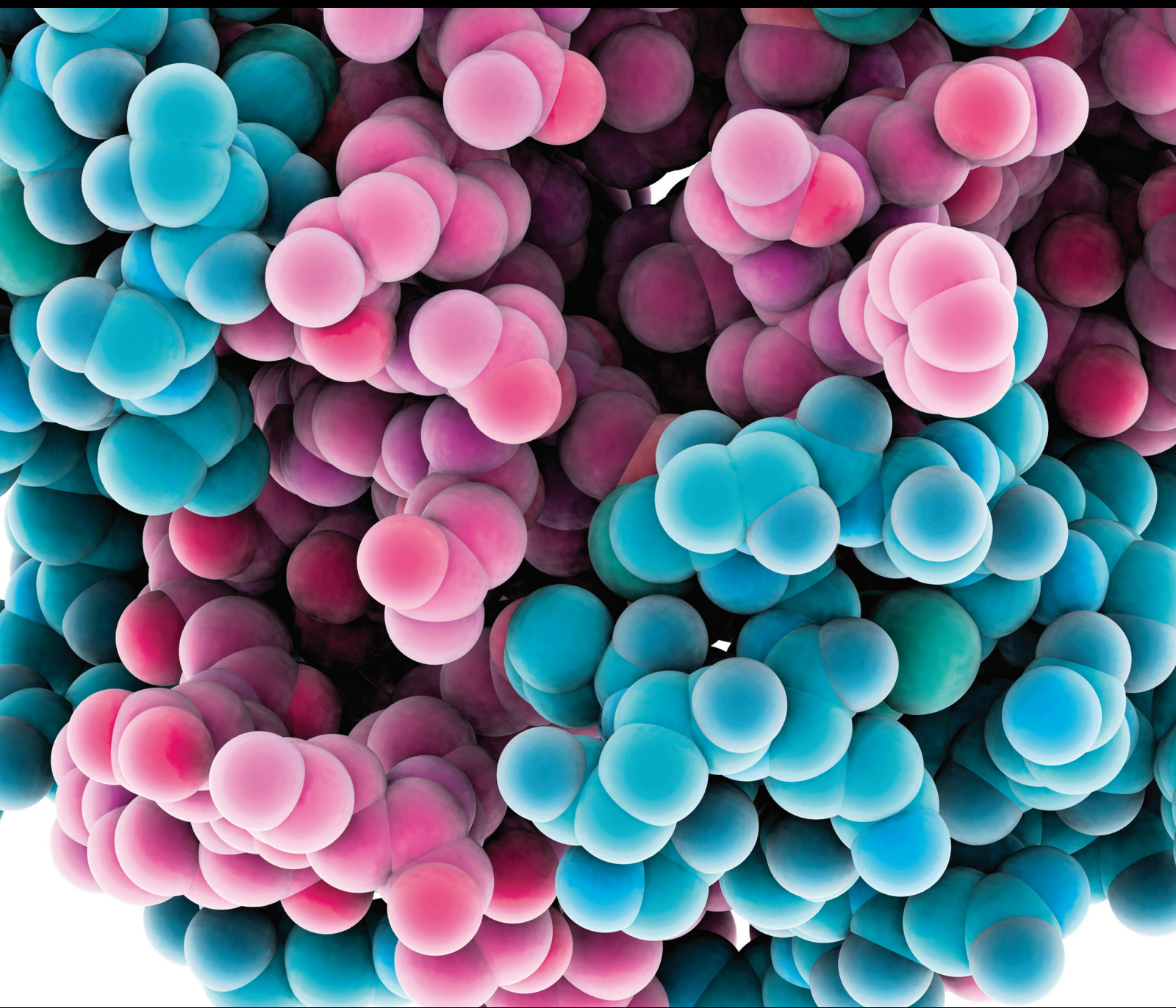
Journal of Diabetes Research

Diabetes Prevention, Early Intervention, and Nondrug Therapy

Lead Guest Editor: Ruozhi Zhao
Guest Editors: Amy L. Hui, Xinghai Yao, Chi Zhang, and Feixia Shen



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Editorial Board

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 5184250, 2 pages
https://doi.org/10.1155/2017/5184250

Editorial

Diabetes Prevention, Early Intervention, and Nondrug Therapy

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Received 24 July 2017; Accepted 26 July 2017; Published 6 December 2017

The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014; the global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes represents significant public health issue with potentially great financial burden of the government, families, and individuals; research addressing the prevention, early intervention, and nondrug therapy is urgently needed. In this special issue, we selected multiple original articles and two reviews which are aiming to explore diabetes prevention, early intervention, and nondrug therapy from clinical and basic research aspects.

Patients with prediabetes are at high risk for diabetes and cardiovascular disease (CVD). No study has explored whether the intervention could revert prediabetes to normal glycemic status as the primary outcome. Y. Luo et al. applied Beijing Prediabetes Reversion Program (BPRP) and evaluated whether intensive lifestyle modification and/or pioglitazone could revert prediabetic state to normoglycemia and improve the risk factors for CVD as well. Between March 2007 and March 2011, 1945 participants were randomized. At baseline, the individuals were 53 ± 10 years old, with median body mass index (BMI) 26.0 kg/m^2 (23.9 and 28.2 kg/m^2) and glycosylated hemoglobin (HbA1c) 5.8% (5.6 and 6.1%). 85% of the participants were impaired glucose tolerance (IGT), and 15% were impaired fasting glucose (IFG). Parameters relevant to glucose, lipids, blood pressure, lifestyle, and other metabolic markers were similar between conventional and intensive lifestyle intervention group at baseline. BPRP was the first study to determine if lifestyle modification and/or pioglitazone could revert prediabetic state to normoglycemia in Chinese population. Major baseline parameters were balanced between two lifestyle intervention groups. Z. Li et al. investigated the knowledge-attitude-practice (KAP) score in diabetes patients living in urban China regarding medical nutrition therapy (MNT) and explored the influencing factors. This national survey recruited diabetes and prediabetes patients in 40 hospitals across 26 provinces in China. A total of 6441 diabetes patients (mean age: 60.02 ± 13.14 years) completed this survey. The mean HbA1c level was $8.12 \pm 2.12\%$, and the control rate of HbA1c (HbA1c < 7.0%) was 38.92%. Of the total, 53.56% had received MNT education. Over half of the patients had a poor total KAP score as well as poor K-, A-, and P scores. Patients with higher KAP scores had higher control rate of HbA1c ($P < 0.05$) but lower levels of fasting blood glucose (FpG) and 2-hour postprandial blood glucose (2hPG). Gender, occupation, residence, education level, and MNT education could influence the KAP scores ($P < 0.05$). This study showed that diabetes patients in urban China had poor understandings and practices related to MNT. Patients with higher KAP scores exhibited better control of blood glucose. J. Wang et al. assessed the effects of leisure-time physical activity on undetected prediabetes. A total of 8204 subjects were eligible for their analyses. For all subjects, high level of total leisure-time physical activity (OR = 0.78, 95% CI: 0.66–0.94) and low level of vigorous leisure-time physical activity (OR = 0.72, 95% CI: 0.58–0.90) were inversely associated with the risk of prediabetes

in multivariate-adjusted model. For subjects under 45 years of age, high level of total leisure-time physical activity (OR = 0.78, 95% CI: 0.61–0.99) and low (OR = 0.61, 95% CI: 0.45–0.83) and high (OR = 0.72, 95% CI: 0.53–1.00) level of vigorous leisure-time physical activity were associated with a decreased risk of prediabetes. In the 45 to 65 age group, only high level of total leisure-time physical activity (OR = 0.73, 95% CI: 0.57–0.95) had a protective effect on prediabetes. Fulminant type 1 diabetes (FT1D) is considered to be an extremely rapidly progressing disease; previous literature reports that FT1D is a distinct subtype within type 1 diabetes (T1D) and most of the patients are adults. Y. Gu et al.'s multicenter study and cohort design involved sixteen hospitals; the total patient's number is 1470 from hospitalized newly onset T1D from Jan 2004 to Dec 2012. They found that FT1D onset age is much younger than that of classical T1D patients. The hospital-based incidence of FT1D in Chinese children was 1.56% in all new-onset T1D. In the aspects of treatment and prognosis, there was no significant difference between FT1D and classical T1D. Nailfold capillaroscopy is an easy and noninvasive technique used to investigate dermal microvasculature. S. Uyar et al. applied nailfold capillaroscopy and fundoscopic examinations in 216 patients with type 2 diabetes mellitus (T2DM) and 101 healthy control group, evaluated nailfold capillaries in T2DM patients, and determined the association of retinopathy with changes in the nailfold capillaries. They showed that retinopathy was detected 43.05% of diabetic patients ($n = 93$). In logistic regression analysis, tortuosity was shown significant (OR: 2.16; $p = 0.036$). There was also a significant relation between diabetes duration and most of the capillaroscopic findings. They suggest that capillarospcopic imaging could be a useful new technique for assessment of diabetic microvascular changes. Damage to small nerve fibers may develop in the early course of diabetes and can be assessed by sudomotor function testing. SUDOSCAN (Impeto, France) is a recently developed sudomotor function test of the electrochemical skin conductance (ESC) of the hands and feet, which has been used widely in early diagnosis of symmetrical diabetic neuropathy. SUDOSCAN can also be used for the efficient screening of cardiac autonomic neuropathy (CAN) using its proprietary cardiovascular autonomic neuropathy risk score (CAN-RS). D. Wang et al. reported that CAN-RS, a cardiac autonomic nerve dysfunction index calculated by SUDOSCAN, may be a promising index for the lens and vitreous abnormality screening in T2DM patients. T. He et al. demonstrated that ESC measurement is a reliable and feasible method to screen diabetic CAN in the Chinese population with diabetes before further diagnosis with cardiovascular autonomic reflex tests (CARTs). ANGPTL8 (angiopoietin-like protein 8) is a novel protein that primarily expressed in liver and fat. ANGPTL8 plays a role in regulating lipid metabolism in mice and in vitro tests. Y. Yin et al. reported that serum ANGPTL8 concentrations were significantly increased in IGR (impaired glucose regulation) and T2DM. Serum ANGPTL8 might play a role in the pathological mechanism of glucose intolerance. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) polymorphisms serve as the genetic basis of hepatic steatosis (HS) in a normal population and lead to dysregulated glucose metabolism. Q. Pan et al. suggested that PNPLA3 rs1010023 may predispose chronic hepatitis B (CHB) patients with HS, but protects them from glucose dysregulation by attenuating the insulin resistance.

In a review article, Y. Zhao and H. Xing reviewed the relationship between diabetes mellitus (DM) and liver diseases from a relatively comprehensive perspective, including chronic hepatitis, cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation (LT). The liver plays an important role in the regulation of glucose homeostasis. Viral liver diseases due to the presence of liver injury are risk factors of DM. Uncontrolled liver injury or glycemic level would accelerate the progression of DM, resulting in the survival of patients shortened. Treatment of diabetes may be difficult due to liver insufficiency and hepatotoxicity of antidiabetic drugs. Treatment of liver diseases would relatively improve the prognosis of the DM patients. Mesenchymal stem cells (MSCs), an ideal cell source for regenerative therapy with no ethical issues, play an important role in diabetic foot ulcer (DFU). Growing evidence has demonstrated that MSC transplantation can accelerate wound closure, ameliorate clinical parameters, and avoid amputation. Y. Cao et al. reviewed the mechanism of preclinical studies, as well as safety and efficacy of clinical trials in the treatment of DFU. Bone marrow-derived mesenchymal stem cells (BM-MSCs), compared with MSCs derived from other tissues, may be a suitable cell type that can provide easy, effective, and cost-efficient transplantation to treat DFU and protect patients from amputation.

The articles in this issue provide some new ideas for diabetes prevention, early intervention, and nondrug therapy. It is our hope that our readers will enjoy these articles and assist them in their future research.

Acknowledgments

We are very grateful to all the authors and reviewers. As guest editors, we are honored to share this discussion with you.

Ruozhi Zhao
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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 6924637, 6 pages
https://doi.org/10.1155/2017/6924637

Research Article

Fulminant Type 1 Diabetes in Children: A Multicenter Study in China

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Received 30 December 2016; Revised 18 April 2017; Accepted 17 May 2017; Published 26 September 2017

Academic Editor: Ruozhi Zhao

Background. To investigate the hospital-based incidence of FT1D in Chinese children and compare the clinical feature with classical T1DM. *Methods*. A cross-sectional study with sixteen hospitals involved. We obtained 23 FT1D cases as group 1, acute-onset T1DM as group 2, and typical T1DM as group 3. *Results*. The incidence of FT1D was 1.56% in 16 participating hospitals. The mean age at the onset of group 1 was 2.00 (1.08, 6.51) years old, much younger than that of group 2 (6.11 (3.92, 9.50)) and group 3 (6.92 (4.17, 10.03)). In addition, significant differences were found between three groups: mean BMI and flu-like symptoms with fever and abdominal pain. Follow-up comparison of three groups from Beijing Children's Hospital for at least one year showed that there is no significant difference between the three groups in terms of mean HbA1c levels and insulin injection dosages. *Conclusion*. FT1D onset age is much younger than that of classical T1D patients. The hospital-based incidence of FT1D in Chinese children was 1.56% in all new-onset T1DM. For the diagnosis, making FT1D alone into a subtype within type 1 diabetes may be meaningful. However, for the treatment and prognosis, such classification should not be helpful to the clinic.

1. Background

Fulminant type 1 diabetes (FT1D) was first reported by Imagawa et al. in 2000 [1]. Most reported cases are from East Asia and occur during pregnancy or just after delivery [1, 2]. This type of diabetes is defined as a subtype of type 1 diabetes (T1DM) because it is considered as having a different mechanism from classical T1DM [1, 3, 4]. Since the recognition of FT1D, It had been reported in Korean [5–8], Chinese [9], French [10], and US Hispanic patients [11]. However, there are no data on children (below 15 years old). To clarify the more detailed clinical characteristics and difference between FT1D and classical T1DM in children in China, we performed a multicenter study with 16 hospitals.

2. Methods

2.1. Patients. It is a cross-sectional study with a multicenter design including sixteen hospitals. But in terms of Beijing Children's Hospital, it should be a cohort study. All patients were diagnosed within January 2004 to December 2012. One dedicated doctor was in charge of reviewing all the data and 2 other doctors in auditing according to the same criterion. Finally, we got effective data from nine hospitals. Seven cases (30.43%) and 16 (69.57%) cases were from south and north, respectively (see Figure 1).

Groupings: group 1 included 23 fulminant type 1 diabetes cases. The clinical characteristics of FT1D were [10] (1) remarkably abrupt onset; (2) very short (<1 week) duration of diabetic symptoms (thirst, weight loss, and polyuria); (3) occurrence of diabetic ketosis or ketoacidosis soon (approximately 7 days) after the onset of hyperglycemic symptoms (elevation of urinary and/or serum ketone bodies at first visit); (4) plasma glucose level ≧ 16.0 mmol/L (‡288 mg/dL) and HbA1c < 8.5% (Japan Diabetes Society value) at first visit; and (5) urinary C-peptide excretion < 10 μg/d or fasting serum C-peptide level < 0.3 ng/mL (<0.10 nmol/L) and <0.5 ng/mL (<0.17 nmol/L) after intravenous glucagon (or after meal) load at onset. Other findings in FT1D were (1) flu-like symptoms (fever, upper respiratory symptoms, etc.) or gastrointestinal symptoms (upper abdominal pain, nausea and/or vomiting, etc.). The second group consisted of 182 acute-onset type 1 diabetes cases where (1) patients meet the criteria of the International Diabetes Federation (IDF) and International Society of Pediatric and Adolescent Diabetes (ISPAD) for type 1 diabetes; (2) there is presence of ketosis or ketoacidosis at the onset of diabetes; and (3) the onset of diabetic symptoms was less than 30 days. Group 3 consists of 879 typical type 1 diabetes cases who had diabetic symptoms within 30–100 days. The study program was approved by the ethical committee of the Beijing Children's Hospital.

