports were excluded

1.3. Data Extraction. Two investigators independently read the literature and extracted information from the eligible literature based on exclusion and inclusion criteria. In case of ambiguity, a consensus was reached on whether to extract the paper data through discussion with the third investigator. For each paper, the following information was collected: (i) author's name, (ii) year of publication, (iii) ethnicity and country of the study population, (vi) number of included cases and controls, and (vii) genotype data [3]. The literature screening process is shown in Figure 1.

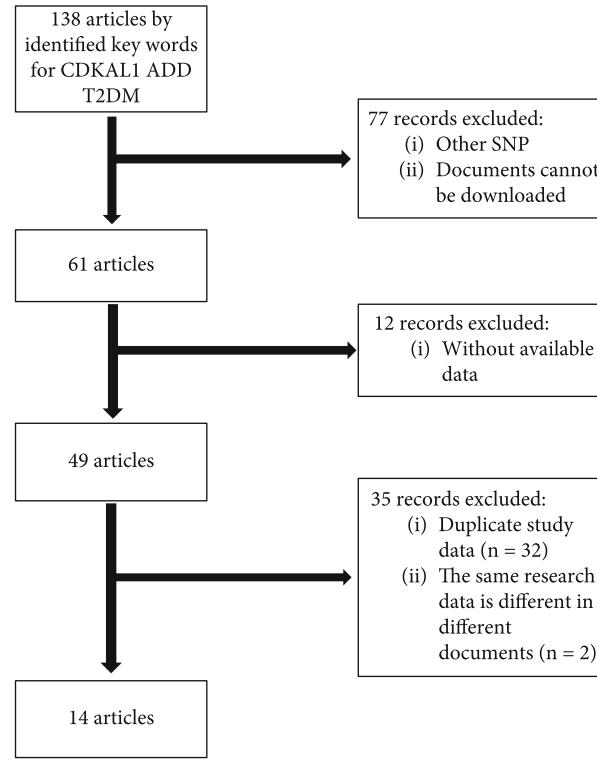

Figure 1: Literature screening flow chart.

1.4. Statistical Methods. Review Manager 5 software was used to complete the meta-analysis. Stata software was used to complete the Egger test.

2. Results

2.1. Baseline Characteristics of Included Studies. We obtained articles on the relationship between CDKAL1 rs10946398 diversity and T2DM susceptibility from PubMed and CNQ. After reading the title, year, author, and abstract of the papers, we conducted the first screening. The second screening was performed by reading the full text and analyzing whether the data was statistically significant. Finally, 14 literatures were included. A total of 14 datasets were obtained for meta-analysis by reading through the full text to filter the data required for recording. A total of 30288 subjects were included in the meta-analysis, including a total of 11016 in the T2DM patient group and 19272 in the control group. Eight of the datasets were from the Asian study population: 3 from China, 1 from India, 1 from Korea, 1 from Japan, 1 from Iran, and 1 from the United Arab Emirates; 6 were from the non-Asian study population: 3 from the USA, 2 from Russia, and 1 from Mexico. Information on

Table 1: Association of CDKAL11 rs10946398 polymorphism with T2DM susceptibility.

First author	Region	Race	BMI		Age (yr)		Controls						Cases					
			Controls	Cases	Controls	Cases	N	AA	AC	CC	A	C	N	AA	AC	CC	A	C
Horikoshi (2007)	Asian	Japanese	23.8 ± 3.7	24.3 ± 3.9	69.5 ± 6.8	63.1 ± 9.5	861	280	423	158	983	739	852	239	434	179	912	792
Joshua P (2008)	Non-Asian	American	NA	NA	NA	NA	1054	184	513	357	881	1227	993	147	470	376	764	1222
Y. Liu (2008)	Asian	Chinese	24.5 ± 3.2	25.3 ± 3.4	58.1 ± 9	63.8 ± 9	1822	588	862	372	2038	1606	1903	707	903	293	2317	1489
Herder (2008)	Non-Asian	American	27.7 ± 4.3	30.9 ± 5.0	61.6 ± 9.7	59.9 ± 7.9	1438	705	604	129	2014	862	433	177	200	56	554	312
Eun Seok (2009)	Asian	South Korea	NA	NA	37.4 ± 9.3	42.6 ± 9.1	444	134	220	90	488	400	145	31	72	42	134	156
Cheng Hu (2009)	Asian	Chinese	23.57 ± 3.25	24.04 ± 3.51	57.39 ± 12.37	61.21 ± 12.62	1785	613	866	306	2092	1478	1850	578	912	360	2068	1632
M. Cruz (2010)	Non-Asian	Mexico	27.50 ± 3.55	29.25 ± 4.76	43.60 ± 6.63	53..44 ± 7.42	548	270	229	49	769	327	519	242	225	52	709	329
Ganesh (2010)	Asian	India	Women 24.90 (21.10–28.60) Men 23.20 (20.20–25.70)	Women 26.70 (24.20–29.20) Men 23.80 (22.00–26.00)	50 (44–60)	53 (45–62)	1006	628	334	44	1590	422	1020	589	372	59	1550	490
Dimitry A (2011)	Non-Asian	Russian	26.9 ± 4.8	28.3 ± 5.9	59.9 ± 7.9	26.9 ± 4.8	767	367	330	70	1064	470	769	333	337	99	1003	535
Jessican (2012)	Non-Asian	American	29.5 ± 7.6	33.7 ± 7.6	48.6 ± 13.0	46.0 ± 12.3	567	105	278	184	488	646	1150	175	547	428	897	1403
Aleksey G Nikitin (2017)	Non-Asian	Russian	28.7 ± 4.8	30.5 ± 5.0	54.4 ± 11.0	60.0 ± 10.2	443	297	124	22	718	168	862	500	293	69	1293	431
Oswald Ndi Nfor (2018)	Asian	Taiwanese women	NA	NA	47.60 ± 10.80	55.56 ± 9.19	8934	3707	4061	1166	11475	6393	974	353	441	180	1147	801
Mariam Al Ali (2019)	Asian	Emirati	NA	NA	NA	NA	264	137	99	28	373	155	153	86	57	10	229	77
Kazem Vatankhah Yazdi (2020)	Asian	Iranian	23.07 ± 1.03	24.00 ± 1.23	65.5 ± 7.3	65 ± 7.5	106	46	50	10	142	70	162	31	104	27	166	158

Table 2: Heterogeneity test.

CDKAL1	Group	A fixed-effects model			A random-efforts model			Heterogeneity		
		OR (95% CI)	Z	p	OR (95% CI)	z	p	X^2	I^2 (%)	PQ test
A vs. C	Total	0.89 [0.86, 0.92]	6.17	$p < 0.00001$	0.85 [0.77, 0.94]	3.25	$p = 0.001$	76.82	83%	$p < 0.00001$
	Asian	0.92 [0.88, 0.97]	3.36	$p = 0.008$	0.87 [0.75, 1.02]	1.73	$p = 0.08$	65.37	89%	$p < 0.00001$
	Non-Asian	0.83 [0.78, 0.88]	5.88	$p < 0.00001$	0.83 [0.77, 0.88]	5.55	$p < 0.00001$	5.59	11%	$p = 0.35$
AA vs. AC+CC	Total	0.77 [0.73, 0.82]	9.15	$p < 0.00001$	0.77 [0.70, 0.85]	5.42	$p < 0.00001$	149.89	92%	$p < 0.00001$
	Asian	0.83 [0.77, 0.88]	7.68	$p < 0.00001$	0.75 [0.64, 0.88]	3.61	$p = 0.0003$	29.52	76%	$p = 0.0001$
	Non-Asian	0.79 [0.72, 0.86]	4.99	$p < 0.00001$	0.79 [0.72, 0.87]	4.97	$p < 0.00001$	84.86	96%	$p = 0.58$
AA+AC vs. CC	Total	0.86 [0.80, 0.92]	4.47	$p < 0.00001$	0.81 [0.69, 0.94]	2.68	$p = 0.007$	33.42	61%	$p = 0.001$
	Asian	0.91 [0.83, 0.99]	2.23	$p = 0.03$	0.85 [0.66, 1.10]	1.21	$p = 0.23$	59.98	86%	$p < 0.00001$
	Non-Asian	0.78 [0.70, 0.87]	4.39	$p < 0.0001$	0.78 [0.70, 0.87]	4.38	$p < 0.0001$	1.70	0	$p < 0.00001$

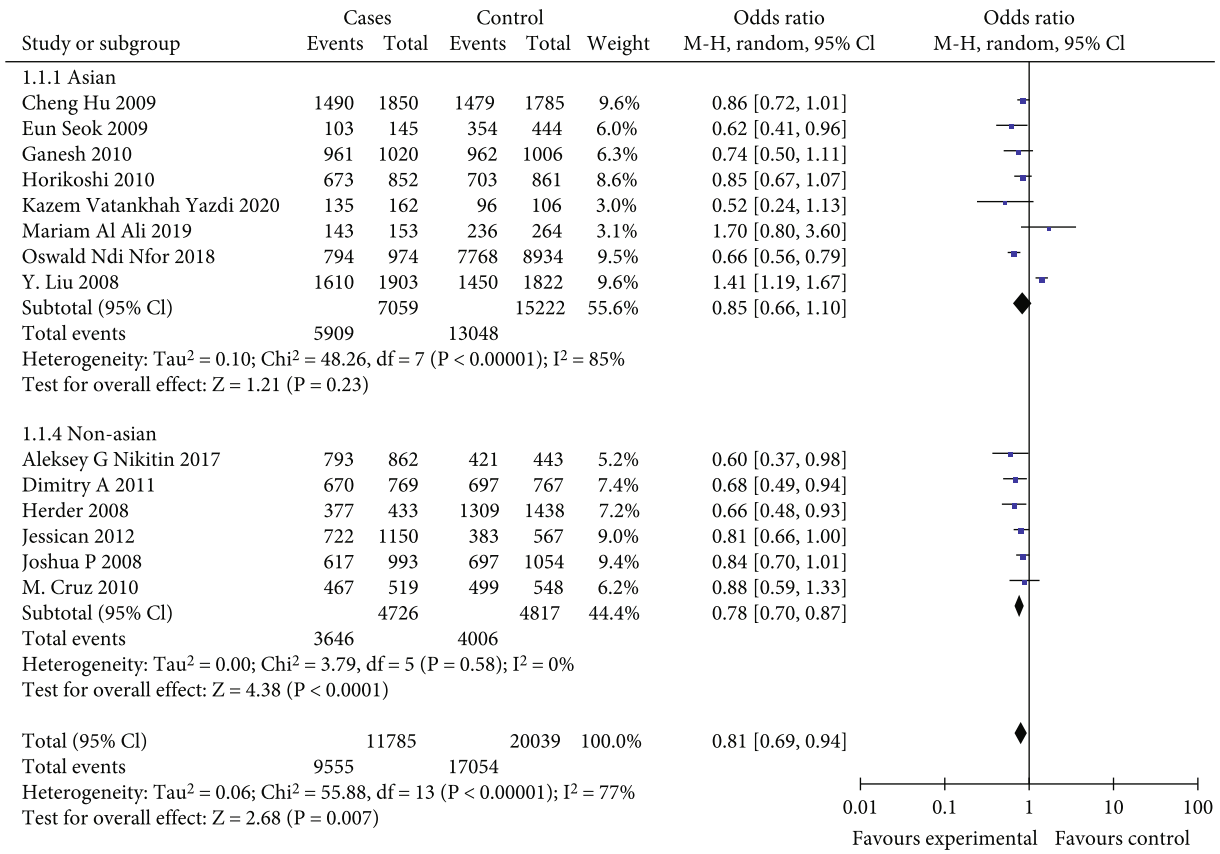

Figure 2: Forest plot of meta-analysis of the A vs. C allele model associated with T2DM at CDKAL1 rs10946398 locus.

the first author, study year, sample size, ethnicity, BMI, mean age of control and case groups, and risk allele frequency for each study is shown in Table 1.

3. Results of Meta-Analysis

In the evaluation of the relationship between the CDKAL1 rs10946398 gene and T2DM susceptibility, a total of 14 studies were included in the meta-analysis after literature data search, screening, and verification. In order to analyze the association between CDKAL1 rs10946398 polymorphism and susceptibility to T2DM, we analyzed the relationships between A and C alleles, AA+AC and CC genotypes, AA and AC+CC genotypes in T2DM patients and controls. Since 8 studies were from Asia and 6 were from non-Asia, we stratified the Asian and non-Asian populations.

We examined heterogeneity separately for the study populations, using I^2, to assess the magnitude of heterogeneity (if $I^2 < 50\%$, a fixed-effects model was used; if $I^2 \geq 50\%$, a random-effects model was used to combine the data). Because our data were randomly selected and we wanted to reflect the overall situation with a small sample size, only

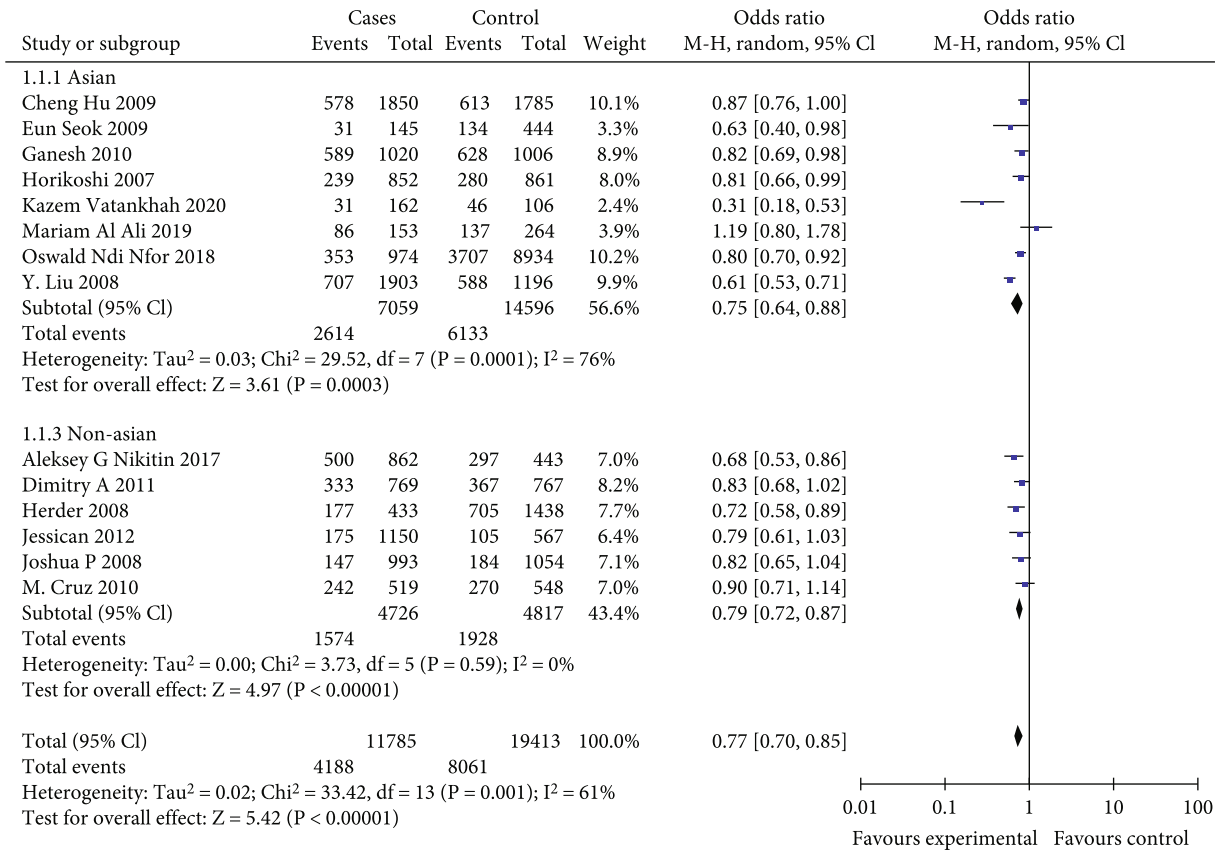

Figure 3: Meta-analysis of a T2DM-associated AA vs. AC+CC genotype model at CDKAL1 RS10946398 locus forest map.

the allelic genetic model and the recessive genetic model in non-Asian populations show that $I^2 < 50\%$, so we used the random-effects model (see Table 2).

In the total population, the differences were statistically significant ($p < 0.01$) for the allelic genetic models (OR = 0.85, 95%CI = 0.77-0.94, $p = 0.001$), the dominant genetic models (OR = 0.77, 95%CI = 0.70-0.85, $p < 0.00001$), and the recessive genetic models (OR = 0.81, 95%CI = 0.69-0.94, $p = 0.007$). The results are shown in Figures 2–4.

In the Asian population, the differences were statistically significant ($p < 0.01$) for dominant genetic model (OR = 0.75, 95%CI = 0.64-0.88, $p = 0.0003$). But the allelic effect model (OR = 0.87, 95%CI = 0.75-1.02, $p = 0.08$) and the recessive genetic model (OR = 0.85, 95%CI = 0.66-1.10, $p = 0.23$) were not statistically significant ($p > 0.01$). The results are shown in Figures 2–4.

In non-Asian populations, the differences were statistically significant ($p < 0.01$) for the allelic genetic model (OR = 0.83, 95%CI = 0.77-0.88, $p < 0.00001$), the dominant genetic model (OR = 0.79, 95%CI = 0.72-0.87, $p < 0.00001$), and the recessive genetic model (OR = 0.78, 95%CI = 0.70 -0.87, $p < 0.0001$). The results are shown in Figures 2–4.