2.2. Index of Clinical Characteristics and Biochemical Analysis. Data at admission clinically included onset age, sex, hyperglycemic symptom duration, family history of diabetes in first-degree relatives, influenza-like symptoms, blood pressure, and body mass index. Laboratory tests included blood glucose, glycosylated hemoglobin (HbA1c), arterial pH, bicarbonate, β-hydroxybutyric acid, electrolytes, aspartate aminotransferase, alanine aminotransferase, total cholesterol, and triglyceride. Glutamic acid decarboxylase antibodies (GADAb), insulin autoantibodies (IAA), and islet cell antibodies (ICA) in serum samples were determined with the enzyme-linked immunosorbent assay method. And fasting plasma C-peptide and 2-h postprandial C-peptide levels were determined using the electrochemiluminescence immunoassay method after the resolution of diabetic ketoacidosis.

Some data were missing in other 7 hospitals; we followed up a comparison of three groups of Beijing Children's Hospital.

2.3. Statistical Analysis. Use SPSS17.0 software. Analysis of variance or Kruskal–Wallis H test was used. Group comparisons were done by using least significant difference test. Frequency comparisons were done by using Fisher's exact test. All continuous variables with a normal distribution are expressed as means ± standard deviation. All tests were two-sided, and a $P < 0.05$ was required for statistical significance.

3. Results

3.1. General Information. There were 23 patients diagnosed with FT1D since 2004–2012, 9 males and 14 females. The incidence of FT1D was 1.56%. Mean age at the onset of group 1 was 2.00 (1.08, 6.51) years old, which was much younger than that of group 2 at 6.11 (3.92, 9.50) years old and group 3 at 6.92 (4.17, 10.03) years old. Significant differences were found between three groups in the mean BMI 16.12 (14.51, 19.55) versus 15.01 (13.54, 17.29) and 14.87 (13.61, 16.64). Abdominal pain was observed in twelve patients (52.2%), much more than that in group 2 (17.6%) and group 3 (7.5%) (see Table 1).

3.2. Biochemical Analysis. Mean plasma glucose in group 1 was higher (25.10 (20.35, 30.00)) than that in group 2 (22.99 (17.99, 30.89)) and group 3 (21.78 (15.70, 28.96)). Similar results were found in triglycerides: 1.24 (0.86, 1.59) versus 1.45 (0.90, 2.52) and 1.10 (0.75, 1.83). There was also significantly lower arterial blood pH and lower plasma bicarbonate concentrations ($P = 0.001$) (see Table 1).

For incidence of acute complications of the three groups, a significant difference was found in group 1 in low T3 syndrome, higher than the other two groups ($P = 0.018$). Other acute complications such as serious DKA, HHS, rhabdomyolysis, myocardial damage, and encephaledema were not observed as different between the three groups (see Table 2).

3.3. Follow-Up Results. Follow-up comparison of three groups from Beijing Children's Hospital showed that there is no significant difference in these three groups neither in mean HbA1c levels nor in insulin injection dosages. Particularly, three groups of patients used minimal insulin injection dosages to maintain plasma glucose level in honeymoon period (see Table 3).

4. Discussion

The prevalence of FT1D worldwide is different. 19.4% of acute-onset type 1 diabetes was revealed in Japan [12] and 7.1% of newly diagnosed type 1 diabetic patients in Korea [6]. Luo et al. [13] reported 53 cases of FT1D from 24 hospitals nationwide in China, and the percentage of FT1D was 14.9%. However, the patients in the above research were older children and adults. The minimum age of patients enrolled in those researches was 12 years old. The mean age at onset was 35 years in females and 43 years in males; 91.3% of patients were adults and pregnancy is associated with female fulminant type 1 diabetes [10]. In this study, we collected 23 FT1D patients younger than 15 years who were children from different provinces of China. The

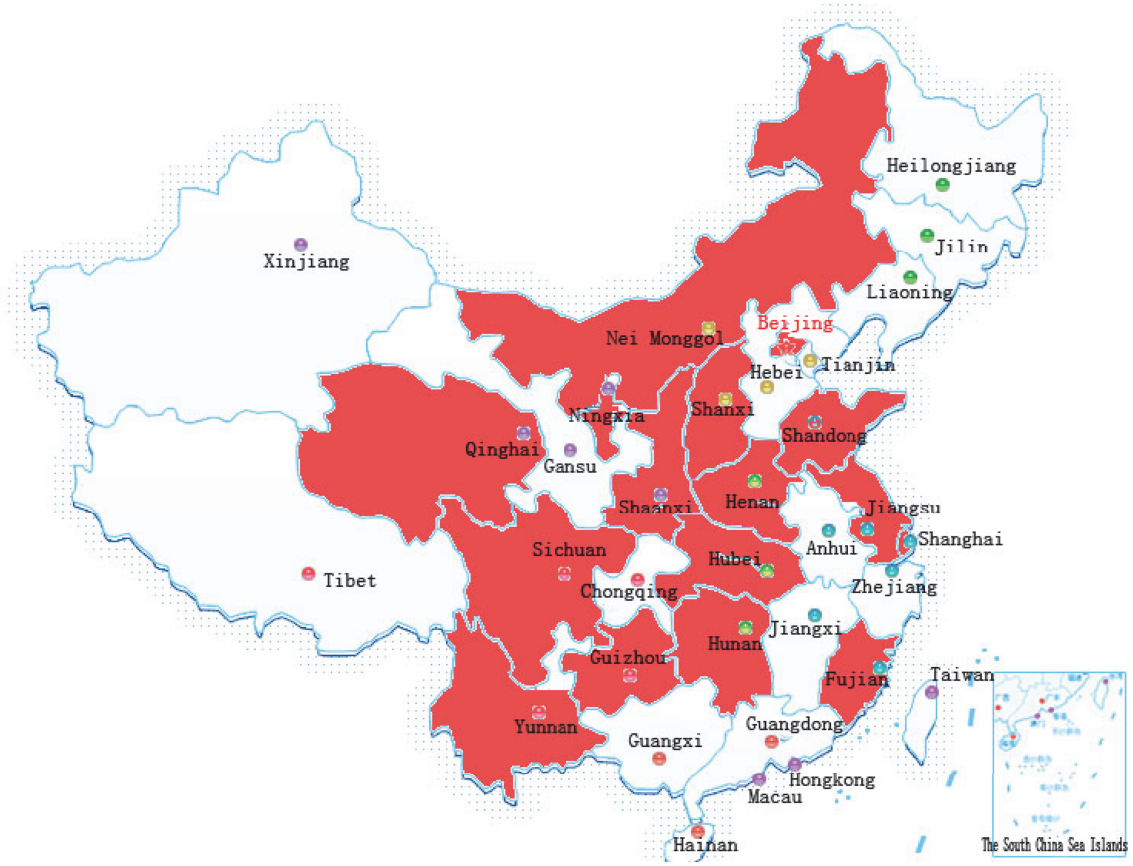

Figure 1: The location of each hospital of this study in China.

incidence of FT1D was 1.56% among all newly T1DM patients, much lower than in adults reported, but it is similar to those Korean studies which reported that the frequency of FT1D was 1.33% under the age of 16 years [8]. The mean age at onset was 2 years (1.08, 6.51). It is quite younger than that in adults, suggesting that the disease occurs in younger age groups of children.

FT1D patients displayed a more diverse clinical manifestation, including flu-like symptoms and gastrointestinal discomforts. In our study, flu-like symptoms and fever were observed in seventeen cases (73.9%), much more than acute-onset type 1 diabetes group (10.4%) and typical type 1 diabetes (4.5%). Also, abdominal pain was observed in twelve patients (52.2%), much more than acute-onset type 1 diabetes group (17.6%) and typical type 1 diabetes group (7.5%). These features are similar to those reported in Koreans and Japanese [6, 14]. Specially, FT1D and acute-onset type 1 diabetes are both acute-onset diseases, but FT1D with flu-like symptoms and abdominal pain was much more frequent than acute-onset T1D. This result indicated that the contribution of viral infection is important to predispose or trigger FT1D. Otherwise, this also seems to explain why the disease occurs in younger age groups of children.

Pathogenesis of FT1D is not clear. Early studies indicated no evidence of islet autoimmunity in FT1D, while recent findings have increasingly suggested that islet autoimmunity is involved in its development [15–17]. Autoantibodies against the β-cell antigens, such as GAD (two patients), insulin cell autoantibodies (one patient), and islet cell antoantibodies (one patient), were found in these twenty-three FT1D patients. This result accounts for 20% positive and also has a similar literature report [18–20]. It was given that T-cell-mediated autoimmunity played the destruction role of the pancreatic β-cells [21, 22]. It is possible that β-cell-specific Th1 immunity, together with low-grade humoral immune responses, further disposes patients to the development of FT1D. In addition, genetic factors also play a role in the pathogenesis. The HLA class II genes, especially HLA-DQ and HLA-DR, have been associated with a high susceptibility of individuals to autoimmune type 1 diabetes [22]. The DR4-DQ4 genes are also associated with the development of FT1D in Japan, and higher frequencies of HLA-DRB1*0405-DQB1*0401 or HLA-DQA1*0303-DQB1*0401 and HLA-DQA1*0302-DQB1*0303 haplotypes are observed in Japanese fulminant patients [23–27].

Recently, hypothesis of FT1D is thought to be a coefficient of viral infection and genetic factors [28]. Viral infections will accelerate antiviral immune reactions of CTLA-4, which induce β-cell death [3]. Moreover, environmental insults such as viral infections are related to alter immune responses in periphery and around the islet [29]. Cytokines such as interleukin and tumor necrosis factor-α recruit additional T cells, macrophages, and NK cells to the islets, and their signaling transductions have direct cytotoxic effects on

Table 1: Clinical features and laboratory tests of the three groups.

	F1TD (9/14)	Acute onset T1DM (83/99)	Classical T1DM (375/504)	F	P
Duration of hyperglycemia (days)	7 (2, 10)	7 (5, 8)	15 (10, 25)	234.736	0.000#
Age (year)	2.00 (1.08, 6.51)	6.11 (3.92, 9.50)	6.92 (4.17, 10.03)	14.048	0.001#
BMI (kg/m^2)	16.12 (14.51, 19.55)	15.01 (13.54, 17.29)	14.87 (13.61, 16.64)	4.621	0.099
Family history	0	12	32	—	—
Flu-like symptoms	17	19	40	57.702	0.000#
Abdominal pain	12	32	66	29.12	0.000#
Plasma glucose (mmol/L)	25.10 (20.35, 30.00)	22.99 (17.99, 30.89)	21.78 (15.70, 28.96)	9.765	0.008#
HbA1C (%)	7.2 ± 1.2	11.7 ± 2.1	11.8 ± 2.4	43.909	0.000#
Peptide C (ng/mL)	0.21 (0.09, 0.29)	0.31 (0.11, 0.54)	0.34 (0.13, 0.58)	8.464	0.015#
Diastolic blood pressure (mmHg)	60 (60, 75)	60 (60, 70)	60 (60, 70)	0.619	0.734
Systolic pressure (mmHg)	90 (70, 103)	100 (90, 110)	100 (90, 110)	5.026	0.081
pH	7.23 (6.98, 7.34)	7.28 (7.14, 7.38)	7.32 (7.19, 7.40)	14.346	0.001#
HCO_3 (mmol/L)	8.20 (3.10, 16.85)	13.10 (7.85, 18.90)	16.30 (8.90, 22.10)	16.657	0.000#
k (mmol/L)	4.41 (4.00, 4.91)	4.10 (3.74, 4.60)	4.12 (3.74, 4.51)	3.792	0.150
Na (mmol/L)	135.6 (131.0, 138.0)	135.0 (131.1, 136.9)	135.0 (131.6, 138.0)	2.602	0.272
Cl (mmol/L)	105.0 (98.0, 107.0)	103.2 (100.0, 107.5)	103.2 (99.2, 106.8)	0.27	0.874
Plasma osmolal pressure (Mosm/L)	284.8 (276.7, 293.1)	288.8 (281.3, 297.0)	289.4 (282.8, 296.6)	2.817	0.244
BUN (mmol/L)	4.20 (2.70, 5.84)	4.48 (3.39, 6.17)	4.60 (3.50, 5.88)	1.437	0.488
CHO (mmol/L)	3.59 (3.24, 5.05)	3.99 (3.32, 4.98)	4.12 (3.44, 4.90)	1.015	0.602
TG (mmol/L)	1.24 (0.86, 1.59)	1.45 (0.90, 2.52)	1.10 (0.75, 1.83)	14.545	0.001#
AST (U/L)	30 (22, 33)	21 (15, 27)	22 (18, 29)	8.159	0.017#
ALT (U/L)	16 (12, 23)	15 (12, 18)	15 (12, 20)	0.808	0.668
Insulin dosage of ketoacidosis treatment (IU)	11.93 (7.42, 31.35)	13.20 (8.40, 25.89)	14.43 (9.26, 28.00)	1.333	0.513
Time of ketoacidosis treatment (hour)	15.0 (4.5, 21.5)	9.3 (6.0, 15.6)	10.0 (6.0, 16.5)	1.043	0.594

The statistical method used was analysis of variance. # represents $P < 0.05$.

Table 2: Acute complications of three groups.

	Serious DKA	HHS	Low T3 syndrome	Rhabdomyolysis	Myocardial damage	Encephaledema
Group 1	4	0	4	0	1	0
Group 2	19	1	10	0	3	0
Group 3	76	2	89	0	30	0
x^2	5.065	0	8.06	—	1.986	—
P	0.079	—	0.018#	—	0.371	—

#There exist differences between group 1 and group 2 and between group 1 and group 3.

Table 3: Follow-up comparison of three groups from Beijing Children's Hospital.

	Course of disease: one month		Course of disease: six months		Course of disease: one year	
	HbA1C (%)	Insulin dosage (IU/kg/d)	HbA1C (%)	Insulin dosage (IU/kg/d)	HbA1C (%)	Insulin dosage (IU/kg/d)
Group 1 ($n = 5$)	7.9 ± 1.2	0.61 ± 0.19	7.1 ± 1.0	0.58 ± 0.12	7.5 ± 1.1	0.63 ± 0.13
Group 2 ($n = 25$)	8.2 ± 1.2	0.51 ± 0.32	7.8 ± 2.1	0.58 ± 0.31	7.5 ± 1.6	0.49 ± 0.31
Group 3 ($n = 65$)	8.3 ± 1.4	0.59 ± 0.35	7.3 ± 1.6	0.58 ± 0.33	7.6 ± 1.7	0.58 ± 0.35
x^2	0.170	0.555	0.889	0.000	0.013	0.726
P	0.844	0.576	0.415	1.000	0.987	0.487

β-cells. The discovery of infiltrating around and in the islet indicates that FT1D may experience a similar pathogenic process as classic type 1 diabetes [30]. In addition to this, Aida et al. [31] studied the in situ status of innate and adaptive immunity of enterovirus-induced FT1D. RIG-I was strongly expressed in β-cells in pancreas infected with enterovirus. T lymphocyte receptors (TLR3) were expressed in mononuclear cells that infiltrated islets. Interferon-α (IFN-α) and IFN-β were strongly expressed in islet cells. Major histocompatibility complex (MHC) class I, IFN-γ, interleukin-18, and Cytotoxic C motif ligand 10 were expressed and colocalized in affected islets. Serum levels of IFN-γ were markedly increased in patients with T lymphocyte receptor [32]. These findings demonstrate the presence of specific innate immune responses to enterovirus infection in T lymphocyte receptor. Therefore, the diagnosis of idiopathic diabetes in FT1D is still to be determined. More discussion and accumulation of cases are essential to conclude whether autoimmunity is involved in FT1D.

FT1D patients had significantly lower arterial blood pH and lower plasma bicarbonate concentrations. Meantime, the serum triglyceride was higher than that in typical type 1 diabetes but not seen in other groups. Otherwise, there is no obvious difference of three groups in acute complications. All these results indicate that FT1D patients display a more diverse clinical manifestation. Significant higher mean BMI was found in FT1D. This related higher BMI phenomenon may be associated with short duration of body weight loss.

There is no significance different in these three groups from Beijing Children's Hospital neither in mean HbA1c levels nor in insulin injection dosages when followed up. Particularly, there is almost the same minimal insulin dosage per day to maintain plasma glucose level in honeymoon period. Therefore, FT1D are similar as acute-onset diabetes and typical type 1 diabetes in insulin treatment and prognosis in children. Indeed, there are some special cases reported which presented another profile, such as Yamashita et al. [33] who reported a woman after acute pancreatitis and FT1D developed simultaneously. She experienced transient complete remission of diabetes and eventually had mild diabetes with non-insulin-dependency and impaired insulin secretion.

In conclusion, this study showed that the incidence of FT1D below 15 years old was very low, the incidence was 1.56%, and the age of FT1D onset in childhood is much younger. Considering the incidence of this disease and no significant difference of FT1D and type 1 diabetes (mean HbA1c levels, injection dosages, and minimal insulin injection dosages to maintain plasma glucose level in honeymoon period), we elicit that for the diagnosis, making FT1D alone into a subtype within type 1 diabetes may be meaningful. However, for the treatment and prognosis, such classification should not be helpful to the clinic, while for the limitation of a small size in following up, more follow-up work should be done.

Disclosure

Yi Gu, Yi Wang, Pin Li, Haiyan Wei, Linqi Chen, Qianqi Liu, Yu Liu, Qiaozhi Yang, Xinran Cheng, and Lanjie He are co-first authors. Liya Wei, Zhiying Zhu, Yongxing Chen, Fengyun Wang, Xing Shi, Yuxian Cheng, Yan Wei, and Jianing Yu are co-second authors.

Conflicts of Interest

The authors have no conflict of interest in this study.

Authors' Contributions

Yi Gu, Yi Wang, Pin Li, Haiyan Wei, Linqi Chen, Qianqi Liu, Yu Liu, Qiaozhi Yang, Xinran Cheng, and Lanjie He contributed equally to this work. Liya Wei, Zhiying Zhu, Yongxing Chen, Fengyun Wang, Xing Shi, Yuxian Cheng, Yan Wei, and Jianing Yu contributed equally to this work.

Acknowledgments

The authors are grateful to the following pediatrics departments for providing patients with fulminant type 1 diabetes: The Second Xiangya Hospital of Central South University, Inner Monglia Medical University affiliated hospital, First Affiliated Hospital of Kunming Medical University, Xi'an Children's Hospital, Qinghai Woman's and Children's Hospital, Shanxi Children's Hospital, Fuzhou Children's Hospital, and Wuhan Woman and Children Medical Center. This research was supported by two funds: The National Key Research and Development Program of China (the prevalence of diabetes in and nutritional affects on children and adolescents) (no. 2016YFC1305304) and multi-qualified doctors training based on diabetes education and data platform construction (no. 2015400688764J010).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 8293207, 7 pages
https://doi.org/10.1155/2017/8293207

Research Article

Increased Serum ANGPTL8 Concentrations in Patients with Prediabetes and Type 2 Diabetes

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Received 31 December 2016; Accepted 16 May 2017; Published 7 September 2017

Academic Editor: Ruozhi Zhao

The objectives of the study were to investigate serum ANGPTL8 concentrations in different glucose metabolic statuses and to explore the correlations between serum ANGPTL8 levels and various metabolic parameters. Serum ANGPTL8 levels were determined using ELISA in 22 subjects with NGT (normal glucose tolerance), 74 subjects with IGR (impaired glucose regulation), and 33 subjects with T2DM (type 2 diabetes mellitus). Subjects with IFG, IGT, CGI, and T2DM had higher levels of serum ANGPTL8 than subjects with NGT. Serum ANGPTL8 was positively correlated with FPG, fasting C-peptide, and postprandial C-peptide and negatively correlated with BETA/IR when adjusted for age and BMI. Multivariate analysis suggested FPG and fasting C-peptide as independent factors associated with serum ANGPTL8 levels. Serum ANGPTL8 concentrations were significantly increased in IGR and T2DM. Serum ANGPTL8 might play a role in the pathological mechanism of glucose intolerance.

1. Introduction

ANGPTL8 (angiopoietin-like protein 8), also called TD26, RIFL (refeeding-induced fat and liver), lipasin, betatrophin, and C19orf80 (chromosome 19 open reading frame 80), is a novel protein that is primarily expressed in the liver and fat. ANGPTL8 plays a role in regulating lipid metabolism in mice and in vitro tests. Ren et al. showed that RIFL-null mice had lower serum triglyceride levels than the wild-type mice [1]. In Quagliarini et al.'s study, plasma TG (triglyceride) level did not change in mice expressing ANGPTL3 alone, whereas coexpression with ANGPTL8 resulted in hypertriglyceridemia, despite a reduction in circulating ANGPTL3 [2]. Zhang reported that obesity increases liver lipasin, whereas fasting reduces its expression in fat. Lipasin overexpression in mice increases serum triglyceride levels [3]. Despite its role in regulating lipid metabolism, whether ANGPTL8 is related to glucose metabolism was controversial. Elevated circulating ANGPTL8 levels were found in subjects with DM in Espes et al.'s reports [4–6], while in Gómez-Ambrosi et al.'s, ANGPTL8 levels declined [7, 8].

To address this question, the study evaluated the association of ANGPTL8 with different glucose metabolic statuses, including IFG (isolated impaired fasting glucose), IGT (isolated impaired glucose tolerance), and CGI (combined glucose intolerance). We demonstrated a significantly elevated serum ANGPTL8 level in IGR subjects, suggesting that ANGPTL8 might play a role before developing into diabetes mellitus.

2. Materials and Methods

2.1. Study Subjects. This study was part of an epidemiologic study of diabetes, thyroid disease, and osteoporosis, which took place in the countryside of Sijing, Shanghai, China. From July 2012 to March 2013, 6184 subjects participated in the survey. We made a follow-up in 494 subjects from

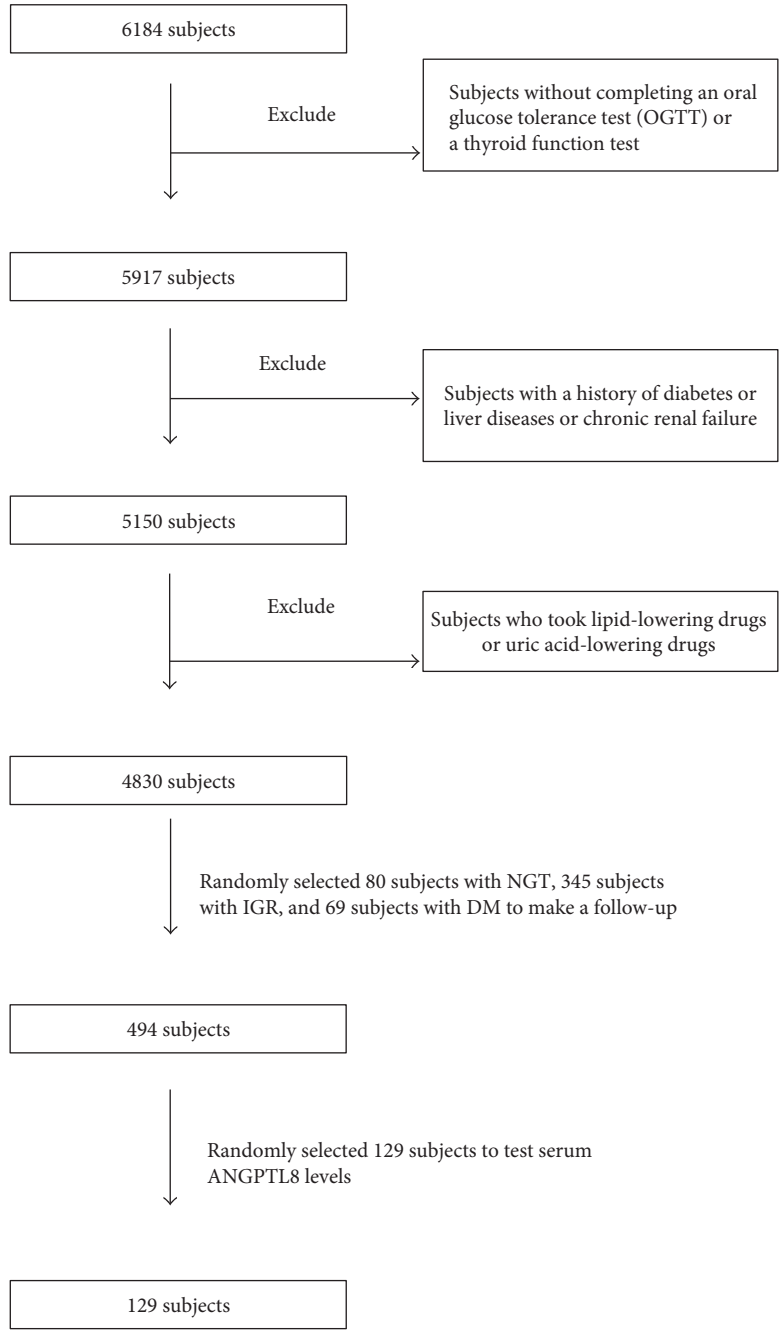

Figure 1: Process of exclusion and inclusion.

October to November in 2014. The 129 subjects which were randomly selected including 22 subjects with NGT, 74 subjects with IGR (30 with IFG, 32 with IGT, and 12 with CGI), and 33 subjects with T2DM (type 2 diabetes mellitus) were included to examine serum ANGPTL8 levels. The diagnosis of T2DM and prediabetes was based on the criteria in the American Diabetes Association's 2003 guidelines. None had gestational diabetes, active hepatitis/liver cirrhosis, chronic renal failure on hemodialysis, congestive heart failure, or other known major diseases. Subjects who were treated with lipid-lowering drugs or uric acid-lowering drugs were also excluded to avoid the possible confounding effects of medications (Figure 1). The study was approved by the medical ethics committee of Shanghai General Hospital. All participants gave written informed consent to participate in this research.

2.2. Anthropometric and Biochemical Measurements. Blood samples were collected after at least 10 h of overnight fasting. Participants with no history of diabetes were given a standard 75 g glucose solution, whereas for safety reasons, participants with T2DM were given a steamed bun that contained approximately 80 g of complex carbohydrates. Blood samples were drawn zero, and 120 min after, the glucose or

carbohydrate load was ingested to measure glucose concentrations. Plasma glucose, cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were assessed enzymatically by an automatic biochemistry (HITACHI 7600) analyzer with WOKO reagent (SANWA INTL CO.). Glycosylated hemoglobin (HbA1c) was detected by high-performance liquid chromatography (Hemoglobin Analyzer D-10; Bio-Rad Laboratories Inc., Shanghai, China). Fasting serum insulin (FINS), postprandial serum insulin (PINS), fasting C-peptide, and postprandial C-peptide were tested using an electrochemiluminescence analyzer (Roche Cobas e170). Blood pressure (BP) was measured three times while the subject sat calmly. Weight (without shoes and any heavy clothing) and height were measured. Waist circumferences were measured at the narrowest point between the lowest rib and the uppermost lateral border of the iliac crest, and the hips were measured at their widest point. Serum ANGPTL8 concentrations were determined using a validated ELISA kit (Phoenix Pharmaceuticals, Phoenix, USA; catalog number EK-051-55) with intra- and interassay coefficients of variation being <10% and <15%, respectively.

2.3. Definitions. Systolic BP (SBP) and diastolic BP (DBP) were calculated as the mean of the three measurements. Body mass index (BMI) was calculated using the following formula: weight (kilograms)/height squared (meters squared). The waist-to-hip ratio (WHR) was calculated using the following formula: waist circumferences/hip circumferences. The homoeostasis model assessment of insulin resistance (HOMA-IR) and the homoeostasis model assessment of beta cell function index (HOMA-BETA) were calculated using the following formulas: HOMA-IR = fasting plasma glucose (FPG) (mM) × insulin (mIU/L)/22.5; HOMA-BETA = 20 × insulin/(FPG − 3.5); BETA/IR = HOMA-BETA/HOMA-IR; $\Delta I_{120}/\Delta G_{120}$ = (PINS − FINS)/(PPG − FPG); and $\Delta I_{120}/\Delta G_{120}$/IR = (PINS − FINS)/(PPG − FPG)/HOMA-IR. According to the 2003ADA recommendations for the diagnosis of diabetes, normal glucose tolerance (NGT) was defined as FPG < 5.6 mM and postprandial plasma glucose (PPG) < 7.8 mM; IFG was defined as 5.6 mM ≤ FPG < 7.0 mM and PPG < 7.8 mM; IGT was defined as FPG < 5.6 mM and 7.8 mM ≤ PPG < 11.1 mM; CGI was defined as 5.6 mM ≤ FPG < 7.0 mM and 7.8 mM ≤ PPG < 11.1 mM; and DM was defined as FPG ≥ 7.0 mM or PPG ≥ 11.1 mM.

2.4. Statistical Analyses. Statistical analysis was performed using SPSS 21.0. Continuous data with a normal distribution were expressed as the means ± SD, and data with a skewed distribution were expressed as medians (interquartile range). One-way ANOVA was used for the normally distributed data, and the Kruskal-Wallis test was used for the nonnormally distributed data or data with different variances. χ^2 test was used to compare the gender differences among the groups. Pearson's correlation was used for the normally distributed data, and Spearman's correlation was used for the nonnormally distributed data to examine the association among variables. Partial correlation was used for the adjustment of age and BMI. Multivariate regression analysis was to assess the associations between ANGPTL8 and the variables. All P values are two tailed. Significance was determined at $P < 005$.

3. Results

3.1. Characteristics of Subjects in NGR, IFG, IGT, CGI, and T2DM. There were no gender differences among different glucose metabolic statuses. Subjects with T2DM were significantly older than subjects with NGT. Subjects with CGI had higher levels of BMI, waist circumferences, WHR, FPG, PPG, FINS, PINS, fasting, and postprandial C-peptide, but lower BETA/IR than subjects with NGT. Subjects with IGT had higher levels of WHR, TG, PPG, and PINS than subjects with NGT. Subjects with IFG had higher levels of waist circumferences, WHR, TG, FPG, and HOMA-IR, but lower BETA/IR than subjects with NGT. Serum levels of ANGPTL8 were significantly higher in subjects with IFG, IGT, CGI, and T2DM when compared to subjects with NGT (1.07 ± 0.52 ng/mL, 0.92 ± 0.57 ng/mL, 1.23 ± 0.48 ng/mL, and 0.85 ± 0.67 ng/mL versus 0.38 ± 0.25 ng/mL, $P < 0.001$) (Table 1).