3.1. Publication Bias. We used Stata software for the Egger test, and the p values of allelic inheritance models (A vs. C), recessive inheritance model (AA+AC vs. CC), and dominant inheritance models (AA vs. AC+CC) were 0.114, 0.307, and 0.304, respectively, which were greater than 0.05, indicating that there was no publication bias. What is more, according to the symmetry of the funnel plot, the existence of publication bias can also be judged. The results are shown in Figures 5–7; it can be found that all points in the funnel plot are distributed symmetrically along both sides of the midline, so there is no bias.

4. Discussion

According to a large number of genome-wide association analyses (GWAS), CDK5 regulation-related protein 1-LIAK 1 (CDKAL1) gene under the action of high glucose toxicity will increase the body's demand for insulin, and pancreatic β cells continue to be activated, which may inhibit the activity of CDK5 in pancreatic β cells. Insulin secretion is reduced by lowering the expression of insulin genes [18, 19]. Because mutations in CDKAL1 may lead to impaired insulin secretion, thus, it increases the risk of T2DM, and CDK5 regulates the related protein 1-LIAK 1 (CDKAL1) gene which is one of the most repeatable risk genes in T2DM [20]. In particular, SNPs rs10946398 and rs7754840 of CDKAL1 have the strongest correlation with T2DM [20].

To study the relationship between the variation of CDKAL1 RS10946398 locus and the susceptibility to T2DM in different populations, 14 sets of data were finally used for meta-analysis through data investigation and screening, and 13 sets of data showed that the CDKAL1

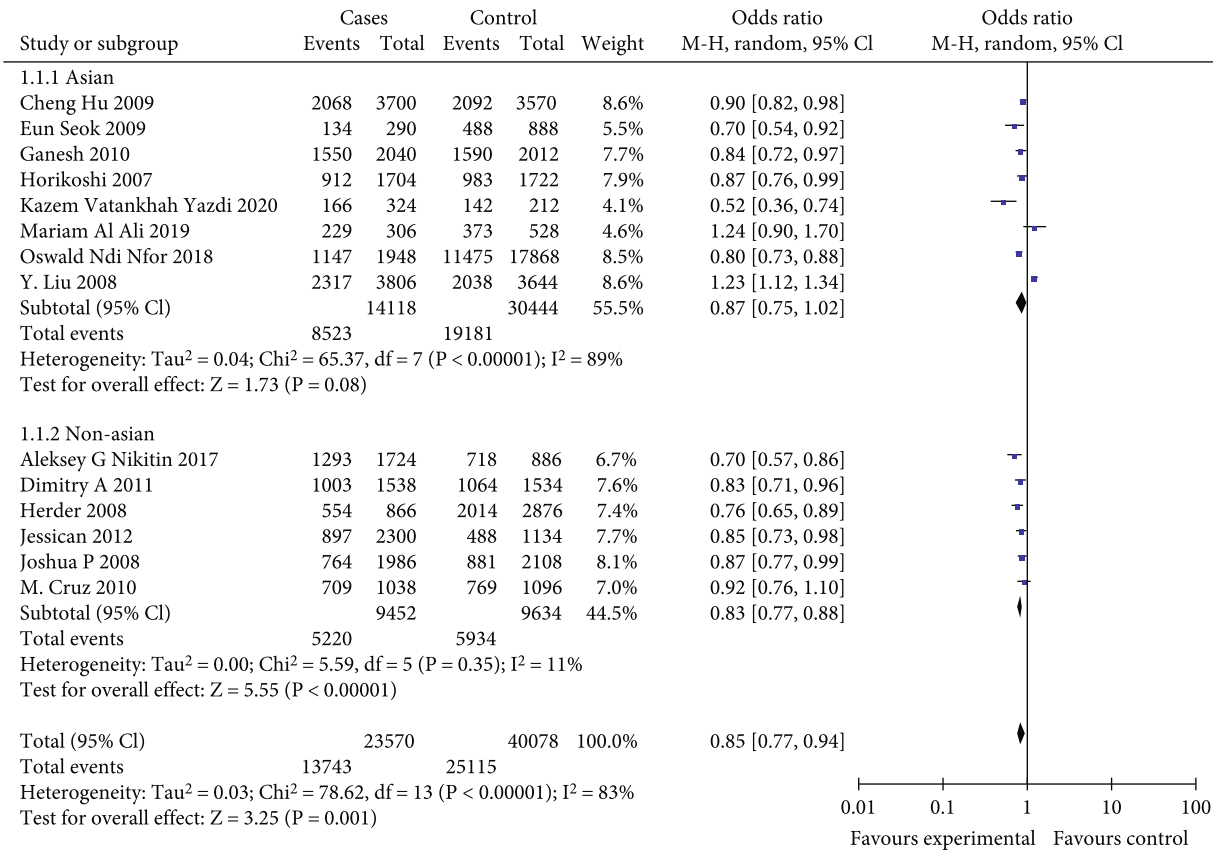

Figure 4: Meta-analysis of a T2DM-associated AA+AC vs. CC genotype model at CDKAL1 RS10946398 locus forest map.

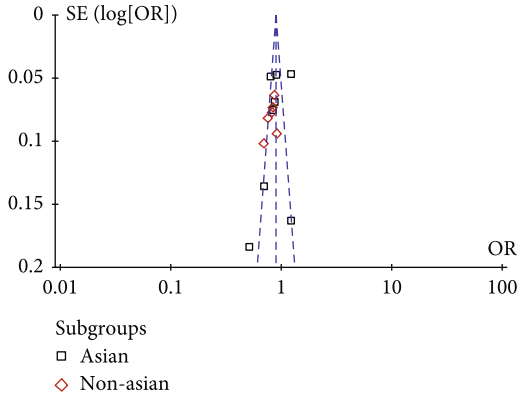

Figure 5: A vs. C allelic funnel plot.

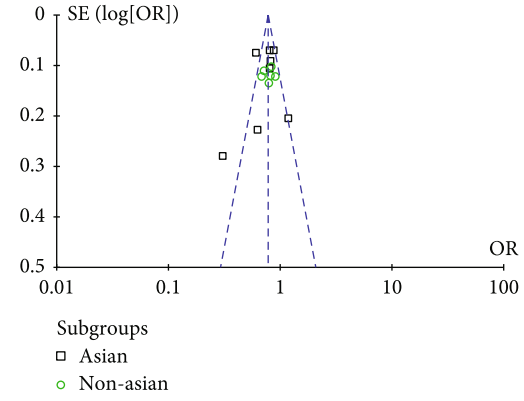

Figure 6: AA vs. AC+CC genotype funnel plot.

RS10946398 locus was significantly correlated with the incidence of T2DM; for example, a study by Nfor et al. showed a significant association between CDKAL1 RS10946398 and T2DM in Taiwanese. CC carriers were more associated with T2DM than AC carriers, and C allele carriers were more associated with type 2 diabetes than A allele carriers [6]. A study by Herder et al. found that CDKAL1rs10946398 was significantly associated with impaired glucose metabolism or β cell function. CDKAL1rs10946398 also plays an important role in the pathogenesis of T2DM in the detected Russian population [12]. Only one set of data showed that CDKAL1 RS10946398 locus was not significantly associated with the pathogenesis of T2DM. The study by Al Ali et al. showed that the CDKAL1 RS9939609 variant in the United Arab Emirates population may not be directly related to the development of T2DM [1]. Therefore, the role of CDKAL1 rs10946398 locus variation in different study populations is different.

In this study, a meta-analysis of the included 14 groups of data concerning the CDKAL1 rs10946398 locus and T2DM was performed by analyzing allelic models (A vs. C), recessive genetic models (AA+AC vs. CC), and dominant genetic models (AA vs. AC+CC) in T2DM patients and controls. Of the 30288 subjects, including 19272

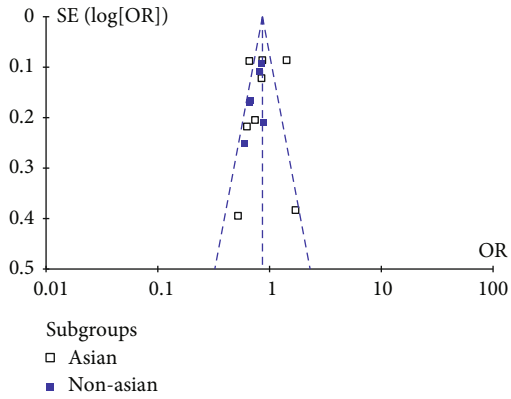

Figure 7: AA+AC vs. CC genotype funnel plot.

controls and 11016 T2DM patients, we found that CDKAL1 RS10946398 gene polymorphism locus is associated with type 2 diabetes mellitus in different ethnic groups, and the degree of correlation is different in different genetic models.

In the Asian population, the differences were statistically significant ($p < 0.01$) for the dominant genetic model (OR = 0.75, 95%CI = 0.64-0.88, $p = 0.0003$). But the allelic effect model (OR = 0.87, 95%CI = 0.75-1.02, $p = 0.08$) and the recessive genetic model (OR = 0.85, 95%CI = 0.66-1.10, $p = 0.23$) were not statistically significant ($p > 0.01$). The risk ratio of the A allele was higher than that of the C allele. In the non-Asian population, the differences were statistically significant ($p < 0.01$) for the allelic effect model (OR = 0.83, 95%CI = 0.77-0.88, $p < 0.00001$), the dominant model (OR = 0.79, 95%CI = 0.72-0.87, $p < 0.00001$), and the recessive model (OR = 0.78, 95%CI = 0.70-0.87, $p < 0.0001$). The risk ratio of the A allele was higher than that of the C allele [21].

We used 14 sets of data for meta-analysis of the locus genetic model (A vs. C) recessive models (AA+AC vs. CC) and dominant models (AA vs. AC+CC). Except for the Asian allelic effect model and recessive gene model ($p > 0.01$), other models were statistically significant ($p < 0.01$). CDKAL1rs10946398 could significantly increase the risk of T2DM in the allele model of Asian and in all models of non-Asian. But this result cannot be attributed to differences in ethnicity; it could also be due to the small sample size. In conclusion, the CDKAL1 rs10946398 gene variant may increase the susceptibility to T2DM.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

NX and TZ designed this study. NX and TZ searched databases and collected full-text papers. NX, WH, and JD extracted and analyzed data. JS provided guidance for statistical analysis. NX wrote the manuscript. NX, LY, NM, XS, and TZ reviewed the manuscript. JS and XS have provided financial support for this work. NX and TZ contributed equally to this work.

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Review Article

Reenvisioning Traditional to Regenerative Therapeutic Advances in Managing Nonalcoholic Fatty Liver Disease in Diabetes Mellitus

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Reports indicate the increasing prevalence of liver disorders in diabetes mellitus (DM) patients. Clinically, it has also been revealed that the existence of nonalcoholic fatty liver disease (NAFLD) enhances the incidence of type 2 diabetes mellitus (T2DM), while T2DM exacerbates NAFLD to extremely severe forms of steatohepatitis, cirrhosis, and hepatocellular carcinoma. This implies the coexistence and bidirectional nature of NAFLD and T2DM, which function synergistically to drive adverse consequences in clinical practice. For treatment of such comorbid state, though the existing practices such as lifestyle management, traditional Chinese medicines (TCM), and pharmaceuticals have offered somewhat relief, the debate continues about the optimal therapeutic impacts. Recent developments in the field of tissue engineering have led to a renewed interest in novel biomaterial alternatives such as stem cells. This might be attributable to their differentiation potential towards hepatic and pancreatic lineage. These cellular therapies could be further complemented by platelet-derived biomaterials, TCM formulations, or any specific drug. Based on these abovementioned approaches, we aimed to comprehensively analyze various preclinical and clinical studies from traditional to regenerative therapeutic approaches in managing concomitant NAFLD and T2DM.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is highly common in diabetes mellitus (DM), a syndrome characterized by altered glucose metabolism [1], and evidence implies that these concomitant pathologies are bidirectional [2]. Specifically, NAFLD participates in the development of type 2 DM (T2DM) by elevating glucose production in the liver and aggravating hepatic insulin resistance [3]. On the other hand, T2DM and insulin resistance stimulate an increase of free fatty acid flux from peripheral tissues to the liver, resulting in the development and progression of NAFLD. A recent United States-based study demonstrated the prevalence of advanced liver fibrosis in patients with T2DM [4]. A twofold higher prevalence of NAFLD (55.5%) in diabetes patients compared to the general population has also been reported [5]. This coincides with epidemiological characteristics of NAFLD from 1999 to 2018 in China showing a notable increasing trend of obesity [6]. A recent meta-analysis of NAFLD in China reported a 51.83% prevalence of NAFLD in patients with diabetes compared to 30.76% of healthy ones [7]. Notably, this prevalence was also found higher among obese (66.21%) than nonobese population (11.72%). Based on the abovementioned evidence, the bidirectional interaction between NAFLD and DM could be inferred.

The presence of chronic liver disease in diabetes patients significantly increases the risk of glucose intolerance and insulin resistance, which renders them vulnerable to liver fibrosis, cirrhosis, and hepatocellular carcinoma [8, 9]. Thus, it seems critical and challenging to reduce morbidity and mortality in patients with liver disease and DM, which may further complicate due to drug metabolism in the liver and risk of hepatotoxicity [10]. Notwithstanding, the treatment of DM linking liver disorders through glucose-lowering agents such as metformin, pioglitazone, GLP-1 receptor agonists, and SGLT-2 seems advantageous [9]. For most of the patients, metformin if tolerated has been recommended as an appropriate first-line therapy, excluding those having advanced liver disorder, who might be susceptible to enhanced risk of lactic acidosis [11]. According to Khan et al., metformin for chronic liver disease patient likely to be safer, with reduced dose of 1500 mg daily, and may be withdrawn in the case of declining liver or renal function [12] Specifically, long-term pioglitazone therapy has been reported safe and efficacious for the patients with T2DM and NAFLD [13]. In a similar trend, DPP-4 inhibitors like sitagliptin have been suggested effective and safe for DM patients complicated by liver injuries [14], whereas the second-line therapies GLP-1 receptor agonists and SGLT-2 inhibitors exhibit positive impact on body weight with reduced risk of hypoglycemia.

Based on limitations such as the risk of lactic acidosis and hypoglycemia associated with these oral hypoglycemic agents has prompted the scientific community to explore other safer and efficacious alternatives [15]. Regular exercise and a controlled diet have also been evidenced as somewhat effective [10]. For decades, the traditional herbs and Chinese medicines (TCM) have been shown to exert therapeutic effects in various disorders with either minimal or no side effects. These may suppress the risk of NAFLD as well as DM [16, 17]. However, to further explore the enhanced therapeutic efficacies, stem cells or platelet-based regenerative alternatives are being examined in various preclinical and clinical studies [18–20]. The stem cells through their differentiation potential towards pancreatic β-cells and hepatocyte lineage may also regulate glucose/lipid metabolism and exert anti-inflammatory actions [21]. Hence, the burden of liver transplantation and related risks could be considerably reduced. Nevertheless, developing regenerative therapy for NAFLD is still at the infant stage. Considering challenges and available therapeutic tools for managing concomitant NAFLD and DM, this article has extensively reviewed preclinical and clinical studies from traditional to advanced regenerative therapeutic interventions.

2. Pathophysiology of Hepatic Disorders and DM

NAFLD is correlated to DM due to shared pathophysiological characteristics like adipose accumulation and insulin resistance (Figure 1(a)) [22]. These characteristics participate in NAFLD progression, by insulin resistance-induced excess synthesis of triglyceride, accumulation, and impaired oxidation of free fatty acid (FFA), and secretion of very-low-density lipoproteins (VLDL) resulting in severe hepatic stress. NAFLD not only contributes to the development of liver cirrhosis and cardiovascular complications but also acts as an etiological factor for cancer initiation and progression. A systemic review and meta-analysis concluded that NAFLD may trigger hepatocellular, colorectal, breast, pancreatic, prostate, and esophageal cancer [23, 24]. Though the exact mechanism underlying NAFLD-induced cancer is not well-established, the possible contributing factors may include unregulated efflux of adipokines, increased levels of IGF-1, insulin, and cytokines (TNF-α, IL-6), accelerated hepatocyte proliferation, lipid peroxidation, oxidative stress, DNA damage, and lipotoxicity [25–29]. The cytokines TNF-α and IL-6 mediate its antiapoptotic impact through activating STAT3 oncogenic transcription factors ensuing carcinoma [30]. Furthermore, the irregular lipid metabolism in NAFLD inhibits the influx of CD4 + T cells resulting in the accumulation of CD8+ T cells in the liver and the development of hepatic cancer [31]. It has been further established that NAFLD could promote the expression of IL-1β, VEGF, and NOD-like receptor C4 in tumor-associated macrophages and accelerate the growth of the liver tumor [32].

Similarly, hepatogenous diabetes (HD) has been evidenced by the progression of irregular insulin clearance and β-pancreatic cell apoptosis [33]. The presence of liver disorders disrupts glucose metabolism due to insulin resistance and impaired sensitivity of pancreatic islet β-cells [34, 35]. Initially, insulin resistance and glucose intolerance occur at the initial stage of HD; however, with its progression, the manifestation of diabetic symptoms becomes clinically distinct. HD is also associated with a low incidence of microangiopathy, reduced response to antiviral treatment, and complicated treatment procedure due to cirrhosis and liver toxicity of drugs. It is also a causative factor for the progression of hepatocellular carcinoma [35, 36], which might be ascribed to polymorphisms in TCF7L2 rs290487 and rs6585194 gene along with the presence of SNPs rs290481, rs290487, and rs29048 at 3′ end of TCF7L2 gene [37]. In addition, HD induces secretagogue of adipokines such as adiponectin, leptin, HGF, TNF-α, TGF-β1, and resistin, resulting in liver fibrosis and inflammation [38–40]. The mortality in HD patients also increases due to immunosuppressive activity and increased risk of infection [41].