3.2. The Relationship between Serum ANGPTL8, Fasting C-peptide, and Metabolic Parameters. We investigated the relationship between serum ANGPTL8 levels and various parameters in all subjects. Serum ANGPTL8 correlated positively with waist circumferences, WHR, FPG, FINS, PINS, fasting C-peptide, postprandial C-peptide, and HOMA-IR, but negatively with BETA/IR. Serum ANGPTL8 still remained positively correlated with FPG, fasting C-peptide, and postprandial C-peptide and negatively correlated with BETA/IR after adjustment for age and BMI (Table 2). Multiple stepwise regression analysis was performed with serum ANGPTL8 as a dependent variable and various parameters including sex, age, BMI, blood pressure, blood lipid, HbA1C, FINS, PINS, fasting C-peptide, and postprandial C-peptide as the independent variables. The result showed that FPG and fasting C-peptide were independently related factors influencing serum ANGPTL8 levels (β for FPG = 0.072, $P = 0.040$; β for fasting C-peptide = 0.120, $P = 0.032$).

We then investigated the relationship between fasting C-peptide and various parameters in all subjects. Fasting C-peptide correlated positively with waist circumferences, TG, FPG, PPG, FINS, PINS, postprandial C-peptide, HOMA-IR, and HOMA-BETA, but negatively with BETA/IR and $\Delta I_{120}/\Delta G_{120}$. Fasting C-peptide still remained positively correlated with FPG, FINS, PINS, postprandial C-peptide, HOMA-IR, and HOMA-BETA and negatively correlated with BETA/IR after adjustments for age and BMI (Table 2).

Furthermore, we, respectively, investigated the relationship between ANGPTL8 levels and lipids in NGT, IGR, and T2DM, and we did not find any correlations between serum ANGPTL8 levels and lipids (Table 3).

4. Discussion

In the recent years, the association of circulating ANGPTL8 concentrations and the hyperglycemic state have become

Table 1: Characteristics of 129 subjects, including a statistical comparison of characteristics of subjects with NGR, IFG, IGT, CGI, and T2DM.

	NGT (22)	IFG (30)	IGT (32)	CGI (12)	T2DM (33)	Value	P	$P < 0.05$
Gender (M/F)	6/16	17/13	14/18	6/6	21/12	8.026	0.091	
Age (years)	54.41 ± 5.59	57.66 ± 5.50	58.09 ± 6.47	58.17 ± 8.33	60.18 ± 5.86	2.923	0.024	d
BMI (kg/m^2)	24.21 ± 3.17	25.44 ± 2.63	25.14 ± 3.18	27.20 ± 3.25	26.53 ± 3.36	2.802	0.029	c
Waist circumferences (cm)	79.89 ± 7.29	85.54 ± 7.10	84.25 ± 7.59	89.12 ± 9.16	87.58 ± 7.61	4.432	0.002	a, c, d
WHR	0.85 ± 0.05	0.90 ± 0.04	0.88 ± 0.04	0.91 ± 0.05	0.90 ± 0.04	3.927	0.005	a, b, c, d
SBP (mmHg)	127.00 ± 13.82	128.07 ± 13.55	130.06 ± 14.63	131.67 ± 13.06	132.79 ± 10.62	0.860	0.490	
DBP (mmHg)	80.82 ± 7.24	80.69 ± 5.91	82.12 ± 7.14	83.67 ± 6.25	84.18 ± 7.63	1.359	0.252	
TC (mmol/L)	1.09 (0.77–2.11)	1.95 ± 1.16	2.13 (1.38–3.05)	1.86 (1.20–3.33)	2.02 ± 0.99	7.540	0.110	
TG (mmol/L)	0.44 ± 0.17	0.65 (0.37–1.10)	0.67 (0.47–1.37)	0.65 (0.43–1.01)	0.67 (0.44–1.13)	12.782	0.012	a, b, d
LDL-C (mmol/L)	0.84 ± 0.59	0.92 ± 0.75	1.06 ± 0.69	1.20 ± 0.90	0.98 ± 0.58	0.714	0.584	
HDL-C (mmol/L)	0.53 ± 0.27	0.57 ± 0.38	0.68 ± 0.36	0.71 ± 0.53	0.65 ± 0.36	0.902	0.465	
HbA1C (mmol/L)	5.52 ± 0.31	5.58 ± 0.28	5.67 ± 0.34	5.71 ± 0.33	6.96 ± 1.66	14.084	<0.001	d, g, i, j
FPG (mmol/L)	4.92 ± 0.35	5.96 ± 0.27	5.17 (5.02–5.42)	6.09 (5.75–6.41)	7.40 (6.71–8.55)	97.295	<0.001	a, c, d, e, g, h, i, j
PPG (mmol/L)	6.72 (5.89–6.95)	6.23 ± 1.09	8.50 (8.12–9.80)	9.25 (8.21–9.79)	14.51 ± 4.26	105.764	<0.001	b, c, d, e, f, g, i, j
FINS (mIU/L)	1.80 (1.37–3.62)	3.45 (1.98–4.99)	3.39 (2.46–6.44)	5.67 (2.98–7.59)	4.36 (2.12–8.65)	18.375	0.001	c, d
Fasting C-peptide (ng/mL)	1.18 ± 0.80	1.50 ± 0.60	1.49 ± 0.84	1.64 ± 0.87	1.86 ± 1.18	2.864	0.026	c, d
PINS (mIU/L)	10.72 (6.13–15.20)	17.54 (6.98–34.68)	35.26 (23.84–51.49)	38.60 (23.52–58.64)	21.22 (11.59–39.48)	31.364	<0.001	b, c, d
Postprandial C-peptide (ng/mL)	4.60 ± 3.55	5.76 ± 3.55	7.73 ± 4.16	8.84 ± 6.04	6.53 ± 3.70	4.191	0.003	c
HOMA-IR	0.39 (0.28–0.81)	0.92 (0.55–1.31)	0.78 (0.55–1.48)	1.37 (0.80–1.92)	1.31 (0.71–2.76)	30.318	<0.001	a, c, d
HOMA-BETA	28.51 (18.22–43.93)	28.42 (15.58–39.25)	40.71 (30.44–71.99)	40.21 (19.75–61.17)	20.46 (10.84–46.72)	9.942	0.041	i
BETA/IR	52.10 ± 24.68	25.85 ± 2.87	52.14 (43.16–58.78)	28.52 (23.64–34.99)	15.59 (10.43–20.84)	30.318	<0.001	a, c, d, e, g, h, i
$\Delta I_{120}/\Delta G_{120}$	4.58 (1.47–7.59)	7.78 (−4.11–29.83)	9.56 (5.34–12.76)	10.53 (5.81–18.71)	2.35 (1.02–5.08)	20.733	<0.001	i, j
$\Delta I_{120}/\Delta G_{120}$/IR	9.51 (3.21–17.00)	11.15 (−3.47–20.54)	10.58 (6.08–16.19)	8.04 (3.64–15.94)	1.86 (0.81–3.36)	26.967	<0.001	d, g, i, j
ANGPTL8 (ng/mL)	0.38 ± 0.25	1.07 ± 0.52	0.92 ± 0.57	1.23 ± 0.48	0.85 ± 0.67	25.974	<0.001	a, b, c, d

M: male; F: female; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FINS: fasting serum insulin; PINS: postprandial serum insulin; HOMA-IR: homoeostasis model assessment of insulin resistance; HOMA-BETA: homoeostasis model assessment of beta cell function index. Pairwise comparisons: a, NGT versus IFG; b, NGT versus IGT; c, NGT versus CGI; d, NGT versus T2DM; e, IFG versus IGT; f, IFG versus CGI; g, IFG versus T2DM; h, IGT versus CGI; i, IGT versus T2DM; and j, CGI versus T2DM.

Table 2: Correlations between serum ANGPTL8 and metabolic parameters in 129 subjects.

(a)

	Model	Age		BMI		Waist circumferences		WHR		SBP	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ANGPTL8	1	0.067	0.453	0.139	0.117	0.191	0.031	0.195	0.027	0.112	0.208
	2	—	—	—	—	0.132	0.142	0.140	0.120	0.118	0.188
Fasting C-peptide	1	−0.115	0.199	0.387	0.000	0.404	0.000	0.251	0.004	0.047	0.600
	2	—	—	—	—	0.174	0.052	0.096	0.287	−0.029	0.751

(b)

	Model	DBP		TC		TG		LDL-C		HDL-C	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ANGPTL8	1	−0.014	0.872	0.035	0.692	0.093	0.296	0.058	0.512	0.041	0.648
	2	−0.040	0.659	0.014	0.875	−0.087	0.335	0.039	0.662	0.039	0.669
Fasting C-peptide	1	0.140	0.117	0.163	0.067	0.401	0.000	−0.013	0.884	0.016	0.862
	2	0.010	0.913	0.147	0.102	0.152	0.090	−0.012	0.893	0.025	0.781

(c)

	Model	HBA1C		FPG		PPG		FINS	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ANGPTL8	1	−0.094	0.290	0.253	0.004	0.017	0.846	0.334	0.000
	2	0.052	0.567	0.198	0.027	0.066	0.468	0.116	0.197
Fasting C-peptide	1	0.011	0.898	0.241	0.006	0.215	0.015	0.881	0.000
	2	0.035	0.699	0.176	0.049	0.138	0.126	0.829	0.000

(d)

	Model	Fasting C-peptide		PINS		Postprandial C-peptide		HOMA-IR	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ANGPTL8	1	0.218	0.013	0.271	0.002	0.196	0.026	0.355	0.000
	2	0.204	0.022	0.093	0.305	0.177	0.049	0.135	0.134
Fasting C-peptide	1	—	—	0.701	0.000	0.665	0.000	0.874	0.000
	2	—	—	0.389	0.000	0.642	0.000	0.781	0.000

(e)

	Model	HOMA-BETA		BETA/IR		$\Delta I_{120}/\Delta G_{120}$		$\Delta I_{120}/\Delta G_{120}/IR$	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ANGPTL8	1	0.140	0.113	−0.252	0.004	0.127	0.153	−0.063	0.478
	2	0.033	0.713	−0.275	0.002	−0.023	0.799	−0.059	0.514
Fasting C-peptide	1	0.673	0.000	−0.240	0.006	0.331	0.000	−0.119	0.182
	2	0.605	0.000	−0.299	0.001	0.141	0.117	0.012	0.894

Model 2: adjusted for age and BMI. — means the analysis was not performed.

topics of interest. Previous reports have shown controversy in DM. Some showed elevated circulating ANGPTL8 concentrations in subjects with DM in comparison with normal subjects [4–6], while others not. Besides, few had invested the levels of ANGPTL8 in IGR subjects, a high-risk population for T2DM. In this study, we investigated serum ANGPTL8 concentrations in different glucose metabolic statuses including NGT, IFG, IGT, CGI, and T2DM.

IGR, or called prediabetes, is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold [9]. It can be classified into three states: IFG, IGT, and CGI. IGR remains a state of high risk

Table 3: Correlations between serum ANGPTL8 levels and lipids in NGT, IGR, and T2DM, respectively.

	TG		TC		HDL-C		LDL-C	
	r	P	r	P	r	P	r	P
NGT	−0.010	0.966	0.029	0.898	−0.049	0.829	0.048	0.830
IGR	−0.073	0.534	−0.108	0.362	−0.019	0.871	−0.071	0.550
T2DM	0.033	0.856	0.031	0.865	0.042	0.815	0.194	0.279

for developing diabetes with yearly conversion rate of 5%–10% [10]. In our study, we found that IFG, IGT, CGI, and T2DM subjects had significantly higher serum ANGPTL8 levels than NGT, but serum ANGPTL8 levels among IGT, CGI, and T2DM had no significant differences. This suggested that ANGPTL8 might act as a predictive marker in prediabetes and diabetes mellitus.

C-peptide is a cleavage protein released during insulin production from its precursor proinsulin. C-peptide is used as a maker to indicate the level of endogenous insulin reserve and β-cell function. Our study showed that fasting C-peptide positively correlated with FPG, FINS, PINS, postprandial C-peptide, HOMA-IR, and HOMA-BETA and negatively correlated with BETA/IR after adjustment for age and BMI. This suggested that islet β-function increased in insulin-resistant subjects in the early stage of the hyperglycemic state including IGR and newly diagnosed DM. Moreover, we found that after adjustment for age and BMI, serum ANGPTL8 remained positively correlated with FPG, fasting C-peptide, and postprandial C-peptide and negatively correlated with BETA/IR. Taken together, ANGPTL8 levels increased with the level of FPG and increased with the level of fasting C-peptide because of insulin resistance. Serum ANGPTL8 might play a role in the pathological mechanism of glucose intolerance.

Multiple studies have identified that ANGPTL8 plays a role in the regulation of lipid metabolism in mice [2, 3, 11]. Zhang found that lipasin overexpression by adenoviruses in mice increases serum triglyceride levels and a recombinant lipasin inhibits LPL activity [3]. Fu et al. suggested that mice injected with the effective antibody or with lipasin deficiency had increased postprandial cardiac LPL activity and lower TAG levels in the fed state [12]. In addition, Quagliarini et al.'s group observed that ANGPTL8 expression in the livers of mice causes hypertriglyceridemia that is exacerbated by coexpression of ANGPTL3 [2]. But in a human being, the results were controversial. The study performed by Chung et al. suggested that ANGPTL8 concentrations were positively associated with triglycerides (TGs) [13]. A similar result was also observed in Gao et al.'s case-control study [14]. But in Espes et al.'s study, the correlation between ANGPTL8 and TG in patients with T2DM was negative [4].

In our study, we did not find any association between serum ANGPTL8 and lipid profile. This was in accordance with Guo et al.'s study in obese individuals [15] and Hu et al.'s in newly diagnosed type 2 diabetes [6].One reason for the discrepancy as Fu et al. described was the differences in ANGPTL8 antibodies, being against the N-terminus or the C-terminus, in ELISA kits used in different studies [16]. The other reason for the discrepancy may be that the lipid levels in our subjects were concentrated in a small range because of small sample size, not scattered in a larger range that might reflect the real lipid levels in population.

In conclusion, the findings of our cross-sectional study suggested that serum ANGPTL8 might play a role in the hyperglycemic state. However, the exact mechanisms of ANGPTL8 action in the hyperglycemic state have not yet been studied.

The present study had several limitations. First, the study was a cross-sectional design. Although we found elevated serum ANGPTL8 in prediabetes and T2DM and the association between serum ANGPTL8 and other metabolic parameters, it was hard to decide the cause-and-effect relationship between them. Second, our analyses were based on a single detection of blood ANGPTL8. Third, the small sample size of this study might lead to selection bias. We will expand the sample size to further explore the relationship between ANGPLT8 and hyperglycemia in further studies.

5. Conclusion

We found that serum ANGPTL8 concentrations were significantly increased in IGR and T2DM. Serum ANGPTL8 were positively correlated with FPG and fasting C-peptide and negatively correlated with BETA/IR after adjustment for sex, age, and BMI. Serum ANGPTL8 might play a role in the pathological mechanism of glucose intolerance.