Notably, viral infections such as Coxsackievirus B, rotavirus, mump virus, the rubella virus, and cytomegalovirus may cause T1DM [42], whereas hepatitis C viral infection enhances the risk of T2DM with the escalated frequency of fibrosis, cirrhosis, and hepatocellular carcinoma [43]. Though the association of DM with the severe progression of hepatic injury and carcinoma along with other complications poses the therapeutic challenge, the research advances from traditional to regenerative treatment regimens (Figure 1(b)) indicate considerable successes, which have been extensively reviewed in our next sections.

3. Treatment Strategies for Concomitant Liver Disease and Diabetes

3.1. Lifestyle Management. As NAFLD and DM are associated with food habits, type and pattern of fat consumption,

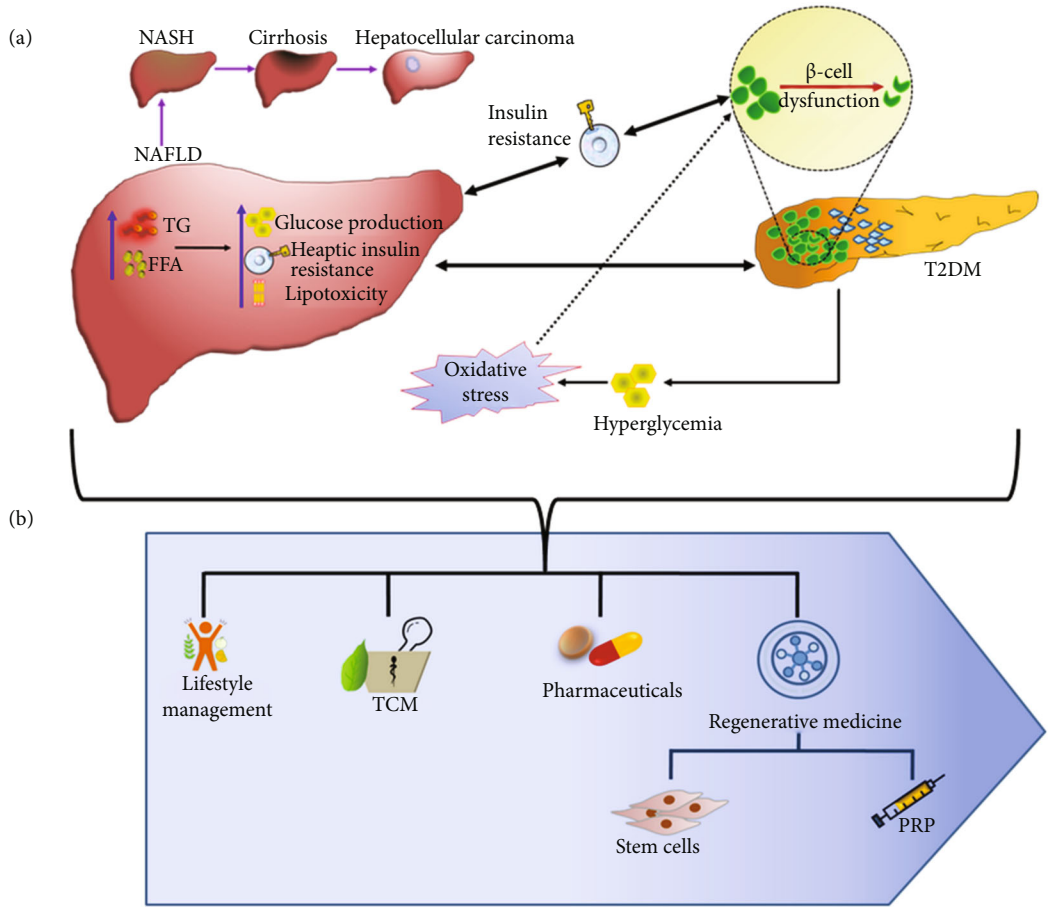

Figure 1: Association between the pathophysiological duo of NAFLD and DM and its treatment. (a) The mechanistic insight underlying NAFLD and DM. NAFLD participates in the development of T2DM by enhancing glucose production and insulin resistance in the liver. T2DM and systemic insulin resistance induces the initiation and progression of NAFLD by increasing levels of FFA and TG from peripheral tissues to the liver. If remain untreated, NAFLD further progresses from NASH, cirrhosis, to hepatocellular carcinoma. (b) Journey of therapeutic alternatives from traditional to regenerative medicines, including lifestyle management, TCM, pharmaceuticals, stem cells, and PRP. NAFLD: nonalcoholic fatty liver disease; FFA: free fatty acid; TG: triglycerides; NASH: nonalcoholic steatohepatitis; T2DM: type 2 diabetes mellitus; TCM: traditional Chinese medicines; PRP: platelet-rich plasma.

exercise and daily life activity, and careful management of these lifestyle-related factors are imperative [44–46]. Therefore, the recommendation of lifestyle management is considered as a first-line therapeutic approach for NAFLD, DM, liver infection, and other severe liver disorders [47]. Regarding NAFLD, the recommended guidelines for dietary changes include reduction of saturated fat intake to <7% of total calories, trans-fat and dietary cholesterol < 200 mg per day, and total fat at 25%-35% of total calories [48]. According to the American Association for the Study of Liver Diseases (AASLD), reducing body weight by at least 3%-5% may improve hepatic steatosis, while body weight reduction to ≥7% could improve histological characteristics of NASH including fibrosis [49]. Similarly, the Korean Association for the Study of the Liver (KASL) has also recommended a body weight reduction of 7%-10% for improving NAFLD [50]. A long-term clinical follow-up of lifestyle-related intervention for 6 years showed a significantly reduced risk of DM [51]. This might further suppress insulin resistance and hence the occurrence of NAFLD and its progression to other severe liver diseases. It is also known that a BMI higher than 21 is associated with an increased risk of DM [52]. Therefore, the initial weight loss and exercise seem crucial in controlling DM [53, 54], and clinical studies have demonstrated that combined lifestyle intervention and metformin-mediated weight loss significantly reduced the incidence of DM [55, 56]. In addition to regular exercise, a very low energy diet is also effective in weight loss, glycemic control, and regulation of lipid metabolism among overweight T2DM patients [57]. In addition, a Mediterranean diet rich in fruit and vegetable may prevent DM by antioxidative stress, anti-inflammatory, and anti-insulin resistance activities [44, 58]. Thus, well-planned lifestyle changes may be an effective preventive tool for DM and its complications.

3.2. Pharmaceutical Interventions. The pharmaceutical intervention is the most common approach to control the progression of NALFD and DM [59]. A reduction of 1% glycated hemoglobin has the potential to diminish 35% microvascular complications and 25% diabetes-related mortality [60]. Thiazolidinediones or glitazones are the agonists of peroxisome proliferator-activated receptors (PPAR),

which play an important role in glucose and lipid homeostasis, in addition to suppressing inflammation and fibrogenesis [61]. Hence, these agents could inhibit the accumulation of hepatic triglyceride, a hallmark of the development of NAFLD. In various clinical trials, the approved glitazones have not been only reported to benefit diabetes but also improve histological lesions of NASH [13, 62, 63]. Metformin is a primary drug to lower blood glucose and glycated hemoglobin (HbA1c) in T2DM [64]. It could also modify gut microbiota and actuate mucosal AMP-activated protein kinase in hepatocytes, the combined effect of which could lower the levels of lipopolysaccharides [65, 66]. Besides, insulin is widely used biologics to address the therapeutic requirement among T1DM patients, and recent progress towards the development of oral insulin has opened the way to overcome repeated pain exposure by injectable insulin [67]. Notwithstanding, the repeated long-term exposure to external insulin may slowly develop insulin resistance. Sulfonylureas and meglitinides are second-line drugs, which stimulate β-cells to secrete insulin mediated by pancreatic ATP-sensitive potassium channels [68, 69]. However, long-term exposure to sulfonylureas and meglitinides might cause weight gain, hypoglycemia, and deterioration in their efficacy [64]. In the line, thiazolidinediones, sodium-glucose co-transporter-2 inhibitors, and dipeptidyl peptidase-4 inhibitors are other considerable groups of drugs for DM treatment. Glucagon-like peptide-1 (GLP-1), a peptide-based alternative, has also been found effective due to its insulin enhancing, glucagon lowering, and appetite-reducing potential [70]. The GLP-1 also lowers the risk of endothelial dysfunction, myocardial ischemia, and renal failure [64, 71]. Though there seems to be a wide spectrum availability of therapeutic agents for DM treatment, in the light of adverse reactions associated with their long-term use, the urgent search for other suitable alternatives is a pressing need.

3.3. The Spectrum of Traditional Chinese Medicine (TCM). For many generations, TCMs have been implicated in the treatment of various disorders due to their considerable efficacy with minimum or no adverse effect. With the advent of time, TCM-based DM treatment has been changed following social environment and lifestyle [72]. Clinical evidence reveals that Chinese herbs tian qi and tang-min-ling may significantly reduce the fasting blood glucose and glycosylated hemoglobin levels and improve insulin resistance and function of pancreatic β-cell [73, 74]. Therefore, in this article, we have comprehensively reviewed TCM decoctions/concoctions and independent herbs in offering therapeutic relief from concomitant DM and NAFLD.

3.4. Rehmannia Six Formula (RF). RF, a concoction of six herbs, i.e., *Rehmannia glutinosa*, Fructus Corni, *Dioscorea* sp. (*D. alata*, *D. opposita*, *D. batatas*), *Poria cocos*, *Alisma sp.* (*A. orientalis*, *A. plantago aquatica*), and *Paeonia suffruticosa*, may effectively regulate blood glucose level through its strong antioxidant and anti-inflammatory actions [75]. This implies that RF could be beneficial in addressing DM and its complications by suppressing insulin resistance. The earlier onset and prolonged duration of T2DM may portend the possibility of developing NAFLD [76]; thus, the TCMs controlling NAFLD may be combined with novel herbal formulation to establish therapy for concomitant NAFLD and DM. Similarly, the other form of RF, i.e., Liuwei Dihuang decoction, may improve steatosis-associated histologic changes in the liver by inhibiting insulin resistance by regulating PI3K/Akt signaling pathway [77].

3.5. Shenling Baizhu San. Shenling Baizhu San is a promising TCM alternative for suppressing NAFLD via targeting glycerophospholipid and glycerolipid along with inhibition of SIRT1 in rat liver [78]. It could also regulate the expression of miRNAs such as miR-155-5p, miR-146b-5p, miR-132-3p, and miR-34a-5p to undermine the progression of NAFLD [79]. In a combinatorial approach, Shenling Baizhu San when mixed with Chaihu-Shugan-San effectually lowered the serum concentration of TNF-α and IL-6 [80]. This is indicative of suppressed inflammatory profile and improved lipid metabolism through regulating the expression of molecules involved in the p38 MAPK signal pathway in the rat model of NAFLD progression.

3.6. Lingguizhugan, Xiaochaihu, and ShengMai-Yin and Ganmaidazao: Decoctions. In a study, Lingguizhugan decoction of *Poria*, *Ramulus Cinnamomi*, *Rhizoma Atractylodis Macrocephalae*, and *Radix Glycyrrhizae* ameliorated phenotypic properties of NAFLD rats by regulating pathways of insulin resistance and lipid metabolisms such as PI3K-Akt and AMPK [81]. Lingguizhugan could also mitigate NAFLD by suppressing the expression of *INSIG1* and *LPIN1* genes, indicating decreased oxidative stress, cholesterol biosynthesis, and triglyceride accumulation in the liver [82]. Among various decoctions, the Xiaochaihu, a combination of seven TCMs, is reported to regulate immunity metabolism and oxidative stress [83]. Further, the modified Xiaochaihu decoction could ameliorate age-associated NAFLD by downregulating mRNA/protein levels of core targets in lipid metabolism and inflammation-related pathways such as fatty acid synthase, acetyl-CoA carboxylase, IL-6, and nuclear factor-β [84]. Both abovementioned studies are an indication of the therapeutic role of Xiaochaihu decoction against the pathophysiological duo of NAFLD and DM.

In a seminal study, a combined ShengMai-Yin and Ganmaidazao decoction (SGD) showed pharmacological efficacy against T2DM with NAFLD in mice by retarding serum levels of glucose, total cholesterol, triglycerides, free fatty acids, adipocyte size, and liver lipid deposits [85]. Further, SGD can improve liver metabolism through elevating the levels of PPARα, HSL, and PI3K/Akt and decreasing sterol regulatory element-binding protein-1 and fatty acid synthase, resulting in reduced lipid biosynthesis and increased insulin sensitivity. In a rat model of T2DM and NAFLD, another tangganjian decoction efficiently controlled lipid and glucose metabolism by regulating insulin receptor substrate (IRS) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathways [86].

3.7. Berberine. Berberine, a kind of isoquinoline alkaloid obtained from dry roots of *coptidis rhizome*, has been demonstrated to suppress insulin resistance and triglycerides in the liver of NAFLD rats by upregulating levels of IRS-2 [87]. It targets sirtuin 3 (SIRT3)/adenosine 5′-monophosphate- (AMP-) activated protein kinase (AMPK)/acetyl-CoA carboxylase (ACC) and ameliorate progression of NAFLD [88]. It is of note that in diabetic mice, berberine could activate AMPK and regulate lipid metabolism [89]. These two studies indicate the dual therapeutic actions of berberine against NAFLD and DM. In an important report, berberine also mediated its NAFLD-associated therapeutic effect by inhibiting reactive oxygen species (ROS), inflammation, lipid accumulation, tumor necrosis factor-alpha (TNF-α) expression, and phosphorylation of nuclear factor kappa B (NF-κB) p65 [90]. Further, berberine can inhibit liver triglyceride synthesis and activate AMP-activated protein kinase (AMPK) and sterol regulatory element-binding protein-1c (SREBP-1c) pathway leading to attenuated hepatic steatosis [91].

3.8. Other Herbal Species. Among various TCMs, the *Amomum xanthioides* is an important herb with hepatoprotective, gastrointestinal protection, and antidyslipidemic effects [92]. The administered ethyl acetate extracts of *Ammonium xanthioides* in high-fat induced NAFLD mice suggested that the extract may efficiently regulate the weight of adipose tissue and lipid profiles by targeting lipid metabolic markers such as SREBP-1, PPAR-α, and AMP-activated protein kinase [93]. Additionally, *Trapa quadrispinosa*, a TCM with an antidiabetic effect, has been found effective in subduing NAFLD through targeting signaling pathways of insulin resistance and lipid metabolisms such as AMP-activated protein kinase (AMPK)/acetyl-CoA carboxylase (ACC)/sterol regulatory element-binding protein (SREBP)/insulin receptor substrate-1 (IRs-1) and protein kinase B (Akt) [94]. Supporting the above studies, the extract of *Lonicera caerulea* suppressed lipid biosynthesis and triglyceride accumulation in both NAFLD mice and HepG2 hepatocyte cell line by activating AMPK/ACC signaling pathways [95]. The seed coat of *Euryale ferox*, a traditional oriental medicine rich in polyphenol, has been found effective to reduce lipid accumulation, oxidative stress, and liver injury through regulating the expression of malondialdehyde, alanine aminotransferase, aspartate aminotransferase, IRS-1, CYP2E1, and superoxide dismutase in mouse model of high-fat-induced NAFLD [96]. In streptozotocin-induced diabetic rats, the extract of *Euryale ferox Salisb* effectively increased the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH), and normalized lipid profile [97]. The presence of 2β-hydroxybetulinic acid 3β-caprylate and pentacyclic triterpene in *Euryale ferox* Salisb has been found responsible for an antidiabetic, antioxidant, and protective role for hepatocytes and pancreatic cells [98–100]. Likewise, the extract of *Folium Mori* has been demonstrated to efficaciously control hyperglycemia, hyperlipidemia, and insulin resistance by regulating IRS-1/PI3K/-Glut-4 signaling pathway in diabetic mice [101]. Collectively, this accumulated body of evidence implies the potential of TCM in offering relief from concomitant liver disease and DM.

4. Innovative Avenues in Regenerative Therapy

4.1. Stem Cell-Based Repair and Regeneration. The application of stem cells in addressing the repair and regeneration of injured tissues is accountable for their differentiation potential into target cells under specific conditions. Of various stem cell types, the bone marrow stem cells (BMSCs), adipose-derived stem cells (ADSCs), and umbilical cord-derived stem cells (UCMSCs) have been employed for diabetes-associated disorders (Figure 2).

Additionally, stem cells have been synergistically applied with different treatment approaches such as TCMs and oxidative agents (Figure 3).

4.2. BMSC-Mediated Therapeutic Bioengineering. In diabetic mice, the BMSC and BMSC-conditioned medium have been shown to repair and regenerate damaged hepatocytes by reducing the infiltration of bone marrow-derived cells, lipid accumulation, insulin resistance, and expression of proinflammatory cytokines [102]. The transplanted MSCs in diabetic mice may suppress fatty liver states by reducing low-density lipids and inflammatory cytokines and elevating Sirt1 and heme oxygenase-1 levels [103]. BMSCs through their paracrine actions may increase the levels of heme oxygenase-1 resulting in a reduction in neutrophil influx, inflammation, and hepatocyte apoptosis [104]. These cells also possess the capacity to reverse weight gain, expansion of subcutaneous adipose tissue, and inhibit steatosis, lobular inflammation, fibrosis via immunomodulation, and immunosuppression, including the suppression of CD4+ T cells [105]. Therefore, BMSCs have been suggested to possess the clinical potential for the treatment of NAFLD. Interestingly, a seminal study showed that supplementation of *Ginkgo biloba L.* extract during BMSC therapy could reduce oxidative stress and blood glucose levels of diabetic rats [106]. This research indicates that a combinatorial approach of cellular therapies and TCMs could offer an improved therapeutic efficacy.