Conflicts of Interest

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

Authors' Contributions

Yanhua Yin and Xiaoying Ding contributed equally to this work and are co-first authors.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (no. 30800562), Key Projects of Shanghai Municipal Health Bureau Research Fund (no. 201440033), Shanghai Jiao Tong University Research Funding on Medical and Engineering Interdisciplinary Projects (YG2015ZD08, YG2015MS30), Key Programs for Science and Technology Development of Songjiang District

(15SJGG54), Songjiang District Health Bureau of Climbing Medical Program (0702N14003), and Shanghai Shen Kang Hospital Development Center for Chronic Disease Prevention and Control Project (SHDC12015304).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 8948452, 10 pages
https://doi.org/10.1155/2017/8948452

Research Article

Influencing Factors of Knowledge, Attitude, and Practice regarding Medical Nutrition Therapy in Patients with Diabetes: A National Cross-Sectional Study in Urban China

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Received 12 December 2016; Accepted 5 March 2017; Published 16 August 2017

Academic Editor: Ruozhi Zhao

To investigate the knowledge-attitude-practice (KAP) score in diabetes patients living in urban China regarding Medical Nutrition Therapy (MNT) and explore the influencing factors, this national survey recruited diabetes and prediabetes patients in 40 hospitals across 26 provinces in China. A self-designed questionnaire was used to collect the data and assess the knowledge, attitude, and practice regarding MNT. Logistic regression was used to explore the factor influencing KAP scores. A total of 6441 diabetes patients (mean age: 60.02 ± 13.14 years) completed this survey. The mean glycosylated hemoglobin (HbA1c) level was 8.12 ± 2.12%, and the control rate of HbA1c (HbA1c < 7.0%) was 38.92%. Of the total, 53.56% had received MNT education. Over half of the patients had a poor total KAP score as well as poor K, A, and P scores. Patients with higher KAP scores had higher control rate of HbA1c ($P < 0.05$) but lower levels of fasting plasma glucose (FPG) and 2-hour postprandial blood glucose (2h-PG). Gender, occupation, residence, education level, and MNT education could influence the KAP scores ($P < 0.05$). This study showed that diabetes patients in urban China generally had poor understandings and practices related to MNT. Patients with higher KAP scores exhibited better control of blood glucose.

1. Introduction

Diabetes is a common chronic metabolic disease that greatly affects people's health and quality of life. It is estimated that about 415 million individuals aged 20–79 years lived with diabetes in 2015, while another 318 million were diagnosed with impaired glucose tolerance. In addition, the number of patients with diabetes aged 20–79 years is expected to increase to 642 million by 2040 [1]. With improvements in living standards and changes of life styles, more and more individuals are diagnosed with diabetes in developing countries. The incidence rate of diabetes in China was as high as 11.6% in 2013 [2]. In 2015, over 109.6 million individuals were living with diabetes in China, which made China the first leading country with the highest number of adult diabetes patients in the world. In addition, the medical cost of diabetes in China was estimated at over 51 billion US dollars [1]. A nationwide survey in patients with type 2 diabetes in 2010 showed that the control rate of blood glucose was only 32.18% (glycosylated hemoglobin [HbA1c] < 7.0%) [3]. Therefore,

it is urgent to take effective measurements to prevent and manage diabetes in China [4]. Medical Nutrition Therapy (MNT), namely, applying special nutritional interventions for specific diseases in clinical practice, was first proposed by the American Diabetes Association (ADA) in 1994, which recommended that equal attention should be paid to nutrition therapy and drug therapy [5]. In 2006, the ADA urged that all diabetes patients should receive individualized MNT under the guidance of special clinicians or dietitians, which could help them achieve the ideal treatment target [6]. The *China Medical Nutrition Therapy Guideline for Diabetes* was issued in 2011 [7], which pointed out that MNT is the foundation for the treatment for diabetes. In 2013, a panel of experts from the area of endocrinology and nutrition developed and issued the first *China Expert Consensus of Medical Nutrition Therapy for Diabetes* [8], which again highlighted that MNT is the foundation of diabetes management. In addition, the consensus also pointed out that, for the blood glucose control in subjects with obesity, metabolic syndromes, prediabetes, diabetes, pregnancy with gestational hyperglycemia, and pregnancy in the perioperative period, MNT has several advantages including higher feasibility, safety, and effectiveness and thus could improve the prognosis and reduce the medical expenses. Therefore, MNT has already been considered as an essential tool for the management of diabetes. Previous studies have already shown that applying MNT in diabetes management can substantially improve the rate of blood glucose control [9, 10]. Furthermore, individualized MNT, according to the specific conditions of each patient, could further bring benefits to both medical and economic aspects [11].

MNT has already been applied in China for several years and received extensive attention from health care professionals. However, the application of this tool is still not optimal, due to its dependence on the communications between clinicians and patients and requirement of high cooperation of the patients. Several studies have already investigated the application of MNT in Chinese diabetes patients in recent years [12–14]. However, the sample sizes of all these studies are relatively low, and thus the results still need to be validated. In addition, the knowledge, attitude, and practice regarding MNT, as well as the factors that could affect the application of MNT in China are still unclear. Therefore, we first conducted this national survey to assess the knowledge, attitude, and practice regarding MNT among diabetes patients in urban China and to explore the influencing factors.

2. Materials and Methods

2.1. Study Design. A multicenter cross-sectional study was conducted by the Diabetes Care and Education Study Group of the Chinese Diabetes Society in 40 tertiary, secondary, and community hospitals across 26 cities in China. Fixed-point continuous sampling was adopted to recruit patients with diabetes and prediabetes. The patients were mainly included from the outpatient department, while the ones from the inpatient department accounted for no less than 10% of all the patients included.

2.2. Patients. The inclusion criteria for the patients were as follows: (1) age $\geq$ 18 years; (2) being diagnosed with impaired glucose tolerance or diabetes for 1 year or more or being with gestational diabetes mellitus; (3) consciousness and ability to correctly understand and respond to the questions; and (4) willingness to participate in the study and signing informed consent form. Patients with one or more of the following items were excluded: (1) age < 18 years; (2) inability to complete the survey due to physical or psychological disorders; (3) refusing to participate in the survey; (4) refusing to sign the informed consent form; and (5) being ineligible due to other conditions assessed by the investigators.

2.3. Questionnaire Design. A structured questionnaire was developed by a panel of experts consisting of clinicians, nurses, dieticians, and other investigators. Each question in the questionnaire was developed from clinical practices and was closely associated with current MNT education in China. The questionnaire was discussed and modified several times before being applied in the survey. The preliminary survey was conducted before the initiation of this study to help modify the questionnaire and improve the validity and reliability of the questionnaire. The Cronbach's alpha values were 0.679, 0.717, 0.412, and 0.440 for the total KAP score, K score, A score, and P score, respectively.

The questionnaire contains two parts. The first part of the questionnaire collects the demographic and disease information, as well as physical examination data, including gender, age, height, weight, occupation, education level, residence, blood glucose level, and duration and type of diabetes. The second part of the questionnaire includes the questions on KAP regarding MNT (Appendix). Knowledge (K) of MNT is assessed by 6 questions (Q4, Q5, Q8, Q9, Q10, and Q15), attitude (A) is assessed by 3 questions (Q11, Q12, and Q20), and practice (P) is assessed by 10 questions (Q2, Q3, Q6, Q7, Q13, Q14, Q16, Q17, Q18, and Q19). Question 1 is an independent question, which assesses whether the patient had been educated about MNT before. For the questions Q11, Q12, Q13, Q14, Q19, and Q20, 1 point is obtained when the answer is "No," while for the other questions, 1 point is obtained when the answer is "Yes." The scores for K, A, and P are obtained by adding the scores of all the questions in each section, and the highest scores possible are 6, 3, and 10, respectively. The total score for the questionnaire is 19, which is calculated by adding the scores of K, A, and P together. The higher score indicates that the patient has better KAP regarding MNT.

The total KAP score and the scores of K, A, and P are considered poor when the score is less than or equal to the median value (the median values for total KAP, K, A, and P score were 9, 3, 1, and 5, resp.).

2.4. Data Collection and Measurements. The questionnaire survey was conducted by trained investigators (clinicians, education nurses, or dieticians) assigned by each of the participating hospitals, through face-to-face interviews with the patients.

Physical examinations were performed by experienced nurses. The following data were collected during the physical examinations: height, weight, waist circumference, hip circumference, systolic blood pressure, diastolic blood pressure,

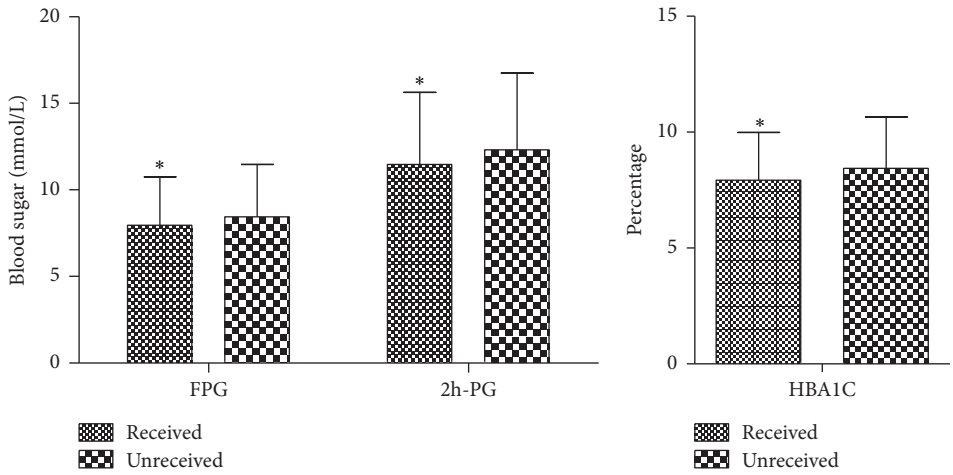

Figure 1: *Influence of the MNT education on blood glucose levels.* * Significant difference compared to the unreceived MNT group, $P < 0.001$.

fasting plasma glucose (FPG), 2-hour postprandial blood glucose (2h-PG), and glycosylated hemoglobin (HbA1c). All these data were measured by standard methods that have been widely accepted and applied in clinical practice within 3 months prior to the study initiation.

2.5. Quality Control. Over 6000 patients were included in this study to provide a sufficient sample size. Strict quality control was applied throughout the processes of data collection and processing. The investigators were uniformly trained several times before the study to minimize the bias and improve the validity and reliability of the questionnaire. Repetitive checks were executed by special investigators after the questionnaires were completed. In addition, double entry and validation of the data were performed within 72 h after the questionnaires were completed.

2.6. Ethical Considerations. This study was approved by the Ethics Committee of Peking University First Hospital and was performed strictly according to the Declaration of Helsinki. All the participants were informed of the contents and other essential information, and written informed consent forms were obtained from all patients before the survey was started. This study was also registered in the Chinese Clinical Trial Registry (number ChiCTR-OCS-14005204).

2.7. Statistical Analyses. SAS 9.2 (SAS 9.2; SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses in this study. Quantitative data are described with means ± standard deviation (SD), while qualitative data are described as frequencies and percentages. Independent t-test or Wilcoxon rank-sum test was used for the comparisons of quantitative data between two groups, and analysis of variance (ANOVA) or nonparametric test was used for the comparisons of quantitative data among three or more groups. Chi-square test was used for the comparisons of qualitative data among different groups. Multivariate linear regression was used to explore the factors influencing the MNT score. All the statistical analyses were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. General Characteristics. Between May 18, 2014, and August 22, 2014, 6932 patients were recruited in this study, and 6441 completed questionnaires were obtained after deleting those with missing data or logical errors, yielding an effective rate of 92.9%. Importantly, 94.67% of these patients had type 2 diabetes, while only 5.32% of the patients had type 1 diabetes, prediabetes, or other types of diabetes. Among these patients, 3061 were females and 3380 were males, and their mean age was 60.02 ± 13.14 years; 37.98% of the patients were over 65 years of age. The mean disease duration was 9.36 ± 7.22 years, and 62.04% of the patients had the disease for a duration of 1–10 years. The mean body mass index (BMI) of the patients was 24.76 ± 3.67 kg/m^2, and 56.67% of the patients were overweight or obese (BMI ≥ 24 kg/m^2). Only 18.00% of the patients had an education level of college or higher. 58.42% of the patients were retired, and 53.56% of the patients had been educated about MNT before (Table 1).

The mean FBG of the patients was 8.15±2.91 mmol/L, and 21.2% of the patients had aFBG level lower than 6.1 mmol/L. The mean 2h-PG of the patients was 11.78±4.28 mmol/L, and 12.1% of the patients had a 2h-PG lower than 7.8 mmol/L. The mean HbA1c of the patients was 8.12±2.12%, and the control rate among them was 38.92% (HbA1c < 7.0%).

3.2. KAP Score. A relatively poor KAP score was obtained from 54.54% of the patients. In addition, 62.79%, 61.85%, and 56.16% of the patients had poor K, A, and P scores in this study (Table 2).

3.3. Association between Total KAP Score and Blood Glucose Control. The FPG, 2h-PG, and HbA1c levels in the patients educated about MNT before were significantly lower than those in patients not educated before (Figure 1). In addition, the FPG, 2h-PG, and HbA1c levels in the patients with total KAP scores of 10–19 were significantly lower than in the ones with the scores of 0–9 (Table 3).

3.4. Influencing Factors of KAP Score. Chi-square test showed that total KAP scores of the patients were influenced by

Table 1: General characteristics of the patients.

Category	Subcategory	Data
Total number		6441
Age (y)		60.20 ± 13.14
	18–65, *n* (%)	3995 (62.02)
	65-, *n* (%)	2446 (37.98)
Gender	Female, *n* (%)	3061 (47.53)
	Male, *n* (%)	3380 (52.47)
BMI (kg/m^2)*		24.76 ± 3.67
	<24, *n* (%)	2791 (43.33)
	24-, *n* (%)	3650 (56.67)
Duration (y)		9.60 ± 7.22
	1–10, *n* (%)	3995 (62.04)
	10-, *n* (%)	2444 (37.96)
Educational status	Middle school or below, *n* (%)	2690 (41.76)
	Senior high school, *n* (%)	1517 (23.55)
	Junior college, *n* (%)	1075 (16.69)
	Undergraduate or above, *n* (%)	1159 (18.00)
Occupation	Retiree, *n* (%)	3763 (58.42)
	In service, *n* (%)	2005 (31.13)
	Others, *n* (%)	690 (10.71)
Diagnosis	Type 1 diabetes, *n* (%)	161 (2.50)
	Type 2 diabetes, *n* (%)	6098 (94.67)
	Other types of diabetes, *n* (%)	33 (0.51)
	Prediabetes, *n* (%)	149 (2.31)
MNT education experience	Yes, *n* (%)	3450 (53.56)
	No, *n* (%)	2991 (46.44)
FBG (mmol/L)		8.15 ± 2.91
	<6.1* (%)	21.2
2h-PG (mmol/L)		11.78 ± 4.28
	<7.8* (%)	12.1
HbA1c (%)		8.12 ± 2.12
	<7.0* (%)	38.92
BP (mmHg)		130.25 ± 16.89/77.54 ± 10. 3
	<140/90* (%)	77.35

*The reference values for HbA1c and the classification of BMI were according to the directions of "China Guideline For Type 2 Diabetes" (2013) [15].

Table 2: Distribution of the MNT, knowledge, attitude, and practice scores.