4.3. ADSC-Mediated Therapeutic Bioengineering. Compared to BMSCs, the ADSCs are the preferred choice owing to their ease of isolation and comparable efficacy. These have also been explored in addressing regenerative therapeutic needs for liver fibrosis, NAFLD, and liver cirrhosis [107–110]. In the T2DM rat, the transplanted ADSCs assuaged hyperglycemia and insulin resistance as well as liver fibrosis through suppressing TGF-β1 levels and phosphorylation of SMAD3 [107]. ADSCs may also ameliorate liver fibrosis by upregulating hepatocyte growth factor (HGF) and downregulating levels of α-smooth muscle actin [111, 112]. Apart from monotherapy of ADSCs, their treatment with antioxidants such as melatonin and glutathione strongly inhibits oxidative stress and liver fibrosis [113]. In the rat model of T2DM and liver fibrosis, an elevated reparative and regenerative influence of ADSCs was found with

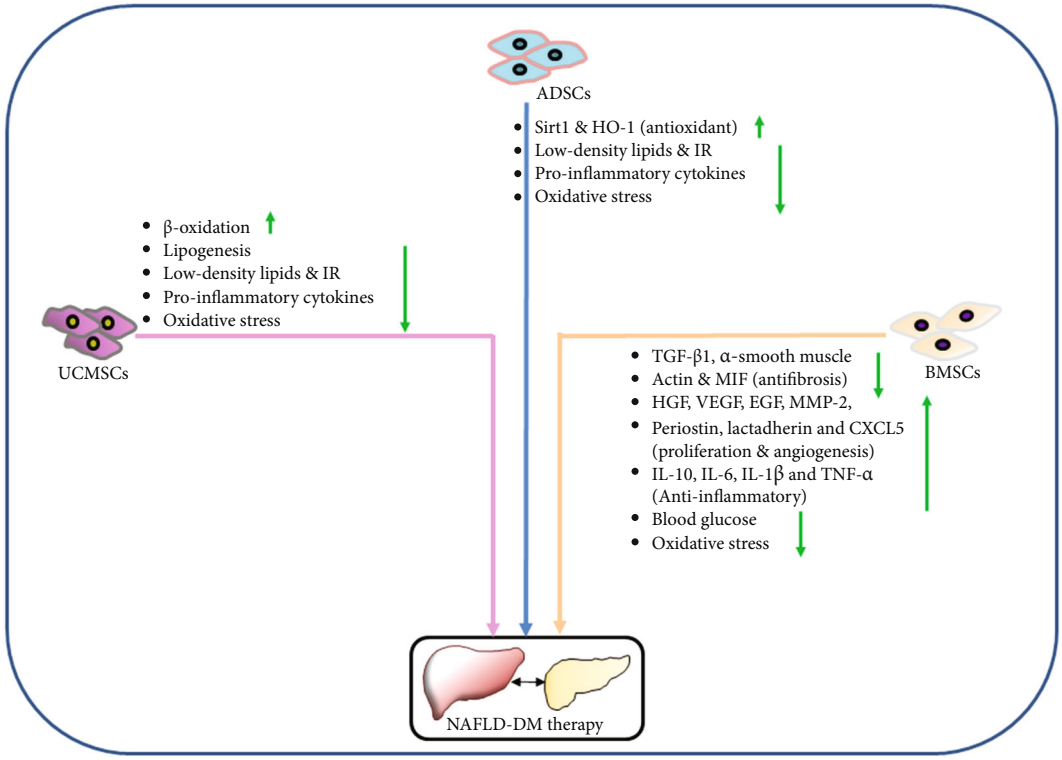

Figure 2: Stem cell-based regenerative therapies for NAFLD in T2DM. (a) BMSC, ADSC, and UCMSC-based regenerative therapies. NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; BMSCs: bone-marrow stem cells; ADSCs: adipose-derived stem cells; UCMSCs: umbilical cord-derived stem cells; HO-1: heme oxygenase-1; IR: insulin resistance; TGF: transforming growth factor; IL: interleukin; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor; EGF: epidermal growth factor; MMP-2: matrix metalloproteinase-2.

oral consumption of resveratrol, which was confirmed through reduced oxidative damage and enhanced survival signaling [114]. Moreover, the regenerative effect of human ADSCs can be improved by cotreatment of lysophosphatidic acid and sphingosine-1-phosphate in the terms of attenuated histologic damage, suppressed oxidative stress, inflammation, fibrosis, and lipid metabolism dysfunction, without tumor formation [115]. As NAFLD and other diabetic complications are associated with hyperglycemia-induced inflammatory effect, the infusion of ADSCs in diabetic rats has shown anti-inflammatory actions by secreting cytokine IL-10, IL-6, IL-1β, and TNF-α [116]. ADSCs could promote proliferation and angiogenesis of hepatocytes by secreting growth factors such as HGF, VEGF, EGF, MMP-2, periostin, lactadherin, and CXCL5 [117]. These cells could also decelerate liver fibrosis by secreting macrophage migration inhibitory factor (MIF) and may regenerate the liver by attenuating acute rejection and reducing inflammatory responses [118]. Remarkably, brown adipose tissue also possesses the potential to lower blood glucose/lipid and suppress oxidative stress and fibrosis and improves lipid metabolism in diabetic mice [119]. This could be achieved by downregulating liver metabolic genes and elevating miRNA-99a through negatively regulating the expression of NOX4. On contrary, a clinical study indicated that though transplanted autologous MSCs, T2DM patients were able to improve the liver function and insulin resistance; the diabetic condition remained unaffected [120].

4.4. UCMSC-Mediated Therapy. In addition to ADSCs and BMSCs, stem cells derived from UCMSCs have also been explored to develop regenerative therapeutic approaches for diabetes and liver-related disorders. In a mouse model of T2DM and NAFLD, the UCMSCs significantly lowered the lipid and LDL content by regulating lipid metabolism genes leading to promoted β-oxidation and suppressed lipogenesis [121]. Moreover, the synergistic application of liraglutide (glucagon-like peptide-1 receptor agonist) and h-UCMSCs may reduce inflammation and oxidative stress through regulating the TLR4/NF-κB inflammation pathway in SD rats with NAFLD and T2DM [122]. These results are also an indication of improved lipid metabolism, insulin resistance, and suppressed liver injury.

It is important to note that regenerative therapeutic efficacy depends on the appropriate homing of injected cells in target tissue or organs. This had been manifested in T2DM mice which showed antidiabetic and antidyslipidemic effects of administered h-UCMSCs with improved liver function migrated after homing to the liver as well as pancreatic islets [123]. Further, the h-UCMSCs-derived exosomes also may improve the structural and functional status of the fibrotic liver through their antifibrotic activity via downregulating

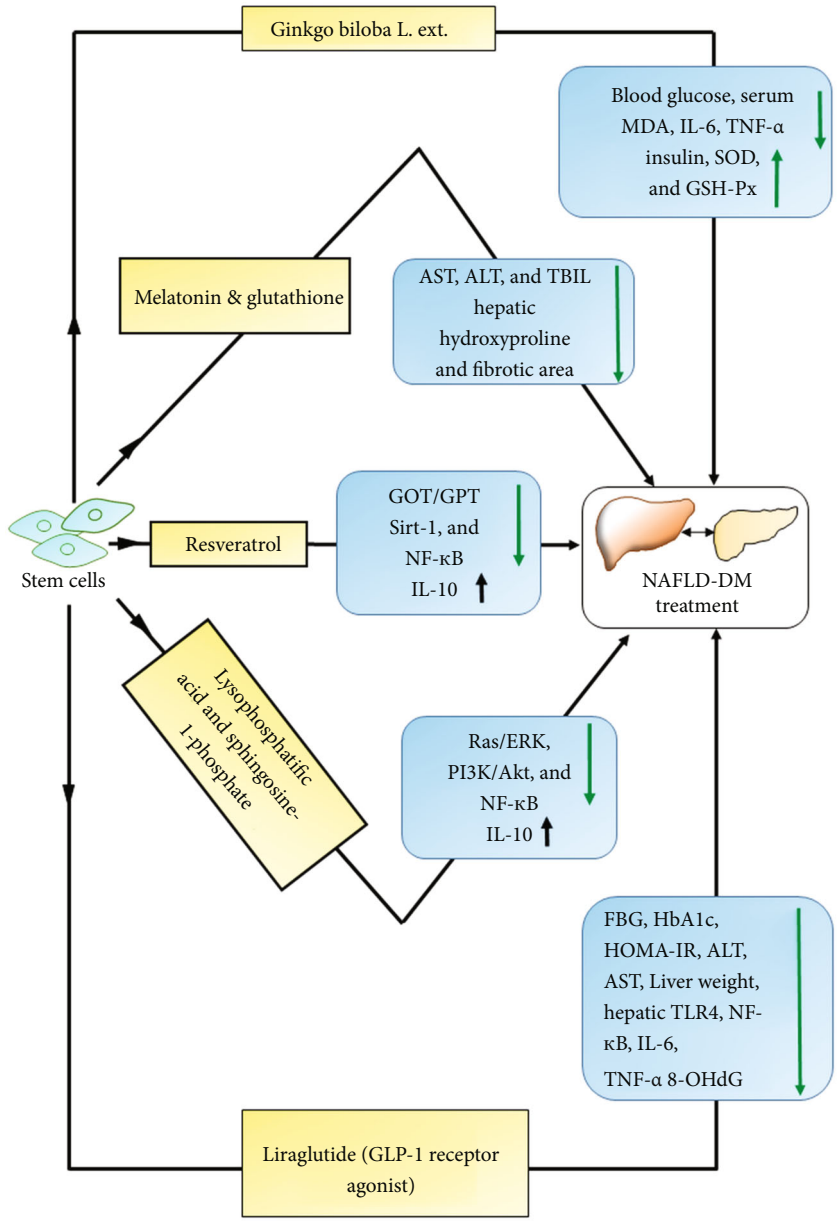

Figure 3: Highlights of novel therapeutic approaches by combining stem cells with the various therapeutic agent (yellow boxes) such as *Ginkgo biloba* extract (TCM), antioxidants (melatonin, reduced glutathione, and resveratrol), bioactive lysophospholipids (lysophosphatidic acid and sphingosine-1-phosphate), and glucagon-like peptide-1 receptor agonist (liraglutide) and their impacts (blue boxes). Up and down arrows indicate the increased and decreased levels, respectively. MDA: malondialdehyde; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; NF-κB: nuclear factor kappa beta; IL-10: interleukin-10; ERK: extracellular signal-regulated kinases; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; FBG: fasting blood glucose; HbA1c: hemoglobin A1C; HOMA-IR: homeostatic model assessment of insulin resistance; TLR4: Toll-like receptor 4; 8-OHdG: 8-hydroxydeoxyguanosine (oxidative stress marker).

the expression of collagen (types I and III) and TGF-β1 [124]. Furthermore, the clinical potential of infused h-UCMSC has already been validated through their hepato-protection and antiviral activity in end-stage liver disease patients without any adverse reactions [125]. A clinical phase I/II study reported that cord blood-derived stem cells could modulate the immune response and restore cytokine balance in T2DM patients [126] and hence could improve insulin resistance mediating concomitant liver disorder and DM. Though the previously discussed stem cells have demonstrated numerous therapeutic outcomes, adverse reactions should be carefully considered before clinical applications. In an important study, a diabetic mouse transplanted with ESCs-derived insulin-secreting cells lowered the glucose level, however, resulted in teratoma formation, which limits its clinical potential in addressing diabetes and related

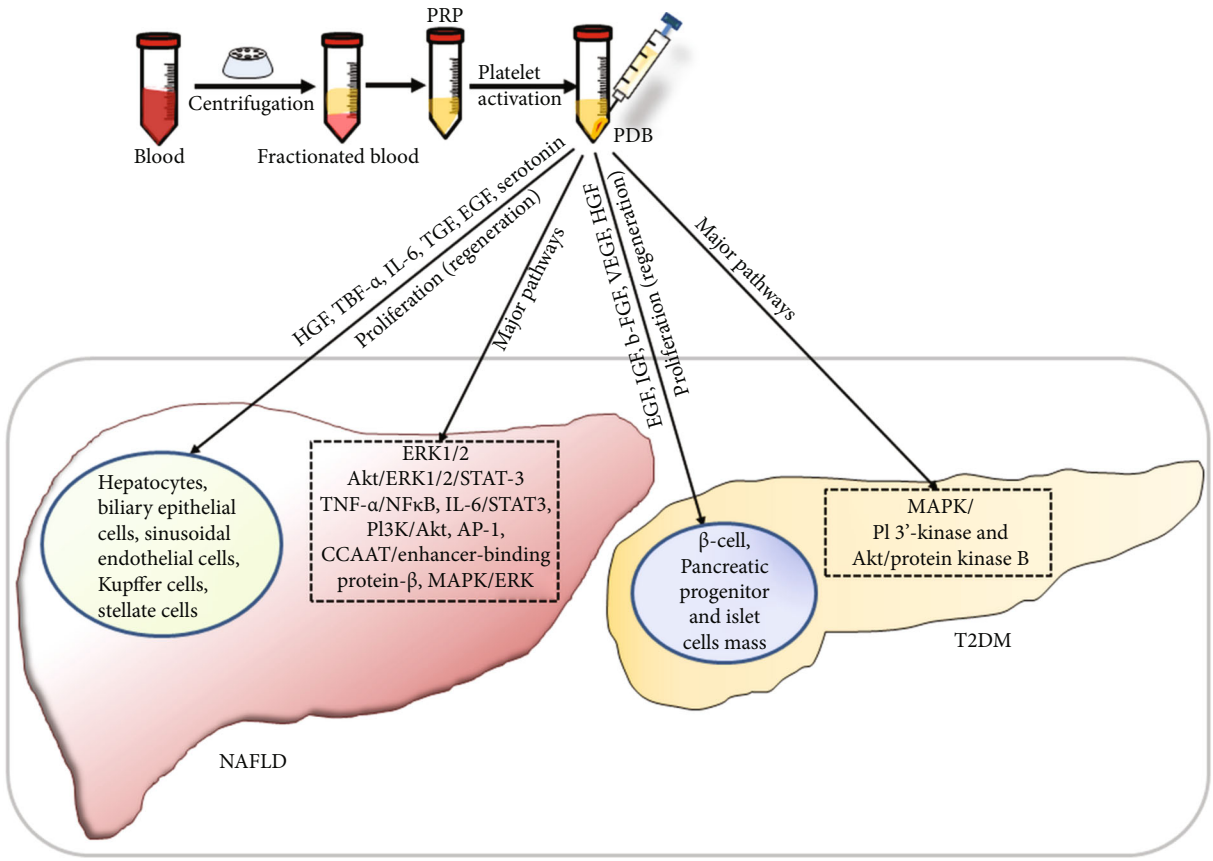

Figure 4: Association between a pathophysiological duo of NAFLD and DM and its treatment. (a) The mechanistic insight underlying NAFLD and DM. NAFLD participates in the development of T2DM by enhancing glucose production and insulin resistance in the liver. T2DM and systemic insulin resistance induce the initiation and progression of NAFLD by increasing levels of FFA and TG from peripheral tissues to the liver. If remain untreated, NAFLD further progresses from NASH, cirrhosis, to hepatocellular carcinoma. (b) Journey of therapeutic alternatives from traditional to regenerative medicines, including lifestyle management, TCM, pharmaceuticals, stem cells, and PRP. NAFLD: nonalcoholic fatty liver disease; FFA: free fatty acid; TG: triglycerides; NASH: nonalcoholic steatohepatitis; T2DM: type 2 diabetes mellitus; TCM: traditional Chinese medicines; PRP: platelet-rich plasma.

complication [127]. Besides this, mitigating the growth of drug-resistant cancer stem cells in diabetes patients is a major challenge for regenerative therapy [128]. Thus, more preclinical and clinical studies are required to completely establish the role of stem cells in providing a safe and effective alternative for liver disease under diabetic conditions.

4.5. Scope of Platelet-Derived Biomaterial (PDB) Therapy in Liver Disease with DM. Owing to its contained PDBs, the platelets have played a significant role in regenerative medicine. These PDBs are present in platelet's α-granules in the form of epidermal growth factor (EGF), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF), platelet factor-4 (PF-4), transforming growth factor-β (TGF-β) along with other releasates such as fibronectin, and vitronectin promote cellular regeneration (Figure 4) [129, 130]. PDB may stimulate vascularization, angiogenesis, fibroblast differentiation, and graft adhesion and improve microenvironment and epithelialization leading to wound healing [131]. The platelets hasten liver regeneration by stimulating the proliferation of hepatocytes, biliary epithelial cells, liver sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells [132]. This is mainly achieved by intercellular interactions between various growth factors and cytokines, such as HGF, tumor necrosis factor-α, interleukin-6, TGF, and EGF. The antifibrotic activity of platelets is mediated by deactivating hepatic stellate cells through adenosine-cyclic adenosine 5′-monophosphate signaling pathway. Platelets also inhibit hepatocyte apoptosis by downregulating *Akt* and upregulating *Bcl-xL* signaling pathways, respectively.