Category	Subcategory	Frequency (*n*)	Percentage (%)
KAP score	0–9 (poor)	3513	54.54
	10–19 (good)	2928	45.46
Knowledge score	0–3 (poor)	4044	62.79
	4–6 (good)	2397	37.21
Attitude score	0-1 (negative)	3984	61.85
	2-3 (positive)	2457	38.15
Practice score	0–5 (poor)	3617	56.16
	6–10 (good)	2824	43.84

gender, BMI, education level, occupation, residence, and MNT education (Table 4). The total KAP scores were significantly higher in female patients than in male patients (OR, 1.24; 95% confidence interval [CI], 1.10–1.40; $P = 0.001$), higher in patients with a BMI < 24 kg/m^2 than in those with a BMI ≥ 24 kg/m^2 (OR, 1.17; 95% CI, 1.05–1.31; $P = 0.007$), higher in patients with senior high school (OR, 1.65; 95% CI, 1.42–1.91; $P < 0.001$), junior college (OR, 2.30; 95% CI, 1.95–2.72; $P < 0.001$), or undergraduate education level or above (OR, 2.92; 95% CI, 2.47–3.45; $P < 0.001$) compared with those of individuals with a junior middle education level or below, higher in the patients who had retired than in the ones in service (OR, 1.55; 95% CI, 1.34–1.79; $P < 0.001$), and higher in those who received MNT education than in those who did not (OR, 5.06; 95% CI, 4.50–5.69; $P < 0.001$). However, no significant association between disease duration and total KAP score was found (Table 5).

Consistent with the total KAP score, the results of this study also showed that the K scores were significantly higher

Table 3: Comparison of FBG, 2h-PG, and HbA1c between KAP score groups.

KAP score	FPG		2h-PG		HbA1c	
	Mean ± SD	CR‡	Mean ± SD	CR‡	Mean ± SD	CR‡
0–9	8.58 ± 3.16	19.58	12.66 ± 4.65	11.07	8.48 ± 2.21	31.85
10–19	7.69 ± 2.50*	25.41	11.01 ± 3.90*	17.48	7.77 ± 1.96*	46.59

SD, standard deviation, mmol/L; CR, controlling rate (%); *$P < 0.001$ compared to the f KAP score group, 0–9; ‡$P < 0.001$ for significant difference determined by the χ^2 test.

in patients with a BMI $< 24\,kg/m^2$ than in those with a BMI ≥ 24 (OR, 1.21; 95% CI, 1.08–1.37; $P = 0.001$); higher in patients with senior high school (OR, 1.50; 95% CI, 1.29–1.75; $P < 0.001$), junior college (OR, 2.11; 95% CI, 1.78–2.50; $P < 0.001$), or undergraduate education level or above (OR, 2.58; 95% CI, 2.18–3.05; $P < 0.001$) than in those with a junior middle education level or below; higher in retired patients than in the ones in service (OR, 1.51; 95% CI, 1.30–1.75; $P < 0.001$); and higher in patients who received MNT education than in those who did not (OR, 5.45; 95% CI, 4.81–6.17; $P < 0.001$). However, gender, age, and disease duration were not significantly associated with the K score (Table 5).

The A score in this study was significantly associated with only gender, education level, occupation, and MNT education. In detail, the A score was significantly higher in female patients than in the male patients (OR, 0.86; 95% CI, 0.77–0.96; $P = 0.008$). However, the education level was positively associated with the A score. Compared with the patients with a junior middle education level or below, patients with senior high school (OR, 1.22; 95% CI, 1.06–1.41; $P = 0.005$) and undergraduate education level or above (OR, 1.25; 95% CI, 1.07–1.45; $P = 0.005$) had significantly higher A scores. In addition, the A score was also significantly higher in retired patients than in in-service patients (OR, 1.35; 95% CI, 1.17–1.54; $P < 0.001$). However, in contrast to total KAP score and K score, patients who received MNT education had significantly lower A scores than those who did not (OR, 0.87; 95% CI, 0.78–0.97; $P = 0.013$; Table 5).

The P score in this study was significantly influenced by gender, education level, occupation, place of residence, and MNT education. The P score in female patients was significantly higher than that in male patients (OR, 1.23; 95% CI, 1.10–1.39; $P < 0.001$). In agreement with the total KAP score, K score, and A score, education level was also positively associated with the P score. Compared with the patients with a junior middle education level or below, those with senior high school (OR, 1.40; 95% CI, 1.21–1.62; $P < 0.001$), junior college (OR, 2.12; 95% CI, 1.81–2.50; $P < 0.001$), and undergraduate education level or above (OR, 2.65; 95% CI, 2.26–3.12; $P < 0.001$) had significantly higher A scores. In addition, patients who were retired (OR, 1.44; 95% CI, 1.25–1.66; $P < 0.001$) and who received MNT education (OR, 3.45; 95% CI, 3.08–3.86; $P < 0.001$) had significantly higher P scores than those in service and did not receive MNT education, respectively (Table 5).

4. Discussion

To our knowledge, this is the first large-scale study investigating the application of MNT and the influencing factors in patients with diabetes living in urban China. In this national cross-sectional survey with over 6000 patients included, the results showed that over half of the patients with diabetes in urban China received MNT education. However, the knowledge and practice of MNT in the patients with diabetes were suboptimal, and the total KAP score as well as K, A, and P scores was poor in more than half of the patients.

A previous study in China has shown that patients with diabetes had positive attitudes but relatively poor nutrition knowledge and practices [16], which has been confirmed in another study in the patients with diabetes in South Africa [17]. However, these findings were not in agreement with our results. Both the previous studies only included limited patients (162 and 217 patients, resp.); thus, the results could be easily biased. In addition, the questionnaires used in those two studies were not identical to the one used in our study, which could also contribute to the differences with our findings. The K score of the patients with diabetes in this study was slightly higher than the score reported in a previous study in Malaysia [9], suggesting that Chinese patients may have a higher level of MNT knowledge than those in Malaysia. However, the disease duration in our study was evidently longer (9.33±7.13 years versus 6.3±4.9 years). The agony from the disease in such long duration could effectively promote the patients to search for related knowledge and actively seek help from clinicians. The attitudes and practices of MNT were not reported in the Malaysia study, and thus we could not compare the findings of these two studies.

Both individualized and group MNT could effectively improve the control of blood glucose in patients with diabetes. Previous studies showed that after the application of MNT the FPG, postprandial blood glucose, and HbA1c levels all decreased significantly [9, 10]. A study in China also showed that, after 1-year education about MNT in patients with type 2 diabetes, the FPG level was significantly lower than the level before the education [14]. For patients with prediabetes, individualized MNT is effective at reducing the HbA1c level compared with usual care [18]. These findings suggested that improving the knowledge and practice of MNT in patients with diabetes could substantially improve the blood glucose control. In our study, the results showed that blood glucose levels were negatively associated with the KAP scores. Patients with higher KAP scores had lower FPG, 2h-PG, and HbAc1 levels. In addition, the HbAc1 control rate also increased significantly with the KAP score, which was in agreement with previous studies [9, 16]. The mean HbAc1 level in this study was 8.12 ± 2.12%, while the control rate was only 38.92%, which was higher than that reported in a national study in patients with type 2 diabetes performed in 2010 (32.18%) [3]. The increase could be associated with the

Table 4: Factors associated with MNT KAP, knowledge, attitude, and practice, scores [*n* (%)].

Category	KAP score *n* (%)		Knowledge score *n* (%)		Attitude score *n* (%)		Practice score *n* (%)	
	Good	Poor	Good	Poor	Good	Poor	Good	Poor
Gender								
Female	1447 (47.27)	1614 (52.73)	1155 (37.73)	1906 (62.27)	1116 (36.46)	1945 (63.54)	1389 (45.37)	1672 (54.63)
Male	1481 (43.82)	1899 (56.18)	1242 (36.75)	2138 (63.25)	1341 (39.67)	2039 (60.33)	1435 (42.46)	1945 (57.54)
	$X^2 = 7.74$	$P = 0.005$	$X^2 = 0.67$	$P = 0.413$	$X^2 = 7.04$	$P = 0.008$	$X^2 = 5.57$	$P = 0.018$
Age								
18–65	1702 (42.60)	2293 (57.40)	1418 (35.49)	2577 (64.51)	1491 (37.32)	2504 (62.68)	1677 (41.98)	2318 (58.02)
65-	1226 (50.12)	1220 (49.88)	979 (40.02)	1467 (59.98)	966 (39.49)	1480 (60.51)	1147 (46.89)	1299 (53.11)
	$X^2 = 34.60$	$P = 0.000$	$X^2 = 13.33$	$P = 0.000$	$X^2 = 3.03$	$P = 0.082$	$X^2 = 14.89$	$P = 0.000$
BMI								
<24	1326 (47.51)	1465 (52.49)	1100 (39.41)	1691 (60.59)	1041 (37.30)	1750 (62.70)	1266 (45.36)	1525 (54.64)
24-	1602 (43.89)	2048 (56.11)	1297 (35.53)	2353 (64.47)	1416 (38.79)	2234 (61.21)	1558 (42.68)	2092 (57.32)
	$X^2 = 8.36$	$P = 0.004$	$X^2 = 10.18$	$P = 0.001$	$X^2 = 1.50$	$P = 0.221$	$X^2 = 4.60$	$P = 0.032$
Duration (y)								
1–10	1727 (43.23)	2268 (56.77)	1406 (35.19)	2589 (64.81)	1500 (37.55)	2495 (62.45)	1716 (42.95)	2279 (57.05)
10-	1200 (49.10)	1244 (50.90)	990 (40.51)	1454 (59.49)	956 (39.12)	1488 (60.88)	1107 (45.29)	1337 (54.71)
	$X^2 = 21.08$	$P = 0.000$	$X^2 = 18.32$	$P = 0.000$	$X^2 = 1.58$	$P = 0.21$	$X^2 = 3.38$	$P = 0.066$
Education								
Middle school or below	948 (35.24)	1742 (64.76)	768 (28.55)	1922 (71.45)	958 (35.61)	1732 (64.39)	936 (34.80)	1754 (65.20)
Senior high school	719 (47.40)	798 (52.60)	579 (38.17)	938 (61.83)	618 (40.74)	899 (59.26)	664 (43.77)	853 (56.23)
Junior college	590 (54.88)	485 (45.12)	489 (45.49)	586 (54.51)	421 (39.16)	654 (60.84)	574 (53.40)	501 (46.60)
Undergraduate or above	671 (57.89)	488 (42.11)	561 (48.40)	598 (51.60)	460 (39.69)	699 (60.31)	650 (56.08)	509 (43.92)
	$X^2 = 226.36$	$P = 0.000$	$X^2 = 180.62$	$P = 0.000$	$X^2 = 13.27$	$P = 0.004$	$X^2 = 199.80$	$P = 0.000$
Occupation								
Retiree	1888 (50.40)	1858 (49.60)	1535 (40.98)	2211 (59.02)	1508 (40.26)	2238 (59.74)	1782 (47.57)	1904 (50.83)
In service	814 (40.60)	1191 (59.40)	673 (33.57)	1332 (66.43)	704 (35.11)	1301 (64.89)	809 (40.35)	1196 (59.65)
	$X^2 = 50.37$	$P = 0.000$	$X^2 = 30.33$	$P = 0.000$	$X^2 = 14.60$	$P = 0.000$	$X^2 = 27.51$	$P = 0.000$
MNT education								
Yes	2181 (63.22)	1269 (36.78)	1874 (54.32)	1576 (45.68)	1293 (37.48)	2157 (62.52)	1988 (57.62)	1462 (42.38)
No	747 (24.97)	2244 (75.03)	523 (17.49)	2468 (82.51)	1164 (38.92)	1827 (61.08)	836 (27.95)	2155 (72.05)
	$X^2 = 945.00$	$P = 0.000$	$X^2 = 930.22$	$P = 0.000$	$X^2 = 1.41$	$P = 0.236$	$X^2 = 572.91$	$P = 0.000$

Table 5: Association of the factors with MNT knowledge, attitude, and practice scores.

Category	KAP score		Knowledge score		Attitude score		Practice score	
	OR (95.0% CI)	*P*	OR (95.0% CI)	*P*	OR (95.0% CI)	*P*	OR (95.0% CI)	*P*
Gender								
Male	1.00		1.00		1.00		1.00	
Female	1.24 (1.10–1.40)	0.001	1.08 (0.96–1.22)	0.212	0.86 (0.77–0.96)	0.008	1.23 (1.10–1.39)	0.000
Age (y)								
18–65	1.00		1.00		1.00		1.00	
>65	1.02 (0.89–1.17)	0.744	0.92 (0.80–1.06)	0.260	0.99 (0.87–1.12)	0.833	0.99 (0.87–1.12)	0.872
BMI								
>24	1.00		1.00		1.00		1.00	
<24	1.17 (1.05–1.31)	0.007	1.21 (1.08–1.37)	0.001	0.95 (0.85–1.06)	0.341	1.12 (1.00–1.25)	0.050
Duration (y)								
10-	1.00		1.00		1.00		1.00	
1–10	1.03 (0.91–1.16)	0.689	1.03 (0.91–1.17)	0.639	1.02 (0.91–1.14)	0.718	0.92 (0.82–1.04)	0.164
Education								
Middle school or below	1.00		1.00		1.00		1.00	
Senior high school	1.65 (1.42–1.91)	0.000	1.50 (1.29–1.75)	0.000	1.22 (1.06–1.41)	0.005	1.40 (1.21–1.62)	0.000
Junior college	2.30 (1.95–2.72)	0.000	2.11 (1.78–2.50)	0.000	1.17 (1.00–1.36)	0.052	2.12 (1.81–2.50)	0.000
Undergraduate or above	2.92 (2.47–3.45)	0.000	2.58 (2.18–3.05)	0.000	1.25 (1.07–1.45)	0.005	2.65 (2.26–3.12)	0.000
Occupation								
In service	1.00		1.00		1.00		1.00	
Retiree	1.55 (1.34–1.79)	0.000	1.51 (1.30–1.75)	0.000	1.35 (1.17–1.54)	0.000	1.44 (1.25–1.66)	0.000
MNT education								
No	1.00		1.00		1.00		1.00	
Yes	5.06 (4.50–5.69)	0.000	5.45 (4.81–6.17)	0.000	0.87 (0.78–0.97)	0.013	3.45 (3.08–3.86)	0.000

scale-up of comprehensive treatment and self-management of diabetes in China in recent years. However, the control rate in this study was still lower than the results reported in Korea, USA, European countries, and Japan [19, 20]. These findings show that although MNT has been applied in China for about 5 years, the knowledge and practice of MNT in diabetes patients in urban China are still suboptimal, and the controlling rate of blood glucose is still very low.

Many factors could affect the knowledge, attitude, and practice of MNT in patients with diabetes. The findings in this study showed that the total KAP score as well as the K, A, and P scores of the patients was significantly associated with gender, occupation, education level, residence, and MNT education. A previous study showed that female patients tend to have higher total KAP scores and K and P scores but lower A scores than male patients, suggesting that although male patients have more active attitude to MNT than female patients, the practice is still lagging behind.

The findings in this study showed that in-service patients had lower total KAP scores as well as lower K, A, and P scores than retired patients. We speculated that this could be associated with the fact that the in-service patients still have careers to worry about, and thus their work and accompanying social activities restricted them from remaining concerned about the disease conditions [21]. In addition, social activities in China are generally accompanied by banquets, which could further impair the MNT of in-service patients. In contrast, retired patients are past the busiest stages in life generally, and they have enough time and interest to learn the knowledge about diabetes management. The findings in this study also showed that patients who lived in rural areas had significantly lower KAP scores than those who lived in urban areas, which was in agreement with previous findings [22, 23]. This could be associated with the fact that most patients in rural areas had a relatively low education level, which restricted them from clearly and correctly understanding the knowledge about MNT. The findings that patients with lower education level had lower KAP scores also supported this hypothesis. In addition, patients from rural areas generally had a lower income; therefore, they had a heavier work load and mental stress than the ones from urban areas, which not only further restricts them from obtaining MNT-related knowledge, but also impairs their practices in MNT [24]. However, another study in China showed no significant differences in the knowledge, attitude, and practice scores regarding MNT between the diabetes patients from rural and urban areas; however, that study did find that the patients with longer disease duration had higher total KAP score and practice score [16]. The difference from our findings could be associated with the sample size, source of subjects, and the questionnaires used.