As per reports, the majority of PDBs contribute to restoring homeostasis, wound healing, and tissue regeneration via stimulating Akt, extracellular signal-regulated kinase (ERK) 1/2, and IL-6 leading to activation of signal transducers and activator of transcriptions-3 (STAT-3) [133]. The PDBs such as HGF, IGF-1, and VEGF play a crucial role in hepatocyte proliferation through activating Akt/ERK1/2/-STAT-3 signaling pathways [134]. Similarly, platelet-derived serotonin may participate in liver regeneration by stimulating the proliferation of hepatocytes or facilitating the release of growth factors IL-6 at the site of liver injury [135]. Moreover, PDBs could stimulate a cascade of transcription factors and associated signaling pathways (TNFα/NF-κB, IL-

6/STAT3, PI3K/Akt, AP-1, CCAAT/enhancer-binding protein-β, and MAPK/ERK) and induce the proliferation of hepatic cells [136]. Platelet-mitigated liver fibrosis occurs through secretion of adenosine which inactivates hepatic stellate cells due to an increase in intracellular cAMP resulting in downregulation of collagen expression [137]. Platelets may also interact with Kupffer cells and trigger the release of IL-6 and TNF-α which initiates hepatocyte proliferation [133, 138, 139]. This synergy of Kupffer cells and platelets may effectively increase the efflux of regenerative factors in mouse livers [139, 140]. Platelet-derived-extracellular vesicles such as exosomes may play a crucial role in maintaining cellular homeostasis and liver regeneration by releasing promitogenic factors such as IL-6, which stimulates hepatocyte proliferation [141, 142]. In recent years, PDBs have also gained attention from the scientific and clinical community due to their potential to address diabetes and associated complications. In albino rats, platelet-rich plasma (PRP) has been demonstrated to elevate regeneration of β-cell and improved pancreatic islet cell mass [143]. This could be mainly attributed to the release of peptide growth factors such as EGF and IGF which induce mitogen-activated protein kinase- (MAPK-) mediated differentiation of acinar and ductal cells into pancreatic islets [144, 145]. Further, it has been indicated that the encapsulation of β-cell into alginate and poly-L-histidine beads supplemented with PRP improves β-cell viability and insulin secretion [146]. These outcomes may facilitate the generation of more functional implants with primary β-cells or pancreatic islets for DM treatment. The PDBs in the forms of cytokines and signaling molecules could enhance the differentiation potential of stem cells into insulin-secreting cells, which may inhibit insulin resistance. The b-FGF and EGF may promote differentiation of stem cells into islet-like cells and proliferation of Pdx1-positive pancreatic progenitors' cells, respectively, and eventually increase insulin levels [147, 148]. The β-cell proliferation, islet number, β-cell mass, and total insulin secretion (2-fold) could be increased by overexpression of HGF [149], whereas, VEGF-A and islet vascular structure are correlated and important for the expansion of beta-cell mass [150]. Based on the above reports, we infer that PDB could synergistically act on DM as well as NAFLD by restoring insulin secretion and reducing the risk of initiation and progression of liver-associated disorders by suppressing insulin resistance. Therefore, extensive preclinical and clinical studies should be conducted to establish the dual role of PDB for managing concomitant NAFLD and DM.

5. Future Prospects and Conclusion

The pathophysiological association between concomitant liver disorder and DM is highly complicated and therefore poses a challenge in establishing an efficacious therapy. Lifestyle management seems a critical factor to not only reduce glucose production and insulin resistance in the liver but also the systemic insulin resistance caused by T2DM. The TCMs may impart a significant therapeutic impact by suppressing triglyceride synthesis and oxidative stress. Besides, pharmaceutical interventions such as thiazolidinediones or glitazones have been explored, which could benefit both NAFLD and DM by maintaining glucose and lipid homeostasis and suppressing inflammation and liver fibrosis. However, it seems that recent developments in regenerative alternatives including stem cells and platelet-derived biomaterials may provide enhanced therapeutic recovery, owing to their differentiation potential to hepatic and pancreatic lineage. These biological agents could not only suppress hyperglycemia and insulin resistance but also dyslipidemia. Recently explored, the synergistic application of stem cells with TCMs (Ginkgo biloba extract), antioxidants, PDBs, and other bioactive molecules (liraglutide) seems to possess high potential to treat the comorbid state of NAFLD and DM, by combined repairing and regenerative modalities. However, these available pieces of evidence should be extensively investigated for their optimized procedure, efficacious dosage, clinical application, and their safety.

Data Availability

No data were used to support this study.

Conflicts of Interest

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

Authors' Contributions

Lung-Wen Tsai and Yi-Hsiang Lu contributed equally to this work.

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Review Article

Pathophysiology of Physical Inactivity-Dependent Insulin Resistance: A Theoretical Mechanistic Review Emphasizing Clinical Evidence

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The modern lifestyle has a negative impact on health. It is usually accompanied by increased stress levels and lower physical activity, which interferes with body homeostasis. Diabetes mellitus is a relatively common metabolic disorder with increasing prevalence globally, associated with various risk factors, including lower physical activity and a sedentary lifestyle. It has been shown that sedentary behavior increases the risk of insulin resistance, but the intermediate molecular mechanisms are not fully understood. In this mechanistic review, we explore the possible interactions between physical inactivity and insulin resistance to help better understand the pathophysiology of physical inactivity-dependent insulin resistance and finding novel interventions against these deleterious pathways.

1. Introduction

The global prevalence of diabetes mellitus (DM) is growing rapidly [1]. This metabolic disorder is responsible for more than a dozen debilitating complications that negatively affect the quality of life and detrimentally impact various crucial organs such as the kidneys, nervous system, and cardiovascular system [2, 3]. The exact pathophysiology of DM is unclear, but the role of insulin resistance, especially in type 2 DM, is well confirmed [4]. Some lifestyle-dependent factors facilitate the development of insulin resistance, and its incidence is rapidly growing even among young adults [4–6]. Insulin resistance was previously considered an aging problem. But substantial changes in modern lifestyle towards lower physical activity have also increased the prevalence of DM in young adults [6, 7]. Furthermore, there is a positive relationship between long-term physical inactivity and insulin resistance [4, 8]. But the exact linking pathophysiologic pathways are not well understood. The current study is aimed at introducing both confirmed and potential molecular mechanisms by which physical inactivity induces insulin resistance.

2. Insulin Signaling Pathway and Insulin Resistance

Insulin is a peptide hormone released by islets of pancreatic beta cells [5]. This hormone has significant effects on metabolic pathways, and thereby, it is critical for normal homeostasis of the body metabolism [5]. Insulin acts via complicated sequential steps known as insulin signal transduction (IST) which starts by binding insulin to the α chain of insulin receptor (IR), which is a transmembrane tyrosine kinase composed of two chains as α and β [9]. This process stimulates autophosphorylation in the β chain, which in turn recruits different adaptor proteins such as IRSs (insulin receptor substrates), Shc protein (SHC-transforming), and APS protein (adapter protein with a PH and SH2 domain) [10, 11]. These events provide a suitable binding site for IRS-1 (insulin receptor substrate-1) and activate it, which links to PI3K (phosphoinositide 3-kinase) and catalyzes the conversion of PIP_2 (phosphatidylinositol 4,5-bisphosphate) to PIP_3 (phosphatidylinositol 3,4,5-trisphosphate) [11, 12]. In addition, PIP_3 is itself a potent activator for PKB (protein kinase B, also known as Akt), which facilitates glucose entering into the cells by localization of GLUT-4 (glucose transporter type 4) [12, 13] (Figure 1). Any defect in these pathways may lead to impaired insulin-dependent glucose entering the cells, known as insulin resistance in adipocytes and skeletal muscles [4].

3. Pathophysiologic Links between Physical Inactivity and Insulin Resistance

There is considerable evidence to emphasize the relationships between physical inactivity and insulin resistance [14]. However, the exact pathophysiological links are not clear so yet. Thus, in the following paragraphs, we will discuss the possible relationships based mainly on clinical evidence (Figure 2).

3.1. Genes and Proteins Involved in Glucose Homeostasis. As described before, IST is included of a variety of proteins and enzymes which all work together to facilitate glucose entry into the insulin-dependent cells [9]. Any defect in these harmonic processes will potentially reduce insulin sensitivity [9]. People with insulin resistance have a point mutation or dysfunction in their IST elements [15–17]. Mutation in a single gene or dysfunction of an enzyme such as Akt, PI3K, or IRs could potentially result in impaired IST and contributes to the development of insulin resistance and DM [16]. Hence, proper functioning of this pathway plays an important role in insulin sensitivity and, in turn, glucose homeostasis [17].

Physical inactivity negatively affects the expression, translocation, and function of genes/proteins involved in glucose homeostasis [18–20]. On the other hand, aerobic exercise is a potent stimulus for these genes [4, 21]. For example, Glut-4 is the main route of glucose entry for insulin-dependent cells and has a crucial role in IST [22]. As a result, any disturbance in its expression or function could disrupt insulin signaling and results in insulin resistance [22–24]. Vukovich and colleagues in 1996 showed that even six days of physical inactivity reduces insulin action, which was analyzed via the hyperinsulinemic-euglycemic clamp method, through lowering Glut-4 levels in muscles of endurance-trained runners [25]. Also, Alibegovic and coworkers in 2010 demonstrated that physical inactivity-dependent insulin resistance is related to lower levels of Glut-4 expression in skeletal muscles of young men [19]. They measured insulin sensitivity by the hyperinsulinemic-euglycemic clamp method. They found that 9 days of complete bed rest significantly impacts genes involved in insulin signaling, such as Glut-4, HK2 (hexokinase 2), RRAD (Ras-related glycolysis inhibitor and calcium channel regulator), and TXNIPy, which decreases insulin sensitivity in skeletal muscles [19]. Chibalin et al. in 2000 provided other experimental evidence demonstrating that physical activity increases IRS-1, IRS-2, Akt, PI3 kinase, and Glut-4 expression in rats [26]. Moreover, Biensø et al. in 2014 found the same results by assessing the possible role of 7 days of bed rest intervention on the expression of genes involved in glucose homeostasis [18]. They observed that physical inactivity downregulates Glut-4, HK2, GS (glycogen synthase), and Akt proteins and reduces insulin sensitivity which was examined by the euglycemic-hyperinsulinemic clamp method in skeletal muscles of young, healthy men [18].

Glynn et al. reported in 2008 that decreased levels of physical activity are associated with higher IRS-1 serine phosphorylation and lesser insulin sensitivity, while chronic exercise (running wheels for 9 weeks) reverses these changes in skeletal muscles of rats [27]. Bunprajun et al. in 2013 have shown that physical activity prevents insulin resistance by promoting the Glut-4 expression/translocation in middle-aged volunteers [28]. They demonstrated that active individuals have higher Glut-1 and Glut-4 mRNA expression and higher Glut-4 protein levels in skeletal muscles than sedentary individuals [28]. This evidence suggests that exercise has a pivotal role on Glut-4 expression, translocation, vesicular trafficking, and function and vice versa; lower physical activity (lack of training or sedentary lifestyle) reverses these processes [25, 29]. So, disturbing the physiologic process of insulin action through suppressing IST elements' expression/function is the main pathway by which physical inactivity may induce insulin resistance in peripheral tissues.

3.2. Beta Cell Insufficiency. Pancreatic beta cells are responsible for insulin synthesis and release, the main hormone in glucose homeostasis, by controlling absorption, digestion, conversion, and storage of carbohydrates [30]. Beta cell insufficiency is a general term mainly referred to as the structural or functional inability of the pancreatic beta cells to fulfill their metabolic activities and impair insulin release in response to meal [31]. It was confirmed that beta cell failure leads to chronic hyperglycemia, which characterizes type 2 DM [31]. Also, inherited abnormalities in beta cell mass or function are important precursors for dysglycemia and type 2 DM [31]. Hence, efficient pancreatic islets are crucial for maintaining normal glucose homeostasis [30, 32, 33]. Furthermore, different levels of beta cell insufficiency are

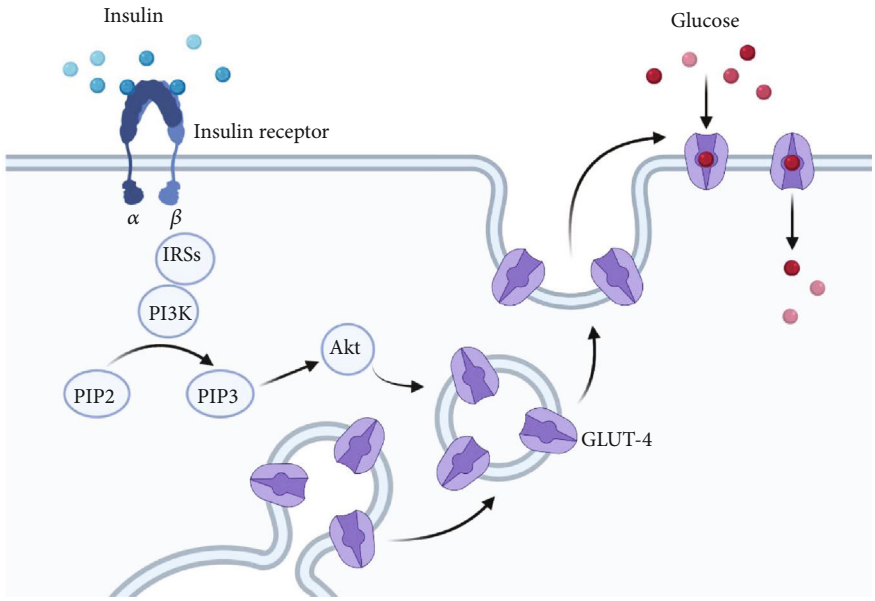

Figure 1: Simple schematic pic of insulin signal transduction (IRSs = insulin receptor substrates; PI3K = phosphoinositide 3-kinase; PIP2 = phosphatidylinositol 4,5-bisphosphate; PIP3 = phosphatidylinositol 3,4,5-trisphosphate; Akt = protein kinase B; Glut-4 = glucose transporter type 4).

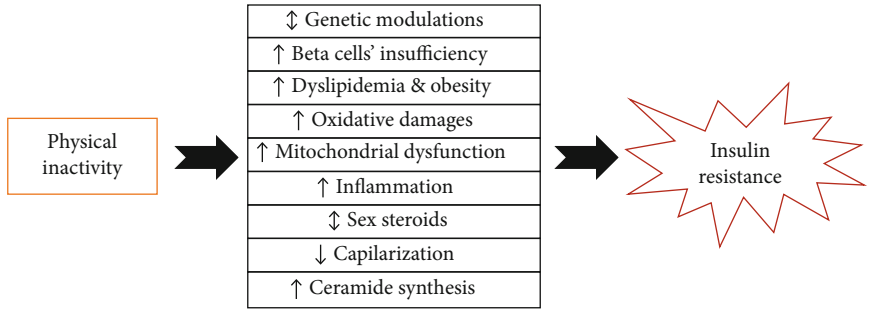

Figure 2: Possible links between physical inactivity and insulin resistance.

commonly seen in patients with T1DM or T2DM, which have lower levels of circulatory insulin [32, 34, 35]. Therefore, any pathologic factor inducing islet cell dysfunction may be a potential threat to glucose homeostasis [30].

We have strong evidence indicating physical inactivity is closely associated with islet cell insufficiency [36]. An inactive lifestyle can give rise to insulin resistance by increasing islets' workload and lowering their efficiency through various pathways such as ER (endoplasmic reticulum) stress, mitochondrial dysfunction, oxidative stress, and inflammation and promoting the apoptosis and death of beta cells [36]. In contrast, physical training and exercise preserve islets' function and restore it, leading to increased peripheral insulin sensitivity [37]. In addition, they can induce beta cell proliferation via elevations in circulating levels of different growth factors such as growth hormone, IGF-1 (insulin-like growth factor 1), and GLP-1 (glucagon-like peptide 1) [38]. It can also prevent or suppress islet apoptosis and thereby increase the functional mass of beta cells [39].

Slentz and coworkers in 2009 reported that inactive subjects have lower beta cell sufficiency than trained individuals [40]. They found that 8 months of inactivity led to a significant rise in fasting plasma glucose, while moderate- to high-intensity exercise restored these changes, improved islet function, and adjusted glucose metabolism [40]. Also, Dela et al. in 2004 revealed that inactive persons have lesser insulin sensitivity and lower beta cell sufficiency in response to an oral bolus of carbohydrates [37]. Similarly, Lee and coworkers in 2015 found that T2DM patients with lower physical activity had reduced islet function and irregular glucose metabolism compared with the aerobic exercise group [41]. Bloem and Chang, in another trial, reported similar findings indicating even short-term exercise improved pancreatic beta cell activity and glucose metabolism than sedentary individuals [42]. Gomes et al. in 2013 demonstrated that inactive obese diabetic rats have lower pancreatic beta cell function compared with the exercise group [43]. Delghingaro-Augusto and colleagues in 2012 found

similar results demonstrating diabetic rats with lower activity have more susceptibility to beta cell failure while exercise improves their function [44]. These studies strongly highlight the strong relationships between physical inactivity and beta cell failure and improvement of islet function with exercise.

3.3. Obesity and Dyslipidemia. Obesity, which is commonly associated with dyslipidemia, is closely related to insulin resistance [45]. It has now been accepted as a significant risk factor for DM since it has detrimental effects on different phases of IST, disrupting physiologic insulin signaling pathways toward impaired peripheral insulin sensitivity [45, 46]. Evidence shows that obesity can induce insulin resistance via ER stress induction, oxidative damage, mitochondrial dysfunction, beta cell dysfunction, dysregulation of adipokines and adiponectins involved in glucose homeostasis, impairing expression/localization/activities of IST elements, and evoking and promoting inflammatory processes [45]. Thus, effective preventive or therapeutic approaches against DM are commonly accompanied by lifestyle modification, keeping body weight in a healthy range and physical fitness [4, 47–49].