The findings in this study showed that the total KAP scores as well as the K, A, and P scores in patients who received MNT education were significantly higher than those in the patients who did not, which is in agreement with the results reported in previous studies [14, 25, 26], suggesting that providing MNT education for diabetes patients could not only improve the related knowledge but also help improve the attitudes and practices of applying MNT and thus finally improving the self-management of blood glucose level.

4.1. Limitations of the Study. As a cross-sectional study, this study could not clarify the causal relationship between the factors and KAP scores on MNT. In addition, the retrospective method for collecting data could also introduce recall bias. However, this study could still provide valuable evidence for helping us to understand the factors influencing the application of MNT in Chinese diabetes patients and thus further provide evidence for further clinical studies and practices. To minimize the bias, investigators were uniformly trained before the survey started. In addition, the preliminary survey, as well as strict quality control in this study, could also help minimize, although not prevent, the bias. This study used a self-designed questionnaire to collect the data, and the Cronbach's alpha values for A and P scores were relatively low. However, the previous adoption of the same questionnaire by many other researchers in China suggests that this questionnaire can reflect well the MNT attitude and behaviors of diabetes patients. Therefore, this questionnaire was still used in our study despite the relatively low alpha values for A and P scores.

In conclusion, this study showed that although over half of the patients with diabetes living in urban China had received MNT education, over half of the patients still had poor understanding and practices of MNT, and the control rate of blood glucose was still very low. Patients with higher KAP scores were with better control of blood glucose. These findings suggest that MNT education is critical for patients with diabetes. However, the method of MNT education should be different, according to gender, BMI, occupation, and education level of the patients.

Appendix

The questionnaire about the knowledge, attitude, and practices of patients with diabetes and prediabetes toward MNT is shown in Table 6.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Zijian Li and Haimin Jin contributed equally to this work.

Acknowledgments

The authors are grateful to Abbott China (Shanghai, China), which provides a grant for the study. Meanwhile, the authors thank China NEEDs Study Group and Diabetes Care and Education Group of the Chinese Diabetes Society for the support. The members of Study Group are as follows: Li Shen from Beijing Tsinghua Changgung Hospital, Wenxia Li from the First Affiliated Hospital of Nanchang University, Jin Huang from the Second Xiangya Hospital of Central South University, Li Yuan from West China Hospital, Qingqing Lou

Table 6

Item	Question
Knowledge	
Q4	Knows the dietary management of diabetes has been upgraded into MNT.
Q5	Knows that a lot of professional associations recommend MNT as the foundation of diabetes prevention and treatment both at home and abroad.
Q8	Understands and remembers MNT recommendations provided by HCPs.
Q9	Knows the total amount of daily food that should be consumed.
Q10	Knows how to allocate the daily intake of food groups.
Q15	Knows the food restrictions or other factors that may induce malnutrition.
Attitude	
Q11	Too frightened to take a meal (or reduce intake) because of concerns about increased post-prandial glycaemia
Q12	Too frightened to eat fruits and sweets because of concerns about increased blood glucose.
Q20	Feels distressed or difficult to adhere to self-management according to the MNT recommendations provided by HCPs (multiple choices). If "yes", the main obstacles for acceptance or adherence to MNT is; (1) no chance to understand; (2) the content is too difficult to be understood; (3) the requirement is too high to adhere to.
Practice	
Q2	Correctly determined their body-weight group (refer to BMI, kg/m^2, low weight ≤18.5; normal 18.6–23.9; overweight 24.0–27.9; obesity ≥28).
Q3	Can calculate the ideal body weight.
Q6	Routinely pay attention to the nutrition status.
Q7	Are routinely provided with MNT recommendations by doctors, clinical dietitians, or nurses.
Q13	Has experienced hypoglycemia due to irregular life style choices.
Q14	Has experienced between-meal hypoglycemia, bedtime hypoglycemia, or nocturnal hypoglycemia
Q16	Routinely follows the recommendations of doctors or clinical dietitians when arranging daily diet,
Q17	Routinely eats more vegetables than meat in order to control blood glucose.
Q18	Routinely increases the intake of snacks as a compensation of the reduction of meals or staple food recommendations.
Q19	Routinely unable to execute the MNT recommendations because of various reasons.

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 4740124, 12 pages
https://doi.org/10.1155/2017/4740124

Research Article

PNPLA3 rs1010023 Predisposes Chronic Hepatitis B to Hepatic Steatosis but Improves Insulin Resistance and Glucose Metabolism

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Received 28 December 2016; Revised 7 May 2017; Accepted 16 May 2017; Published 14 June 2017

Academic Editor: Ruozhi Zhao

PNPLA3 polymorphisms serve as the genetic basis of hepatic steatosis in normal population and lead to dysregulated glucose metabolism. Whether it underlies the hepatic steatosis and glucose homeostasis in chronic hepatitis B patients remains uncertain. Here, we investigated the *PNPLA3* polymorphisms in biopsy-proven chronic hepatitis B patients with (CHB+HS group, $n = 52$) or without hepatic steatosis (CHB group, $n = 47$) and non-CHB subjects with (HS group, $n = 37$) or without hepatic steatosis (normal group, $n = 45$). When compared to the TT genotype, C-allele at *PNPLA3* rs1010023 (CC and TC genotypes) conferred higher risk to hepatic steatosis in chronic hepatitis B patients (odds ratio (OR) = 1.768, 95% confidence interval (CI): 1.027–3.105; $P = 0.045$) independent of age, gender, and body mass index. In contrast to their role in hepatic steatosis, CC and TC genotypes of *PNPLA3* rs1010023 were correlated to significant improvement of homeostasis model assessment index (HOMA-IR) as compared to TT genotype in the CHB+HS group. Downregulated fasting blood glucose also characterized the CHB+HS patients with C-allele at *PNPLA3* rs1010023 (CC/TC versus TT: 4.81 ± 0.92 mmol/L versus 5.86 ± 2.11 mmol/L, $P = 0.02$). These findings suggest that *PNPLA3* rs1010023 may predispose chronic hepatitis B patients to hepatic steatosis but protects them from glucose dysregulation by attenuating insulin resistance.

1. Introduction

By the high prevalence (7.2%, 2006) of HBV infection, chronic hepatitis B (CHB) used to serve as the leading causes of chronic liver diseases (CLDs) in the Chinese population [1, 2]. In contrast, the growing incidence of obesity and metabolic syndrome (MetS), mainly on the basis of western diets and unhealthy lifestyle, leads to a dramatic alternation in the spectrum of CLDs [2]. Hepatic steatosis, with the prevalence of 60–90% in obese patients, has recently replaced chronic hepatitis B to dominate the CLD in China [2]. As a result, hepatic steatosis occurs on the basis of chronic hepatitis B with an increasing annual prevalence from 8.2% (2002) to 13.5%–31.8% (2011) [3, 4].

In contrast to simple chronic hepatitis B, concurrence of chronic hepatitis B and hepatic steatosis demonstrates a significant impact on both insulin sensitivity and glucose metabolism [5–10]. By multivariate analysis, body mass index (BMI) [5–7, 9], fasting insulin [5], homeostasis model assessment index (HOMA-IR) [8–10], and fasting blood glucose (FBG) [6–8] are positively associated with the hepatic steatosis in different ethnicities independent of HBV infection. Glycosylated haemoglobin (HbA1c), another critical biomarker of glucose regulation, is correlated to

indexes of hepatic steatosis, including ultrasonography scores (FLUS) and serum cholinesterase (ChE) [11]. On the other hand, hepatic steatosis exerts a prominent effect on both viral dynamics [12, 13] and sustained response to antiviral therapy [14, 15]. Host metabolic abnormality rather than viral factor is verified to be responsible for these effects [6-7, 16].

Single-nucleotide polymorphisms (SNPs) in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), which encodes adiponutrin in hepatocytes, has recently been proposed to confer the genetic susceptibility of hepatic steatosis. *PNPLA3* rs738409 C>G among these ones induces adiponutrin variant of I148M, an isoleucine-to-methionine substitution with reduced activity of triglyceride (TG) hydrolysis, and predisposes normal populations (Chinese, Japanese, Korean, Filipino, Indian, Turk, Belgian, Mexican, American, and so forth) to hepatic steatosis [6, 17–22]. Except for rs738409, some other *PNPLA3* SNPs (rs2281135, rs139051, and rs2294918) also relate to increased risk of hepatic steatosis in ethnic groups of African, Caucasian, East Asian, and Mexican Americans [23, 24]. However, the role of *PNPLA3* SNPs in patients with concurrent chronic hepatitis B and hepatic steatosis has not been well explored. Actions of *PNPLA3* SNPs in BMI, FBG, and insulin resistance (IR) remain controversial until now [24–28].

We, therefore, investigated the *PNPLA3* polymorphisms by deep sequencing in biopsy-proven chronic hepatitis B patients, with or without hepatic steatosis, from Southern, Central, and Northern China. The interaction between *PNPLA3* SNPs and hepatic steatosis was determined by liver pathology. Biochemical characteristics of FBG, HbA1c, and HOMA-IR were further employed to highlight the metabolic effect of *PNPLA3* SNPs in opinion of IR and glucose metabolism.

2. Materials and Methods

2.1. Study Populations. Forty-five normal controls (normal group), 47 patients with only biopsy-proven chronic hepatitis B (CHB group), 37 patients with hepatic steatosis (HS group), and 52 patients with biopsy-proven chronic hepatitis B and hepatic steatosis (CHB+HS group) were enrolled between January 2012 and June 2013. Subjects of normal, CHB, and CHB+HS groups were recruited from Zhengxing Hospital (Zhang Zhou, Southern China, $n = 28$), Xinhua Hospital (Shanghai, Central China, $n = 67$), and Tianjin Hospital of Infectious Diseases (Tianjin, Northern China, $n = 49$). Patients of HS group were recruited from Xinhua Hospital. Participants with the following were excluded: type 2 diabetes, high alcohol intake (>30 g/d for men and >20 g/d for women), chronic HCV infection, autoimmune hepatitis, Wilson's disease, hereditary hemochromatosis, and hepatic steatosis related to current or previous treatment. The study was approved by the Ethics Committee of Xinhua Hospital. Informed consent was obtained from all subjects. Clinical investigations were conducted in appliance with the principles of Helsinki Declaration (1964).

2.2. Demographic, Anthropometric, and Biochemical Analysis. Demographic (age, gender) and anthropometric information (height, weight, BMI, hipline, and waistline) were characterized for the study population. Blood sample was collected from each patient and control subject after a 12-hour fasting. Biochemical tests were performed for measuring the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase (γ-GT) activity, as well as the level of uric acid (UA), total bilirubin (TBIL), FBG, HbA1c, total cholesterol (TC), TG, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using multichannel automatic analyzer (Hitachi 7600, Tokyo, Japan).

2.3. Assessment of Insulin Sensitivity. Serum samples of different groups were harvested as mentioned above. The level of fasting insulin was quantified by ARCHITECT® insulin assay (Abbott Laboratories, Abbott Park, IL, United States) on ARCHITECT i2000 fully automated immunoassay analyzer (Abbott Laboratories, Abbott Park, IL, United States). HOMA-IR was used to evaluate insulin resistance [29].

HOMA-IR = fasting serum insulin (μIU/ml) × fasting plasma glucose (mmol/L) / 22.5.

2.4. Hepatic Pathologic Analysis. Liver samples of patients were collected by needle biopsy after informed consent. Obtained liver tissues were then fixed in 10% buffered formalin, embedded in paraffin, and sliced for hematoxylin-eosin (H&E) evaluation. Hepatic steatosis was graded from 0 to 3 based on the severity of steatosis at histological examination: S0: <5%, S1: 5–33%, S2: 34–66%, and S3: >66% [30].

2.5. Genotyping of PNPLA3 SNPs. Blood samples obtained from the subjects were centrifuged at 1500 rpm for 10 min immediately after sample collection. The buffy-coat layer was separated and transferred into 1.5 mL centrifuge tubes. Genomic DNA was successively extracted from the concentrated lymphocytes of the buffy coat using QIAamp DNA Mini Kit (Qiagen, Benlo, Limburg, Netherlands). Thereafter, the custom Ion AmpliSeq panel (Life Technologies of Thermo Fisher Scientific, Waltham, MA, United States) of *PNPLA3* was designed, with the overall coverage rate of 89.91%. Emulation PCR of the template was performed using the Ion OneTouch 2 System (Life Technologies of Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's instructions. *PNPLA3* variants were genotyped by DNA sequencing using the Ion 318 Chip (Life Technologies of Thermo Fisher Scientific, Waltham, MA, United States) following the Ion PGM 200 Sequencing kit protocol.

2.6. Association Analysis for PNPLA3 SNPs and Clinical Phenotypes. The association test of PNPLA3 SNPs (rs1010023, rs738409), demographic (age, sex), anthropometric (height, weight, hipline, and waistline), and biochemical parameters (TBIL, DBIL, AST, ALT, GGT, ALP, INS, FBG, TC, TG, HDL, LDL, UA, and BUN), was carried out by logistic regression using PLINK v1.07 [31, 32].

Table 1: Demographic, anthropometric, and clinical data.

	Normal group	HS group	CHB group	CHB+HS group	P value
Age (years)	46.05 ± 6.64	38.98 ± 13.55	36.46 ± 11.93	39.82 ± 13.89	<0.001
Gender	M: 27 (60.00%) F: 18 (40.00%)	M: 27 (72.97%) F: 10 (27.03%)	M: 32 (68.09%) F: 15 (31.91%)	M: 38 (73.08%) F: 14 (26.92%)	0.388
BMI (kg/m^2)	23.12 ± 2.18	27.23 ± 3.84	22.59 ± 2.47	27.57 ± 3.35	<0.001
TC (mmol/L)	4.26 ± 0.75	4.67 ± 1.30	4.46 ± 0.86	4.77 ± 0.83	0.021
TG (mmol/L)	1.00 ± 0.35	1.52 ± 0.61	1.15 ± 0.41	1.73 ± 1.29	<0.001
HDL (mmol/L)	1.32 ± 0.24	1.18 ± 0.37	1.36 ± 0.25	1.19 ± 0.32	0.092
LDL (mmol/L)	2.32 ± 0.38	3.01 ± 0.62	2.19 ± 0.57	2.92 ± 0.87	<0.001
ALT (U/L)	13.91 ± 4.00	50.44 ± 22.56	73.85 ± 52.38	61.19 ± 32.59	<0.001
AST (U/L)	20.19 ± 4.84	27.24 ± 14.47	70.56 ± 49.54	33.74 ± 20.51	<0.001
TBIL (μmol/L)	2.22 ± 0.42	4.62 ± 0.93	21.48 ± 14.14	5.86 ± 2.54	<0.001
GGT (U/L)	15.05 ± 4.94	43.91 ± 20.83	73.79 ± 64.96	64.04 ± 39.57	<0.001
ALP (U/L)	16.31 ± 4.61	61.58 ± 16.17	93.66 ± 35.80	89.11 ± 42.48	<0.001
Insulin (μIU/L)	7.51 ± 1.86	34.37 ± 23.61	8.10 ± 2.37	30.95 ± 23.16	<0.001
HOMA-IR	1.11 ± 0.42	8.25 ± 6.07	1.33 ± 0.42	7.38 ± 5.63	<0.001
FBG (mmol/L)	3.57 ± 1.13	5.14 ± 1.17	4.15 ± 0.45	5.48 ± 1.83	<0.001
Hb1Ac (%)	5.28 ± 0.97	5.82 ± 1.29	5.17 ± 1.01	6.12 ± 1.43	<0.001

CHB: chronic hepatitis B; HS: hepatic steatosis; BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; FBG: fasting blood glucose; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; GGT: γ-glutamyltransferase ALP: alkaline phosphatase; HOMA-IR: homeostasis model assessment index.