Physical inactivity and sedentary behaviors can induce insulin resistance via lowering energy expenditure, dysregulating lipid homeostasis, and enhancing lipid storage [46, 50]. Amati et al. in 2009 demonstrated that physical inactivity-dependent obesity underlies insulin resistance in older athletes [51]. Hamburg and coworkers in 2007 found that only five days of bed rest dysregulates serum lipid profile and induces insulin resistance in healthy volunteers [52]. Also, Davies and coworkers in 2018 conducted a clinical study demonstrating a short-term decrease in physical activity dysregulates lipid profile, changes body composition, and increases lipid content and reduces sensitivity inactive participants [53]. They concluded that insulin sensitivity could be improved by normalizing lipid homeostasis and energy balance [53]. Sjöros and colleagues in 2020 reported that physical activity improves cardiometabolic health and lipid profile toward higher levels of insulin sensitivity in sedentary volunteers [54]. In another study, more physical activity was related to lower BMI (body mass index) and lipid content and higher insulin sensitivity in healthy volunteers [55]. Similarly, abdominal obesity and dysregulated lipid profile were reported as underlying culprits of insulin resistance in physically inactive individuals [56]. It must be noted that active people with endurance training may have higher intramuscular lipid, which has no adverse effects on insulin sensitivity (known as athletes' paradox) [57]. This exception may be due to lower DAG or ceramide levels in trained individuals [57]. In total, obesity and dysregulated lipid profiles are potential links between physical inactivity and insulin resistance.

3.4. Mitochondrial Dysfunction. Mitochondria are a double-membrane intracellular organelle involved in most metabolic pathways and are recognized as the powerhouse of the cells [58]. It has major roles in vital cellular events such as cell death and signaling, thus playing a fundamental role in body homeostasis [58]. Effective mitochondrial volume is vital for insulin signaling and glucose homeostasis, and any impairment in this pathway increases the risk of insulin resistance [24, 59]. Many patients with diabetes have different levels of mitochondrial dysfunction [60, 61]. It is involved in insulin resistance via at least four mechanisms: point mutations in mtDNA (mitochondrial DNA), activation of PKC (protein kinase C), mitochondria-induced oxidative stress, and pancreatic beta cell dysfunction [59]. Altered mitochondrial capacity or reduced mitochondrial genes involved in glucose homeostasis, such as hexokinase II or PPARGC1A (peroxisome proliferator-activated receptor- γ coactivator-1 α), were also reported in many patients with T2DM [19, 62]. Moreover, lower mitochondrial content in skeletal muscles was reported in many patients with T2DM [63, 64]. So, any pathologic factor reducing mitochondrial content or function may be a potent risk factor for insulin resistance [59, 65, 66].

There is strong evidence implying physical inactivity and sedentary behaviors have deleterious impacts on mitochondrial function [67, 68]. These findings show that trained individuals have a higher capacity for mitochondrial performances than sedentary individuals [58]. Abadi and coworkers in 2009 reported that limb immobilization significantly downregulates some mitochondrial proteins like cytochrome c oxidase and citrate synthase and suppresses metabolic machinery of glucose homeostasis [69]. Figueiredo et al. in 2009 demonstrated that long periods of inactivity have a deleterious impact on the mitochondrial respiratory function of skeletal muscles in mice [68]. They observed that lifelong inactivity seriously impairs mitochondrial oxidative capacity by inducing oxidative damages in immobilized tissues [68]. Distefano et al. in 2018 found that inactive subjects have lower mitochondrial oxidative capacity than the exercise group [67]. They suggested that active older adults have a better mitochondrial capacity and concluded that mitochondria are a key therapeutic target for sedentary-related complications and insulin resistance [67]. Alibegovic and colleagues in 2010 provided clinical evidence indicating physical inactivity-dependent insulin resistance is closely associated with significant changes in mitochondrial genes involved in glucose metabolism [19]. They found that only 9 days of bed rest impairs PPARGC1A and CPT1B (carnitine palmitoyltransferase 1B) mitochondrial gene expression via downregulation or increased DNA methylation in young men's skeletal muscles [19]. Bilet and coworkers in 2020 provided further evidence implying limb immobilization promotes insulin resistance via suppressing mitochondrial oxidative capacity in skeletal muscles of healthy young men [70]. Therefore, mitochondrial dysfunction is another possible link between sedentary behaviors and insulin resistance and could be an effective therapeutic target for physical inactivity-induced diabetes.

3.5. Oxidative Stress. Oxidative stress, which refers to an imbalance between free radicals and antioxidants, favoring the free radicals, is a key player in the pathophysiology of insulin resistance [24, 71]. It can significantly disturb normal IST and disrupt physiologic, metabolic pathways toward pathologic events such as a polyol or hexosamine pathways producing harmful byproducts like AGEs (advanced

glycation end products) and MDA (malondialdehyde) [24]. In addition, there is strong evidence suggesting higher levels of free radical species directly attack different elements of IST and disrupt their function and reduce insulin sensitivity [71, 72]. Also, many patients with diabetes have different levels of oxidative stress due to weakened intrinsic antioxidant defenses or hyperproduction of free radicals [73]. Hence, antioxidant therapy in these patients could readjust oxidative balance, improve insulin sensitivity, and normalize whole-body metabolism [74, 75].

We have evidence suggesting that physical inactivity increases oxidative stress [70, 76]. For example, Laufs et al. in 2005 demonstrated that physical inactivity upregulates nox1, p47phox, and p67phox subunits of NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase), increases ROS (reactive oxygen species) generation, and induces oxidative damage in vascular tissue of C57BL6 mice [76]. Also, Alghadir and coworkers in 2016 reported that physically inactive patients, compared with active participants, have higher levels of oxidative damage markers as higher MDA and lower TAC (total antioxidant capacity) in plasma [77]. Moreover, Kozakiewicz et al. in 2019 established that inactive older men have lower SOD (superoxide dismutase), CAT (catalase), and GPx (glutathione peroxidase) activity and higher plasma MDA content than active individuals [78]. Accordingly, Alibegovic and colleagues in 2010 reported that physical inactivity insulin resistance is partly dependent on transcriptional changes inducing oxidative stress such as PPARGC1A and TXNIP (thioredoxin-interacting protein), in which daily physical activity reverses these changes [19]. These findings imply that physical inactivity may be correlated to more oxidative damages, disturbing peripheral insulin sensitivity. However, more investigations are required to confirm these findings.

3.6. Low-Grade Inflammation. Inflammation is closely involved in the pathophysiology of insulin resistance [79]. Different forms of cytokines and proinflammatory mediators, e.g., TNF-α (tumor necrosis factor alpha), MCP-1 (monocyte chemotactic protein-1), and CRP (C-reactive protein), were upregulated in patients with T2DM [79]. Also, animals lacking the proinflammatory mediators were protected against insulin resistance [80]. TNF-α, a widely expressed inflammatory cytokine, impairs insulin signaling via serine phosphorylation of IRS-1 or reduces Glut-4 expression [81]. Moreover, other inflammatory pathways such as IKKβ (a subunit of IκB kinase) and activation of IKKβ/NF-κb and JNK (c-Jun N-terminal kinase), which is a key element in tissue inflammation, are commonly followed by insulin resistance [82]. Activation of the JNK pathway induces serine phosphorylation in IRS-1 in 307, which impairs insulin signaling (47). Also, another potent cytokine of IL-1 (interleukin-1) reduces IRS-1 expression via ERK1/2 (extracellular signal-regulated kinase 1) and IKKβ/NF-κb activation in adipocytes and skeletal muscles (48). Likewise, IL-6 stimulates IRS degradation and so reduces insulin sensitivity [83]. Inflammation can also upregulate Socs1 (suppressor of cytokine signaling) and Socs3, which induce IRS degradation through ubiquitylation [84]. Many patients with T2DM have chronic low-grade inflammation with increased accumulation of immune cells and higher levels of circulating proinflammatory markers impairing normal insulin signaling [24, 85]. Thus, any agent that can elicit inflammatory responses, such as inactivity, may threaten insulin sensitivity and glucose homeostasis [79].

Inactivity increases visceral fat accumulation, stimulating chronic low-grade systemic inflammation and dependent comorbidities such as insulin resistance and DM [86]. Thus, there is a vicious mutual cycle between physical inactivity, obesity, and light systemic inflammation, which drives the internal milieu toward insulin resistance [86]. As we know, adipose tissue has endocrine activities by producing and releasing a wide variety of inflammatory factors such as leptin, NY (neuropeptide Y), interleukins, TNF-α (tumor necrosis factor alpha), resistin, adipokines, and adiponectin [87]. These proteins have complicated cross-talks with immune system elements and modulate their activity [87]. As a result, lower physical activity is commonly followed by more visceral/subcutaneous adipose tissue (higher BMI), resulting in more immune system activity and higher circulatory levels of inflammatory cytokines [87]. Thus, adipose tissue-induced inflammation is a known cause of insulin resistance in obese people [88, 89].

Studies are suggesting a sedentary lifestyle increases inflammatory markers [86, 90]. In an extensive clinical experiment, Hamer and coworkers found that physical inactivity is directly linked to more circulating inflammatory cytokines [91]. They reported that physical activity has a linear relationship with circulating cytokine levels as CRP (C-reactive proteins) and IL-6 (interleukin 6) in healthy volunteers [91]. Also, Phillips et al., in 2017, conducted a clinical study showing sedentary behavior is associated with higher inflammatory cytokines in plasma [92]. They also found that replacing sedentary behaviors with physical activity reduces circulating cytokines and improves insulin sensitivity in obese adults [92]. Højbjerre et al. in 2011 presented further evidence indicating even short periods of physical inactivity in healthy volunteers can induce inflammatory responses and increase the risk of insulin resistance and T2DM [93]. So, it seems that physical inactivity has a potent relationship with insulin resistance via inducing and promoting inflammatory responses.

3.7. Sex Steroids. Sex hormones have dominant impacts on metabolic pathways [94]. These steroids have potent catabolic, anabolic, or releasing effects on main substrates like lipids, proteins, and carbohydrates and could induce or suppress their metabolism in different conditions [94, 95]. Evidence is well confirmed that estrogen (estradiol), progesterone, and testosterone, as the primary sex steroids, have profound effects on most steps of glucose homeostasis such as absorption, glycogenesis, and gluconeogenesis, releasing into circulation and entering into the insulin-dependent cells and thereby providing protective defense against metabolic disorders as well as DM [95]. Testosterone increases Glut-4 expression/localization in adipocytes which in turn increases insulin sensitivity [96]. It also induces insulin sensitivity via

IRS phosphorylation [97]. Similarly, estradiol enhances Glut-4 translocation and induces PI3K/Akt signaling pathway, increasing insulin sensitivity [98, 99]. Lower levels of these steroids are closely associated with insulin resistance [100]. For example, age-related or obesity-dependent insufficiency of sex steroids is the main cause of insulin resistance and the onset of T2DM [95]. So, the level of sex steroids and their release have significant importance in glucose homeostasis.

A sedentary lifestyle may alter circulating sex steroids via several pathways as some adipokines (such as leptin and adiponectin) induce estrogen biosynthesis, reducing adiposity, aromatization of androgens (occurs within peripheral adipocytes), and hepatic synthesis of SHBG [101–103]. There is strong clinical evidence confirming these findings. He and colleagues in 2018 found that plasma-reduced sex steroids in plasma are related to higher adiposity and lower physical activity in women and men [104]. They reported that 20 weeks of aerobic exercise significantly increased sex hormones and sex hormone-binding globulin (SHBG) and reduced abdominal fat [104]. Also, Tin and coworkers in 2020 demonstrated that physical activity is directly correlated to levels of sex steroids as estradiol, testosterone, and SHBG in women [105]. Furthermore, they found that self-reported sedentary time is negatively related to plasma levels of these factors [105]. Thus, we have no direct evidence confirming sedentary behavior induces insulin resistance via sex steroids but have indirect evidence. However, more experiments are required to confirm these findings.

3.8. Capillarization. Capillarization, which refers to the formation of a network of capillaries in an organ or tissue, is an on-demand process directly associated with the level of metabolism rate in the tissue [106]. Increased capillary density in skeletal muscles is an independent factor predicting the level of insulin sensitivity [107]. Animals with higher capillary density demonstrated a higher glucose tolerance and improved glucose metabolism [106]. Treatment with angiogenic agents improves insulin sensitivity and increases glucose tolerance in animals [108]. Also, tissue-specific insulin sensitivity is directly affected by the level of capillarization in that tissue [106]. Similarly, athletes with higher capillary density and increased blood flow in skeletal muscles have better glucose homeostasis than nonathlete individuals [109], although other molecular mechanisms may also be involved [4]. Although the exact involved molecular pathways are not clear so yet, muscle morphology, the level of angiogenesis, and amount of capillary density are independent determinant factors in insulin sensitivity [110].

It has been confirmed that angiogenesis and capillarization in skeletal muscles are influenced by many factors and metabolites released during physical activity and exercise [111]. Therefore, while exercise and training increase capillarization, physical inactivity and sedentary behavior reduce or suppress this process [106]. Furthermore, studies have shown that an increased level of capillarization is related to more insulin sensitivity, especially in skeletal muscles [106, 112, 113]. For example, Snijders et al., in 2017, conducted a clinical study showing capillary density is a determinant factor for insulin sensitivity in skeletal muscles [110]. They found that muscles with a higher capillary network have better glucose tolerance in response to Oral Glucose Tolerance Test (OGTT) [110]. Also, Rodrigues et al. in 2020 reported that GLP-1 (glucagon-like peptide 1) exerts its antidiabetic effects at least partly via an increase of capillarization in adipocytes [112]. In addition, they found that GLP-1-dependent increased vascular network is correlated to more insulin sensitivity in visceral adipose tissues of rats with T2DM [112]. Moreover, Evans and coworkers recently demonstrated that exercise training and physical activity upregulated angiogenic factors such as VEGF (vascular endothelial growth factor), PlGF (placental growth factor), sFlt-1 (soluble fms-like tyrosine kinase receptor-1), and bFGF (basic fibroblast growth factor) and increased capillarization which accompany improvement in insulin sensitivity in skeletal muscles of older men [114]. These findings suggest that a lower level of capillarization may be another link between sedentary behavior and insulin resistance [115].

3.9. Ceramide Level. Ceramide is a family of naturally occurring highly bioactive lipids present abundantly in the lipid bilayer membranes of eukaryotic cells and contribute to many intracellular pathways, such as free radical generation, the release of inflammatory cytokines, apoptotic processes, and gene expression [116]. These lipid molecules are mainly composed of sphingosine and are a significant component of the cellular lipid bilayer membrane by an essential role in maintaining its integrity [117]. Ceramide synthesis occurs in at least three distinct ways as the de novo pathway, the sphingomyelin hydrolysis (degradation), and the salvage (recycling) pathway [8]. In addition to structural roles, more recent studies have suggested a causal relationship between ceramide and metabolic complications as well as insulin resistance [118]. They have shown that ceramide may play a role in pancreatic inflammation, beta cell apoptosis and insulin synthesis, ER stress, adipokine release, mitochondrial stress, IRS-1 phosphorylation, and oxidative stress [119, 120]. Furthermore, treatment with myriocin, an inhibitor of de novo ceramide synthesis, has improved insulin sensitivity [121]. Also, the knockout of ceramide-generating enzymes in animals has increased insulin sensitivity [122, 123]. Therefore, ceramide is now widely accepted as a potent insulin antagonist involved in the pathophysiology of insulin resistance and DM, especially in overweight and obese people [116].

There is strong evidence suggesting physical inactivity increases ceramide production, which induces insulin resistance [124]. Bergouignan and colleagues in 2009 have reported that physical inactivity declines insulin sensitivity via impairing cellular and plasma trafficking and metabolism of lipids as well as ceramides in lean women [125]. They observed that 2 months of bed rest was followed by saturated fat and sphingosine accumulation in myocytes, which impaired insulin sensitivity in participants [125]. Also, Kwon and coworkers in 2015 established that 14 days of inactivity increases ceramide level, dysregulates skeletal muscle insulin signaling, and impairs glucose tolerance in mice [126]. Furthermore, Bergman et al. in 2016 provided direct evidence indicating the increased amount of muscle ceramide during physical inactivity is related to insulin

Table 1: Cellular pathways linking physical inactivity and insulin resistance (IST = insulin signal transduction).

Molecular mechanisms	Effects of physical inactivity	Experimental evidence	Clinical evidence
Genetic modulations	Modulates expression/function of IST elements	[26]	[18, 19]
Beta cells' insufficiency	Induces beta cell insufficiency and reduces pancreatic islet mass	[43, 44]	[37, 40–42, 129]
Obesity and dyslipidemia	Reduces energy expenditure toward dyslipidemia and higher risk of obesity which in turn stimulates insulin resistance	—	[50–53]
Mitochondrial dysfunction	Reduces mitochondrial mass, which in turn impairs insulin expression/secretion/signaling	[68]	[19, 67, 69, 70]
Oxidative damages	Increases free radical species followed by more systemic oxidative stress	[76]	[77, 78]
Inflammation	Onset and progress low-grade inflammatory response, which in turn induce insulin resistance	—	[91–93]
Sex steroids	Modulates sex steroid expression/secretion leading to impaired glucose homeostasis	—	[104, 105]
Capillarization	Reduces the amount of vascular network, which in turn impairs insulin sensitivity	—	[106, 112]
Ceramide synthesis	Increases the amount of ceramide synthesis, which in turn interferes with insulin signaling	—	[125–127]

resistance in obese volunteers [127]. They found that acute exercise reduces sphingolipid synthesis in the recovery period and improves insulin sensitivity in trained volunteers [127]. However, due to some reports about the effect of short-term inactivity on ceramide level and glucose homeostasis, it seems that ceramides need more time to exert pathologic effects and disturb insulin signaling [128].

4. Conclusion

Physical inactivity, a common health risk of the modern lifestyle, is a severe threat to body homeostasis that deviates physiologic metabolism toward injurious pathways. So it is now recognized as a potent underlying cause of insulin resistance and DM, but the interconnections are not fully understood. Our study suggests that physical inactivity is closely related to insulin resistance via at least 9 molecular mechanisms (Table 1) as genetic modulation of IST elements, impairment of pancreatic beta cell function, increase of the risk of dyslipidemia and obesity, mitochondrial dysfunction, increase of oxidative damages, modulating sex hormone expression/function, reduction of the vascular network as capillarization, enhancement of ceramide production, and inducing chronic low-grade systemic inflammation. We have strong clinical evidence regarding the links with these various pathways; however, other unidentified cellular pathways may contribute to this.

Data Availability

There is no raw data associated with this review article.

Conflicts of Interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Characteristics Associated with Early Worsening of Retinopathy in Patients with Type 2 Diabetes Diagnosed with Retinopathy at Their First Visit: A Retrospective Observational Study

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Aims/Introduction. To investigate whether the occurrence of early worsening of diabetic retinopathy in patients with type 2 diabetes diagnosed with simple or preproliferative diabetic retinopathy at their first visit differed according to HbA1c reduction and/or treatment intensification. *Materials and Methods*. Our study design was a retrospective observational study. Subjects with type 2 diabetes diagnosed with either simple or preproliferative diabetic retinopathy by ophthalmologists at their first visit and followed up for 6–18 months thereafter were included and divided into worsening and nonworsening groups. Thereafter, baseline characteristics and changes in HbA1c and therapy over a year were investigated. *Results*. Among the 88 subjects with simple diabetic retinopathy, 16% improved to no retinopathy, 65% retained their simple diabetic retinopathy, 18% worsened to preproliferative diabetic retinopathy, and 1% worsened to proliferative diabetic retinopathy. Among the 47 subjects with preproliferative diabetic retinopathy, 9% improved to simple diabetic retinopathy, 72% retained their preproliferative diabetic retinopathy, and 19% worsened to proliferative diabetic retinopathy. Patients with simple diabetic retinopathy had an odds ratio of 1.44 for worsening retinopathy with a 1% increase in baseline HbA1c. Meanwhile, the odds ratios for worsening retinopathy with a 1% decrease in HbA1c from baseline at 3, 6, and 12 months were 1.34, 1.31, and 1.38, respectively. Among patients with simple diabetic retinopathy, significantly more new interventions were introduced in the worsening group than in the nonworsening group. *Conclusions*. Increased baseline HbA1c, a substantial decrease in HbA1c, and intensified therapy were identified as risk factors for early worsening of diabetic retinopathy in patients with simple diabetic retinopathy at the first visit. Patients should therefore be intimately followed for retinopathy after their first visit.

1. Introduction

Diabetic retinopathy (DR) remains one of the major microvascular complications of diabetes, with severe cases possibly leading to blindness among adult patients. DR has two main stages, namely, nonproliferative (NPDR) and proliferative diabetic retinopathy (PDR) [1]. Accordingly, NPDR progresses from mild, moderate, and then severe [2], whereas the incidence of PDR increases as the baseline retinopathy stage worsens as shown in previous reports [3, 4]. The modified Davis classification [5–7] has commonly been used for grading retinopathy. Simple diabetic retinopathy (SDR) can

be characterized by hard exudates, capillary aneurysms, or abnormal capillary aneurysm lesions. Meanwhile, preproliferative DR (PPDR) can be characterized by intraretinal hemorrhage, definite venous beading, definite intraretinal microvascular abnormalities, or soft exudates.

Studies have documented that the duration of diabetes [4], the high levels of HbA1c, blood pressure, and body mass index (BMI) [8], were risk factors for the onset and progression of DR. Compared with conventional glycemic control, intensive therapy has been known to reduce the progression of retinopathy in patients with both type 1 [9] and type 2 [10–13] diabetes. However, several reports have indicated that intensive therapy and/or substantial HbA1c reduction may be associated with early worsening of DR (EWDR) in patients with both type 1 [9, 14, 15] and type 2 [16–18] diabetes. Moreover, other specific and nonspecific risk factors for EWDR, unrelated to ordinary DR worsening, have been reported, including prolonged duration of diabetes, high baseline HbA1c levels, history of DR [3, 9, 14, 16, 17], and bariatric surgery [19, 20]. Furthermore, no current agreement exists regarding the appropriate timing of HbA1c reduction [21].

The current study retrospectively investigated whether the occurrence of EWDR in patients with type 2 diabetes diagnosed with SDR at their first visit differs according to baseline HbA1c, abruptness in HbA1c reduction, and treatment intensification.

2. Materials and Methods

2.1. Subjects. A total of 2334 patients with type 2 diabetes initially visited the Division of Diabetes and Metabolism, the Institute of Medical Science, Asahi Life Foundation ($n = 2045$), or the Department of Endocrinology and Diabetes, Saitama Medical University Hospital ($n = 289$) for glycemic control or diabetic education from January 2006 through October 2015. The subjects were evaluated for retinopathy either at the Institute of Medical Science, Asahi Life Foundation, or at the Saitama Medical University Hospital. Ophthalmologists observed and sketched the entire area with mydriasis, and retinopathy was classified into four stages of severity according to the modified Davis classification: no retinopathy, SDR, PPDR, proliferative retinopathy (PDR), or photocoagulation. Fundus photographs were obtained when there was a change in fundus findings or when fluorescein angiography was performed. Subjects were classified as SDR by the presence of hard exudates, capillary aneurysm, or abnormal capillary aneurysm lesions, and they were classified as PPDR by the presence of intraretinal hemorrhage, definite venous beading, definite intraretinal microvascular abnormalities, or soft exudates. We included subjects diagnosed with SDR or PPDR following ophthalmologic examinations within 6 months after their first visit and those who underwent fundus examinations two or more times within 6–18 months after their first examination. Among these subjects, those who did not undergo the first fundus examinations prior to HbA1c measurement 3 months after the first visit or the last fundus examination after HbA1c measurement at 12 months were excluded. When differences in the classification of both eyes were present at the first visit of patients, the worse one was used. Worsening retinopathy was defined as disease stage progression after the first visit. For those who visited ophthalmologic clinics several times after their first visit, findings during the period closest to 12 months after their first visit were used.

2.2. Laboratory Tests and Statistical Analysis. Baseline demographic information, such as sex, age, disease duration, blood pressure, BMI, biochemical profiles, and use of statins and antihypertensive agents, was collected as previously described [22]. Previous medications taken before the first visit and new interventions provided were also determined.

Patients were categorized into four groups according to previous medications taken before their first visit: "none," "nonsulfonylurea (non-SU)," sulfonylurea (SU)," and "insulin,", respectively. "None" indicates patients taking neither oral hypoglycemic agents nor insulin. "Non-SU" indicates those taking antidiabetics except for insulin, SU, or glinides. "SU" indicates those taking SU or glinide, including combinations of other hypoglycemic agents, except for insulin. "Insulin" indicates those taking insulin, including any combination of hypoglycemic agents. Patients were also categorized according to new interventions received at their first visit based on the treatment stages of their previous medications. For instance, when "non-SU" patients received other non-SU agents as their new intervention, the new intervention was categorized as "none" given that no treatment intensification on the subsequent stage occurred. Moreover, when patients with no prior medication received insulin and glinide, the new intervention was categorized as "insulin." Therapy intensification was defined as "yes" when any new intervention was confirmed and "no" when no new interventions were received.

HbA1c levels were assessed at the first visit, as well as after 3, 6, and 12 months: baseline HbA1c, 3 M HbA1c, 6 M HbA1c, and 12 M HbA1c, respectively. Thereafter, the magnitude of HbA1c reduction 3, 6, and 12 months after the first visit was determined by subtracting baseline HbA1c levels from 3 M HbA1c, 6 M HbA1c, and 12 M HbA1c, with HbA1c reduction being indicated by "Δ" (Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c, respectively). To assess HbA1c reduction during the follow-up period, the magnitude of HbA1c reduction at 3 and 6 months (Δ3 M HbA1c and Δ6 M HbA1c) was subtracted from that at 12 months (Δ12 M HbA1c): Δ12 M-Δ3 M HbA1c and Δ12 M-Δ6 M HbA1c.

After the aforementioned procedures, multiple logistic regression analysis adjusting for age, sex, BMI, and use of antihypertensive agents was performed by using retinopathy progression as the objective variable and baseline HbA1c, Δ3 M HbA1c, Δ6 M HbA1c, Δ12 M HbA1c, and treatment profiles as explanatory variables in patients with SDR to calculate the odds ratios.

Retinopathy progression was evaluated by dividing patients with SDR into two groups according to retinopathy findings after treatment initiation, namely, worsening and nonworsening groups. Both groups were then compared in terms of other collected variables.

Table 1: Summary of the clinical characteristics of patients with simple and preproliferative diabetic retinopathy.

	SDR			PPDR		
	Total ($n = 88$)	Nonworsening ($n = 71$)	Worsening ($n = 17$)	Total ($n = 47$)	Nonworsening ($n = 38$)	Worsening ($n = 9$)
Age (years)	58 ± 10	59 ± 10	54 ± 11	52 ± 8	52 ± 8	53 ± 9
Sex (% male)	80	76	94	79	82	67
Duration of diabetes (years)	9 < 4, 15>	10 < 4, 17>	7 < 1, 11>	9 < 3, 15>	9 < 4, 15>	7 < 1, 13>
Body mass index (kg/m^2)	25.0 ± 3.8	25.2 ± 3.8	24.1 ± 3.6	25.8 ± 4.9	26.1 ± 5.3	24.6 ± 2.4
Newly diagnosed diabetes (%)	14	13	18	6	5	11
Previous medication (%) None/non-SU†/SU‡/insulin	33/5/45/17	30/3/46/21	47/12/41/0	36/13/36/15	26/16/42/16	78/0/11/11
New intervention (%) None/non-SU†/SU‡/insulin	64/6/15/15	70/6/11/13	35/12/29/24	62/11/4/23	68/11/0/21	33/11/22/33
Therapy intensification (% yes)	36	30	65	38	32	67
Use of statins (%)	28	30	24	36	37	33
Use of antihypertensive agents (%)	53	59	29	51	45	78
Baseline HbA1c (%)	9.1 ± 2.0	8.9 ± 1.9	10.3 ± 1.8	9.5 ± 1.9	9.4 ± 1.9	9.8 ± 2.3
3 M HbA1c (%)	7.4 ± 1.1	7.3 ± 0.9	7.8 ± 1.5	7.6 ± 1.2	7.8 ± 1.3	7.0 ± 0.7
6 M HbA1c (%)	7.2 ± 1.1	7.1 ± 1.0	7.4 ± 1.4	7.3 ± 1.1	7.4 ± 1.0	6.9 ± 1.2
12 M HbA1c (%)	7.1 ± 1.0	7.1 ± 0.9	7.0 ± 1.2	7.4 ± 1.1	7.5 ± 1.1	6.8 ± 0.9
Δ3 M HbA1c (%)	−1.7 ± 1.8	−1.5 ± 1.8	−2.5 ± 1.5	−1.9 ± 1.9	−1.7 ± 1.8	−2.8 ± 2.2
Δ6 M HbA1c (%)	−1.9 ± 2.0	−1.7 ± 1.9	−2.9 ± 2.0	−2.2 ± 1.9	−2.1 ± 1.7	−2.9 ± 2.6
Δ12 M HbA1c (%)	−1.9 ± 2.0	−1.7 ± 2.0	−3.1 ± 1.7	−2.1 ± 1.8	−1.9 ± 1.6	−3.0 ± 2.2
Baseline systolic blood pressure (mmHg)	137 ± 18	138 ± 19	135 ± 16	142 ± 24	142 ± 23	142 ± 29
Baseline diastolic blood pressure (mmHg)	81 ± 14	81 ± 14	83 ± 11	85 ± 15	86 ± 15	81 ± 15
Baseline triglyceride (mg/dL)	146 < 104, 239>	164 < 104, 243>	137 < 89, 163>	124 < 85, 173>	131 < 85, 198>	102 < 78, 148>
Baseline total cholesterol (mg/dL)	210 ± 63	212 ± 64	203 ± 56	208 ± 45	206 ± 49	217 ± 24
Baseline high density lipoprotein (mg/dL)	51 ± 13	52 ± 14	50 ± 12	55 ± 14	54 ± 14	60 ± 11
Baseline low density lipoprotein (mg/dL)	116 ± 36	113 ± 30	126 ± 55	122 ± 41	123 ± 44	120 ± 19
Baseline non-high-density lipoprotein (mg/dL)	159 ± 63	161 ± 65	152 ± 57	153 ± 44	152 ± 47	156 ± 26
Creatinine (mg/dL)	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Uric acid (mg/dL)	5.2 ± 1.2	5.2 ± 1.1	5.1 ± 1.4	5.4 ± 1.3	5.4 ± 1.4	5.5 ± 0.9
Urinary albumin/creatinine ratio (mg/gCre)	12 < 7, 36>	11 < 6, 32>	13 < 11, 50>	29 < 11, 110>	29 < 11, 122>	35 < 16, 99>

Variables are expressed as mean ± standard deviation or median < 1st quartile, 3rd quartile>. †Nonsulfonylurea. ‡Sulfonylurea.

This study was approved by the Ethics Committees of both the Institute of Medical Science, Asahi Life Foundation (approval number 10302-5-A), and Saitama Medical University Hospital (approval number 18123.03). All clinical investigations were conducted in accordance with the tenets of the Declaration of Helsinki. All statistical analyses were performed using JMP version 14.2 (SAS Institute Inc.), with $p < 0.05$ indicating statistical significance during regression analysis.

3. Results

According to the results of the fundus examination, we obtained 109 SDR and 63 PPDR subjects, and 88 SDR and 47 PPDR subjects could be included in the analyses of the comparison between EWDR and patients' characteristics, including HbA1c changes, as per the inclusion criteria. Table 1 summarizes the patients' characteristics at their first visit. Accordingly, patients with SDR and PPDR, 80% and 79% of whom were male, had a mean age of 58 ± 10 and 52 ± 8 years, diabetes duration of 9 (4, 15) and 9 (3, 15) years, BMI (kg/m^2) of 25.0 ± 3.8 and 25.8 ± 4.9, percentage of newly diagnosed diabetes of 14% and 6%, baseline HbA1c of 9.1% ± 2.0% (range 5.9%–13.9%) and 9.5% ± 1.9% (range 5.6%–13.9%), Δ3 M HbA1c of −1.7% ± 1.8% (range −7.5%–1.2%) and −1.9% ± 1.9% (range −7.3%–1.2%), Δ6 M HbA1c of −1.9% ± 2.0% (range −8.8%–1.5%) and −2.2% ± 1.9% (range −6.4%–1.1%), Δ12 M HbA1c of −1.9% ± 2.0% (range

Table 2: Univariate logistic regression analysis for retinopathy progression in patients with simple diabetic retinopathy.

	OR	95% CI	p value	AUC	Cutoff value
Age (+1 years)	0.95	0.90–1.00	0.09		
Sex (males)	5.03	0.92–94	0.13		
Diabetes duration (+1 years)	0.93	0.85–0.99	0.05		
Body mass index (+1 kg/m^2)	0.92	0.79–1.06	0.28		
Newly diagnosed diabetes (yes)	1.48	0.30–5.72	0.59		
New intervention (nonsulfonylurea vs. none)	4.17	0.50–26.8	0.16		
New intervention (sulfonylurea vs. none)	5.21	1.25–21.7	0.02*		
New intervention (insulin vs. none)	3.70	0.81–15.8	0.09		
Therapy intensification (yes)	4.37	1.47–14.2	0.01*		
Use of statins (yes)	0.73	0.19–2.35	0.62		
Use of antihypertensive agents (yes)	0.29	0.08–0.86	0.03*		
Baseline HbA1c (+1%)	1.44	1.10–1.94	0.01*	0.73	9.8
3 M HbA1c (+1%)	1.51	0.92–2.54	0.10		
6 M HbA1c (+1%)	1.25	0.75–2.07	0.39		
12 M HbA1c (+1%)	0.90	0.49–1.60	0.74		
Δ3 M HbA1c (−1%)	1.34	1.00–1.82	0.05	0.71	−1.6
Δ6 M HbA1c (−1%)	1.31	1.00–1.74	0.048*	0.69	−1.5
Δ12 M HbA1c (−1%)	1.38	1.06–1.83	0.02*	0.75	−1.6
Δ12 M-Δ3 M HbA1c (−1%)	2.04	1.12–4.30	0.03*	0.67	−0.6
Δ12 M-Δ6 M HbA1c (−1%)	2.96	1.34–6.96	0.01*	0.73	−0.4
Baseline systolic blood pressure (mmHg)	0.99	0.96–1.02	0.61		
Baseline diastolic blood pressure (mmHg)	1.01	0.97–1.05	0.60		
Baseline triglyceride (mg/dL)	1.00	0.99–1.00	0.16		
Baseline total cholesterol (mg/dL)	1.00	0.99–1.01	0.57		
Baseline high density lipoprotein (mg/dL)	0.99	0.95–1.03	0.70		
Baseline low density lipoprotein (mg/dL)	1.01	0.99–1.03	0.25		
Baseline non-high-density lipoprotein (mg/dL)	1.00	0.98–1.01	0.61		
Creatinine (mg/dL)	0.27	0.02–2.28	0.30		
Uric acid (mg/dL)	0.91	0.57–1.42	0.67		
Urinary albumin/creatinine ratio (mg/gCre)	1.00	0.99–1.00	0.91		

*$p < 0.05$.

−8.4%–0.9%) and −2.1% ± 1.8% (range −6.3%–0.9%), and baseline urinary albumin creatinine ratio of 12 (7, 36) and 29 (11, 110) mg/gCre, respectively. Among subjects with SDR at their first visit, 16% (14 patients) improved to no retinopathy, 65% (57 patients) retained their SDR, 18% (16 patients) worsened to PPDR, and 1% (one patient) worsened to PDR. Meanwhile, among those with PPDR at their first visit, 9% (4 patients) improved to SDR, 72% (34 patients) retained their PPDR, and 19% (9 patients) worsened to PDR.

Logistic regression analysis for the worsening (17 patients) and nonworsening groups (71 patients) revealed an odds ratio of 1.44 for the worsening retinopathy with a 1% increase in baseline HbA1c level (Table 2). Moreover, the odds ratios for worsening retinopathy with a 1% decrease in Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c were 1.34, 1.31, and 1.38, respectively. The odds ratios for worsening retinopathy with a 1% decrease in Δ12 M-Δ3 M HbA1c and Δ12 M-Δ6 M HbA1c were 2.04 and 2.96, respectively. The cutoff values of Δ3 M HbA1c, Δ6 M HbA1c, Δ12 M HbA1c, Δ12 M-Δ3 M HbA1c, and Δ12 M-Δ6 M HbA1c maximized the sum of sensitivity plus specificity −1 (Youden index) for worsening retinopathy were −1.6% (area under the curve 0.71), −1.5% (0.69), −1.6% (0.75), −0.6% (0.67), and −0.4% (0.73), respectively. These cutoff values remained almost constant regardless of the duration of HbA1c decrease. Moreover, those not taking antihypertensive agents exhibited significantly greater retinopathy worsening. No associations were observed between retinopathy progression and sex, statin use, baseline blood pressure, lipid, creatinine, uric acid levels, and urinary albumin creatinine ratio.

The relationship between EWDR and previous medication or new intervention determined using the Cochran–Armitage trend test is presented in Figure 1. Among patients with SDR or PPDR, those who did not receive previous medications tended to have worsening retinopathy. Among patients with SDR, the new intervention tended to significantly intensify in the worsening group, whereas among patients with PPDR, the new interventions did not differ

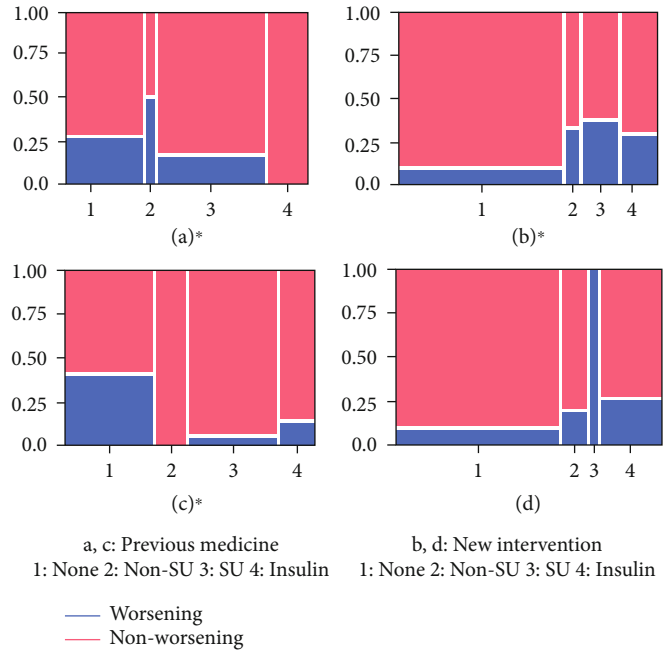

Figure 1: Rates of previous medication and new interventions in a patient with simple diabetic retinopathy (a, b) and preproliferative diabetic retinopathy (c, d). Cochran–Armitage trend test was performed, with $p < 0.05$ indicating statistical significance (∗). The X-axis classifies patients into four categories: none (1), nonsulfonylurea (2), sulfonylurea (3), or insulin (4). The width represents the number of patients classified. The Y-axis indicates the number of patients classified into two categories: worsening (blue) or nonworsening (red). Among patients with SDR, those who received no previous medication tended to have worsening retinopathy (a). New intervention tended to significantly intensify in worsening patients with SDR (b). Among patients with PPDR, those who received no previous medication tended to have worsening retinopathy (c).

between both groups. Multiple logistic regression analysis was then utilized by using retinopathy progression as the objective variable and baseline HbA1c, Δ3 M HbA1c, Δ6 M HbA1c, Δ12 M HbA1c, and therapy intensification as explanatory variables among patients with SDR (Table 3). Accordingly, Δ12 M HbA1c and baseline HbA1c were determined to be significantly associated with EWDR after adjusting for age, sex, BMI, and use of any antihypertensive agents. Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c were not independent of therapy intensification and/or baseline HbA1c for EWDR, and odds ratios of Δ3 M HbA1c and Δ6 M HbA1c were changed from more than 1.00 to less than 1.00. Moreover, the decrease in HbA1c, baseline HbA1c, and therapy intensification were confounded with each other for EWDR.

Figure 2 shows the correlation between baseline HbA1c and ΔHbA1c in worsening and nonworsening patients with SDR. Accordingly, high baseline HbA1c levels were found to be associated with lower Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c similarly between worsening and nonworsening subjects. However, the distribution density showed that the worsening group had greater baseline HbA1c distribution at the high region and greater Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c distribution at the low region compared to the nonworsening group.

4. Discussion

The current study showed that a certain proportion of patients with type 2 diabetes who were diagnosed with SDR or PPDR at the first visit developed EWDR within one year. Moreover, our results showed that reduced HbA1c, intensification of hypoglycemic pharmacotherapy, and baseline HbA1c levels were associated with EWDR in subjects with SDR. Unfortunately, no previous studies have determined the rate of progression from SDR to PPDR, while the incidence of PDR in patients with PPDR observed herein was similar to that presented in previous reports [23, 24].

No current agreement exists on the timing of HbA1c reduction to prevent EWDR [21]. Nonetheless, the present study found that the relationship between EWDR and HbA1c reduction was independent of the timing of HbA1c reduction, considering that the cutoff value of HbA1c reduction over time for retinopathy worsening remained almost constant. Accordingly, our findings suggest that a 1.6% decrease in HbA1c at any time within 12 months since the first visit could be a risk factor for EWDR. No difference in the ratio of baseline HbA1c to HbA1c reduction during hospital visits had been noted between patients with EWDR and those who showed no retinopathy progression. Given the correlation between baseline HbA1c levels and HbA1c decrease, determining which variable contributed more to EWDR remains challenging.

Among patients with SDR, EWDR increased when no previous medication or new pharmacotherapy interventions were introduced. However, the new intervention for EDWR was not independent of HbA1c and ΔHbA1c. Treatment selection may have been confounded with HbA1c reduction. It should be noted that 27% of the subjects who did not

Table 3: Multivariate logistic regression analysis of possible factors for retinopathy progression in patients with simple diabetic retinopathy.

		OR	95% CI	p value
Model Δ3 M-1	Δ3M HbA1c (−1%)	1.30	0.92–1.89	0.14
Model Δ3 M-2	Δ3 M HbA1c (−1%)	0.84	0.43–1.55	0.58
	Baseline HbA1c (+1%)	1.61	0.95–2.97	0.08
Model Δ3 M-3	Δ3M HbA1c (−1%)	1.15	0.78–1.72	0.48
	Therapy intensification (yes)	2.97	0.73–12.8	0.13
Model Δ3 M-4	Δ3 M HbA1c (−1%)	0.81	0.40–1.50	0.51
	Baseline HbA1c (+1%)	1.51	0.87–2.83	0.15
	Therapy intensification (yes)	2.38	0.55–10.3	0.25
Model Δ6 M-1	Δ6 M HbA1c (−1%)	1.24	0.91–1.73	0.17
Model Δ6 M-2	Δ6 M HbA1c (−1%)	0.81	0.44–1.44	0.46
	Baseline HbA1c (+1%)	1.67	0.94–3.07	0.08
Model Δ6 M-3	Δ6M HbA1c (−1%)	1.10	0.77–1.58	0.60
	Therapy intensification (yes)	3.19	0.74–14.6	0.12
Model Δ6 M-4	Δ6 M HbA1c (−1%)	0.75	0.40–1.37	0.36
	Baseline HbA1c (+1%)	1.59	0.88–2.96	0.13
	Therapy intensification (yes)	2.73	0.60–12.4	0.19
Model Δ12 M-1	Δ12 M HbA1c (−1%)	1.42	1.04–2.03	0.03*
Model Δ12 M-2	Δ12 M HbA1c (−1%)	1.25	0.66–2.50	0.50
	Baseline HbA1c (+1%)	1.16	0.59–2.22	0.66
Model Δ12 M-3	Δ12 M HbA1c (−1%)	1.29	0.91–1.90	0.17
	Therapy intensification (yes)	2.48	0.57–11.3	0.23
Model Δ12 M-4	Δ12M HbA1c (−1%)	1.20	0.62–2.43	0.58
	Baseline HbA1c (+1%)	1.09	0.55–2.12	0.81
	Therapy intensification (yes)	2.41	0.54–10.7	0.25
Model baseline HbA1c	Baseline HbA1c (+1%)	1.38	1.02–1.92	0.04*
Model therapy intensification	Therapy intensification (yes)	3.21	0.94–11.7	0.06
Model baseline HbA1c + therapy intensification	Baseline HbA1c (+1%)	1.26	0.90–1.80	0.19
	Therapy intensification (yes)	2.59	0.62–11.2	0.19

*$p < 0.05$.

receive previous medications for SDR were newly diagnosed with diabetes; although it was unanalyzable, this high percentage of the subjects presumably influenced the detected significance of the previous medication. Meanwhile, among patients with PPDR, previous medication, and not therapy intensification, affected EWDR, indicating that the retinopathy onset might have preceded the first diagnosis. This speculation is supported by the considerably short diabetes duration among patients with PPDR having EWDR, who might have experienced rapid progression of retinopathy and/or a long interval of untreated diabetes. Although several studies have reported retinopathy worsening following insulin injection [16, 17, 25], the mechanism underlying such a development has remained unclear [26]. However, previous research has hypothesized [26] that exogenous insulin acts synergistically with vascular endothelial growth factor expressed by the ischemic retina, thereby triggering vascular proliferation and worsening of diabetes retinopathy. Nonetheless, the mechanisms behind EWDR have yet to be elucidated [21]. Studies have shown that numerous cytokines, namely, growth hormones, insulin-like growth factor-1 [27, 28], vascular endothelial growth factor [29], and erythropoietin [30], are involved in DR.

Hypoglycemia often occurs when intensive glycemic control is achieved [12] with reports suggesting a relationship between hypoglycemia and worsening of retinopathy [31, 32]. However, other studies have also reported that hypoglycemia was not associated with retinopathy [33] and that intensive treatment reduced microvascular complications despite increased hypoglycemia [12]. Granting that a relationship exists between EWDR and hypoglycemia, new interventions, such as SU and insulin, could cause EDWR through hypoglycemia owing to their pharmacological mechanisms. However, subjects categorized as non-SU, who received

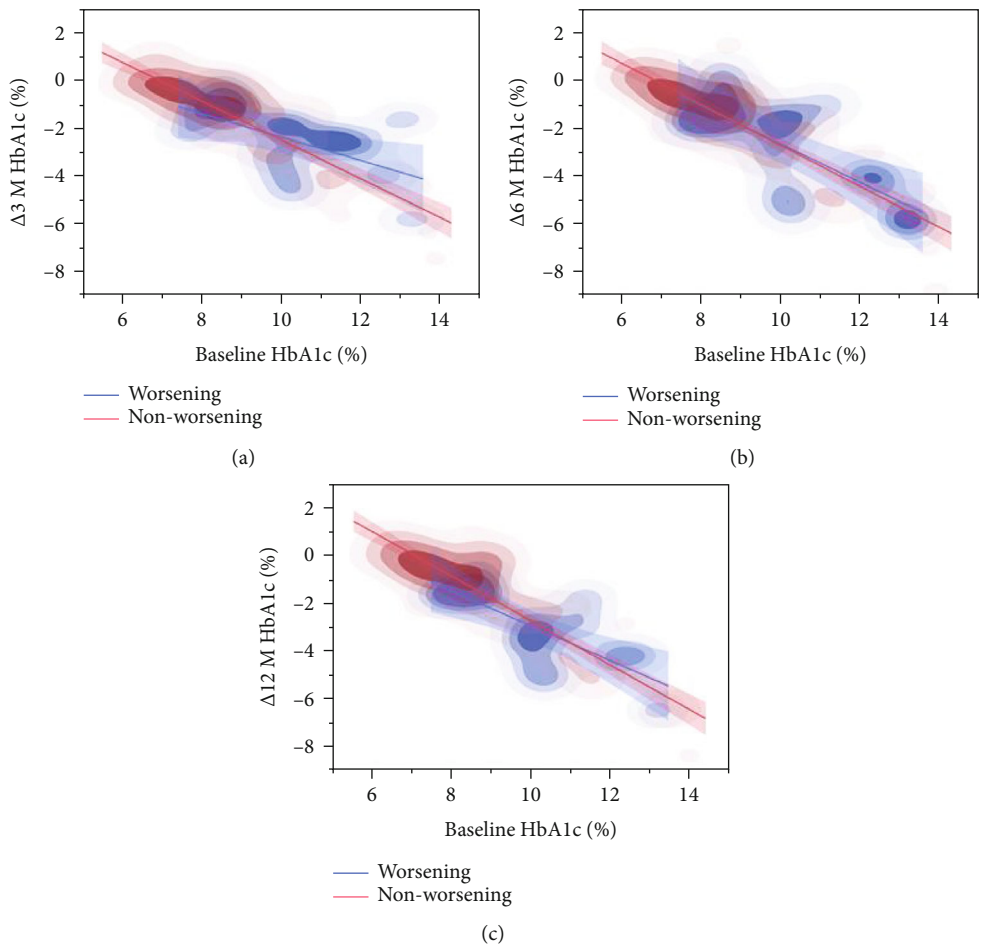

Figure 2: Two-dimensional density plots and regression line of baseline HbA1c and ΔHbA1c comparing the retinopathy worsening (blue) and nonworsening (red) groups in patients with simple diabetic retinopathy (SDR). The X-axis represents baseline HbA1c level, whereas the Y-axis represents Δ3 M HbA1c (a), Δ6 M HbA1c (b), or Δ12 M HbA1c (c). High baseline HbA1c was associated with lower levels of Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 M HbA1c. The worsening group had greater baseline HbA1c distribution at the high region and greater Δ3 M HbA1c, Δ6 M HbA1c, and Δ12 MHbA1c distribution at the low region compared to the nonworsening group.

neither SU nor glinide nor insulin, tended to suffer from EDWR. Therefore, hypoglycemia may not be the only cause for EDWR. A double-blind trial [34, 35] revealed that the GLP-1 analog semaglutide promoted more DR complications compared to placebo among high-risk patients. Given that the GLP-1 analog apparently does not increase hypoglycemia, DR worsening was suggested to have been caused by preexisting DR and the rapid improvement in glycemic control.

Studies [4] and guidelines [36] recommend a retinopathy follow-up interval of 6 months to 2 years among patients who have mild NPDR given the increased incidence of retinopathy requiring treatment when the fundus examination interval exceeds 2 years [37]. Frequent follow-up is required in high-risk groups with more advanced DR and high HbA1c levels [38]. Therefore, patients with high HbA1c levels and SDR at their first visit should be referred to an ophthalmologist within 1 year.

Previous randomized controlled trials (RCTs) [9, 14, 15] involving patients with type 1 diabetes have identified high baseline HbA1c, rapid improvement in glycemic control, history of DR, intensified treatment, long diabetic duration, women, and pregnancy as risk factors for EWDR [39, 40]. Unfortunately, no large RCTs have been conducted on patients with type 2 diabetes [21], although previous non-RCTs [16–18, 41] have shown that high baseline HbA1c, rapid improvement in glycemic control, intensified treatment, previous DR, long diabetic duration, and bariatric surgery were risk factors for EWDR among those with type 2 diabetes [19, 20]. Moreover, a previous report [15] found that baseline HbA1c exceeding 10.1% increased the risk of EWDR in both intensive and conventional treatment groups. Therefore, patients with high HbA1c at their first visit need to be mindful of EWDR regardless of treatment. Although duration of diabetes has been identified as a risk factor for EWDR [15, 41], the current study found no significant relationship between duration of diabetes and EWDR, with 14% (12 patients) of patients with SDR having been newly diagnosed with type 2 diabetes. Therefore, the duration of diabetes could have been underestimated. Similar to the previous studies [21], the current study found that blood pressure or lipids were not associated with EWDR.

Some limitations of the current study are worth noting. Firstly, the number of subjects was relatively small. Due to the limited number of subjects with PPDR, a detailed analysis could not be performed. Moreover, no difference in the characteristics of EWDR was observed between those with SDR and PPDR. A previous study [15] showed that EWDR occurred in 13% and 7.6% of those with type 1 diabetes who received intensive and conventional treatment, respectively. Based on such conditions, at least 962 subjects were required to satisfy a statistical power of 80%. Therefore, the several subanalyses conducted herein might have diminished significance due to the small number of subjects. Secondly, various biases inherent to retrospective studies may have been present. For instance, treatment bias may have occurred among physicians who were already knowledgeable regarding the relationship between rapid improvement in glycemic control and EWDR. However, our dataset comprising clinical practice information from several certified diabetologists across Japan showed that HbA1c reduction was not hindered in accordance with EWDR information. Finally, the short follow-up period is a limitation of this study. If the follow-up period had been 2 years or more, the progress of retinopathy may have been found in more patients.

5. Conclusion

Although initiating or changing therapy can effectively improve glycemic control, rapid glycemic control has been associated with EWDR. The current study identified high baseline HbA1c and a large decrease in HbA1c as risk factors for EWDR among patients diagnosed with SDR at their first visit. As such, patients should be closely followed up for retinopathy within a year after their first visit, regardless of the decline of HbA1c levels and the type of hypoglycemic agents administered.

Data Availability

The data that support the findings of this study are available from Akifumi Kushiyama upon reasonable request.

Conflicts of Interest

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

Acknowledgments

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