2.7. Statistical Analysis. The data are expressed as mean ± SD. Age- and gender-adjusted odds ratios (ORs) were calculated using multivariant logistic regression with genotypes, age, and gender as the independent variables. Chi-square test was used to test differences in genotype distribution. Differences among the groups of genotypes were tested by ANOVA using SPSS version 16.0 (SPSS Inc., Chicago, IL, United States). Differences were considered to be statistically significant at a P value < 0.05.

3. Results

3.1. Anthropometric and Clinical Data. Patients with hepatic steatosis (HS group, CHB+HS group) exhibited BMI much higher than that of the chronic hepatitis B patients and normal controls ($P < 0.001$, Table 1). When compared to those without hepatic steatosis (CHB group, normal group), patients of the HS and CHB+HS group also suffered from the increased levels of TG, TC, LDL, and FBG (all $P < 0.05$) and a trend for decreased HLD (Table 1). Impaired glucose homeostasis and lipid metabolism were then suggested in the CHB+HS patients independent of chronic hepatitis B. For the sake of coexisted chronic hepatitis B, which reflects the chronic hepatic inflammation, there was no significant difference in ALT and AST activities between the CHB+HS and CHB groups.

In both HS and CHB+HS groups, subjects with C-allele at *PNPLA3* rs1010023 (CC and TC genotypes) exhibited BMI much lower than those with T-allele (TT genotype) ($P < 0.05$, Table 2). Similarly, a decreasing tendency of BMI characterized the normal subjects with CC and TC genotypes, instead of TT genotype, of *PNPLA3* rs1010023 (Table 2).

3.2. PNPLA3 rs1010023 Associated with Lipid Metabolism and Hepatic Steatosis in CHB Patients. In normal, HS, and CHB+HS groups, subjects bearing C-allele (CC and TC genotypes) at *PNPLA3* rs1010023 demonstrated fasting TG level statistically lower than those with both T-alleles (TT genotype) (CC/TC versus TT: 0.86 ± 0.22 mmol/L versus 1.06 ± 0.37 mmol/L, $P = 0.03$ (normal group); 1.21 ± 0.46 mmol/L versus 1.72 ± 0.64 mmol/L, $P = 0.04$ (HS group); 1.22 ± 0.41 mmol/L versus 1.96 ± 1.48 mmol/L, $P = 0.04$ (CHB+HS group)) (Table 2). Among these subjects, there was a slight reduction in the TC and LDL levels and a moderate increase in the HDL level (Table 2).

Genotyping for *PNPLA3* polymorphisms, the C-allele at rs1010023 (CC and TC genotypes) was associated with hepatic steatosis in CHB patients (odds ratio (OR) = 1.78, 95% confidence interval (CI): 1.05–3.03; $P = 0.03$) (Table 3). A significant association between hepatic steatosis and *PNPLA3* rs1010023 was further confirmed after adjusting for age, gender, and BMI (OR = 1.77, 95% CI: 1.03–3.11; $P = 0.045$) (Table 3). In addition, CC/TC at *PNPLA3* rs1010023 proved their association with patients of the HS group (Table 3). In contrast to the statistical difference of *PNPLA3* rs1010023 polymorphism among CHB+HS and nonsteatosis groups, there was a similar percentage of C-allele at rs1010023 between the groups of normal and CHB, regardless of age, gender, and BMI adjustment (Table 3).

To evaluate the relationship between *PNPLA3* rs1010023 and pathological features, severity of hepatic steatosis was investigated in both CHB+HS and HS groups. As compared to those with TT genotype at rs1010023, patients harboring CC and TC genotypes at rs1010023 showed no association with significant steatosis (>S1) (Table 4).

Table 2: Demographic, anthropometric, and clinical characteristics of all groups subdivided by *PNPLA3* rs1010023.

Indexes	Normal group			HS group			CHB group			CHB+HS group		
	TC/CC	TT	*P*	TC/CC	TT	*P*	TC/CC	TT	*P*	TC/CC	TT	*P*
Age (years)	44.25 ± 6.33	46.72 ± 6.72	0.270	40.70 ± 12.79	37.82 ± 14.10	0.437	38.20 ± 11.27	35.19 ± 12.44	0.316	43.00 ± 13.35	38.23 ± 14.13	0.312
Gender	M: 5 (41.67%) F: 7 (58.33%)	M: 22 (66.67%) F: 11 (33.33%)	0.175	M: 10 (76.92%) F: 3 (23.08%)	M: 17 (70.83%) F: 7 (29.17%)	0.691	M: 15 (83.33%) F: 3 (16.67%)	M: 17 (58.62%) F: 12 (41.38%)	0.077	M: 18 (78.26%) F: 5 (21.74%)	M: 20 (68.97%) F: 9 (31.03%)	0.453
BMI	22.81 ± 2.61	23.68 ± 2.58	0.320	25.16 ± 3.13	28.89 ± 3.14	0.040	22.67 ± 2.62	22.54 ± 2.424	0.957	25.86 ± 3.02	28.43 ± 3.24	0.029
TC (mmol/L)	4.31 ± 0.76	4.24 ± 0.75	0.766	4.60 ± 0.63	4.74 ± 0.96	0.460	4.30 ± 0.76	4.59 ± 0.93	0.298	4.54 ± 0.94	4.89 ± 0.77	0.260
TG (mmol/L)	0.86 ± 0.22	1.06 ± 0.37	0.030	1.21 ± 0.46	1.72 ± 0.64	0.041	1.07 ± 0.38	1.20 ± 0.43	0.260	1.22 ± 0.41	1.96 ± 1.48	0.037
HDL (mmol/L)	1.35 ± 0.25	1.28 ± 0.21	0.495	1.27 ± 0.71	1.16 ± 0.28	0.667	1.37 ± 0.17	1.35 ± 0.16	0.834	1.20 ± 0.29	1.17 ± 0.39	0.814
LDL (mmol/L)	2.29 ± 0.42	2.43 ± 0.21	0.253	2.97 ± 0.49	3.12 ± 0.90	0.597	2.09 ± 0.49	2.27 ± 0.63	0.329	2.92 ± 0.87	2.92 ± 0.88	0.929
ALT (U/L)	13.88 ± 4.36	14.00 ± 3.03	0.913	48.10 ± 20.33	51.92 ± 24.28	0.654	72.41 ± 66.83	73.74 ± 42.14	0.949	57.64 ± 30.08	63.66 ± 34.47	0.491
AST (U/L)	19.45 ± 5.13	20.45 ± 4.79	0.580	24.87 ± 15.77	28.54 ± 13.92	0.488	59.20 ± 35.36	83.56 ± 60.24	0.099	33.18 ± 22.48	34.13 ± 19.37	0.870
TBIL (μmol/L)	2.24 ± 0.47	2.22 ± 0.40	0.901	4.49 ± 0.71	4.79 ± 1.19	0.587	21.84 ± 10.21	21.22 ± 16.67	0.779	5.60 ± 2.23	6.09 ± 2.84	0.636
GGT (U/L)	13.46 ± 4.18	15.76 ± 5.16	0.138	40.63 ± 22.23	45.79 ± 20.60	0.589	63.82 ± 58.98	84.67 ± 70.68	0.286	60.11 ± 40.65	66.92 ± 39.20	0.548
ALP (U/L)	16.90 ± 4.95	15.33 ± 3.98	0.335	57.15 ± 14.64	65.07 ± 16.97	0.231	95.92 ± 41.60	91.90 ± 31.28	0.714	77.50 ± 32.82	97.09 ± 46.83	0.096

CHB: chronic hepatitis B; HS: hepatic steatosis; BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; GGT: γ-glutamyltransferase; ALP: alkaline phosphatase.

Table 3: Association tests of *PNPLA3* rs1010023 with hepatic steatosis.

	Group				OR (95% CI)			*P* value		
	Normal	HS	CHB	CHB+HS	Unadjusted	Adjusted for age, gender	Adjusted for age, gender, BMI	Unadjusted	Adjusted for age, gender	Adjusted for age, gender, BMI
Normal versus CHB	C: 16.67% T: 83.33%		C: 24.49% T: 75.51%		1.310 (0.647–2.654)	1.356 (0.695–2.643)	1.315 (0.674–2.567)	0.453	0.372	0.422
Normal versus HS	C: 16.67% T: 83.33%	C: 32.43% T: 67.57%			2.302 (1.216–4.358)	2.679 (1.348–5.323)	3.460 (1.209–9.904)	0.010	0.005	0.021
Normal versus CHB+HS	C: 16.67% T: 83.33%			C: 37.04% T: 62.96%	2.048 (1.182–3.550)	2.529 (1.335–4.792)	3.018 (1.318–6.914)	0.011	0.004	0.009
CHB versus HS		C: 32.43% T: 67.57%	C: 24.49% T: 75.51%		2.401 (1.264–4.561)	2.334 (1.169–4.657)	2.774 (1.176–6.543)	0.007	0.016	0.020
CHB versus CHB+HS			C: 24.49% T: 75.51%	C: 37.04% T: 62.96%	1.781 (1.046–3.033)	2.170 (1.128–4.174)	1.768 (1.027–3.105)	0.034	0.020	0.045
HS versus CHB+HS		C: 32.43% T: 67.57%		C: 37.04% T: 62.96%	1.298 (0.714–2.359)	1.347 (0.689–2.816)	1.265 (0.705–2.433)	0.392	0.488	0.421

BMI: body mass index; CHB: chronic hepatitis B; CI: confidence interval; HS: hepatic steatosis; OR: odds ratio; SNPs: single nucleotide polymorphisms.

Table 4: Association tests of *PNPLA3* rs1010023 with steatosis grade.

Steatosis grade	HS group		CHB+HS group	
	TC/CC	TT	TC/CC	TT
≤S1	4 (30.77%)	5 (19.23%)	8 (36.36%)	10 (31.25%)
>S1	9 (69.23%)	21 (80.77%)	14 (63.64%)	22 (68.75%)
P value	0.420		0.695	

CHB: chronic hepatitis B; HS: hepatic steatosis.

3.3. PNPLA3 rs1010023 Increased Insulin Sensitivity. The normal, CHB, HS, and CHB+HS groups were stratified by *PNPLA3* genotypes and then subjected to comparisons on the basis of insulin sensitivity and β-cell function. When compared to those of the normal and CHB groups, patients of both HS and CHB+HS group exhibited upregulated fasting insulin and HOMA-IR ($P < 0.05$) (Table 5).

Dramatically, C-allele (CC and TC genotypes) of *PNPLA3* rs1010023 was correlated with HOMA-IR significantly lower than that of T-allele (TT genotype) in both CHB+HS (CC/TC versus TT: 4.98 ± 3.14 versus 9.98 ± 6.64, $P = 0.031$) and HS groups (CC/TC versus TT: 5.65 ± 3.26 versus 11.15 ± 7.29, $P = 0.045$) (Table 5). Similar observations of serum insulin concentration and HOMA-IR, yet without statistical significance, could be obtained in nonsteatosis subjects (normal group, CHB group) with C-allele at rs1010023 (Table 5). Thus, phenotype of *PNPLA3* rs1010023 may sensitize subjects to insulin and attenuate IR in patients with HS.

3.4. PNPLA3 rs1010023 Improved Glucose Homeostasis. Critical indexes (FBG, HbA1c) that related to glucose metabolism were evaluated in blood samples obtained from normal, CHB, HS, and CHB+HS groups, respectively. As a result, obvious upregulation of FBG and HbA1c characterized the patients of HS and CHB+HS groups (Table 1).

In both HS and CHB+HS group, hepatic steatosis patients bearing CC and TC genotypes of *PNPLA3* rs1010023 were susceptible to decreased level of FBG in comparison to those with TT genotype (CC/TC versus TT: 4.81 ± 0.92 mmol/L versus 5.86 ± 2.11 mmol/L (CHB+HS group), $P = 0.017$; 4.27 ± 0.82 mmol/L versus 5.52 ± 1.11 mmol/L (HS group), $P = 0.003$) (Table 5). There was also mild downregulation of FBG in the normal and CHB groups (Table 5).

Besides, decreasing tendency of HbA1c, yet without statistical significance, characterized the subjects containing C-allele at rs1010023 in groups with or without hepatic steatosis (Table 5). Similar observations in both FBG and HbA1c suggested an improving effect of *PNPLA3* rs101002 on glucose homeostasis, especially in chronic hepatitis B patients with hepatic steatosis.

3.5. PNPLA3 rs1010023 Shared Hepatosteatosis Susceptibility but Not Glucometabolic Effect with rs738409. In opinion to the risk of hepatic steatosis, an intimate association of *PNPLA3* SNPs (rs1010023, rs738409) was revealed with statistical significance ($P = 2.18 \times 10^{-26}$) (Figure 1). Despite their similar effect on steatotic susceptibility, *PNPLA3* rs738409 differed from rs1010023 in its glucometabolic characteristics. When compared to those with GG and GC genotypes, the CC genotype of *PNPLA3* rs738409 conferred no risk to increased serum insulin and HOMA-IR in groups of HS and CHB+HS (Table 6). Consistently, there was no statistical difference in both FBG and HbA1c between subjects carrying G- and C-allele at rs738409 (Table 6). *PNPLA3* rs738409, therefore, does not exert significant impact on insulin sensitivity and glucose metabolism.

4. Discussion

Hepatic steatosis, an important component of metabolic syndrome, is now accepted to introduce the pathological disorders of nonalcoholic fatty liver disease (NAFLD) on the basis of "two-hit" mechanism [33]. Hepatocyte-specific lipid (mainly TG) accumulation reflects the "first hit," which is recently resulted from the western lifestyle with high-fat diet in the Chinese population [34]. Then, hepatic steatosis based on lipid accumulation predisposes subjects to the "second hit" of lipoperoxidation and oxidative stress [34]. Thus, hepatic steatosis serves as the initiation of NAFLD, which ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) with clinical outcomes of liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC) [2]. Physiologically, dietary TG is absorbed and transported to hepatocytes by circulating chylomicrons. Low concentration, yet in steady state, of hepatic TG can be diverted from the cytosolic storage pool in a form of serum very low-density lipoprotein (VLDL), and finally be uptaken by systemic adipose tissue [35]. In contrast, steatosis occurs upon the unbalance of TG metabolism, especially excessive acquisition (i.e., high-fat diet) and decreased disposal (fatty acid oxidation and secretion of TG-rich lipoproteins), in hepatoyctes [36]. Dysregulation of hepatic TG metabolism, therefore, is suggested to introduce hepatic steatosis.

PNPLA3, a single-pass type II membrane protein with patatin-like domain at the N-terminal, has been characterized to be the multifunctional enzyme with both triacylglycerol lipase and acylglycerol O-acyltransferase activities in hepatocytes [37]. The effect of *PNPLA3* on triacylglycerol hydrolysis qualifies itself for a pivotal regulator of TG metabolism in the liver [38]. In the present study, C-allele of a novel *PNPLA3* polymorphism, rs1010023, was uncovered to significantly associate with the susceptibility to hepatic steatosis in chronic hepatitis B patients from Southern, Central, and Northern China. This action was further proved to be independent of age, gender, and BMI after statistical adjustment. In consistent with other steatosis-related SNPs (i.e., rs738409, rs2281135, rs139051, and rs2294918) [17–24], *PNPLA3* rs1010023 seems to be loss-of-function in the aspect of TG hydrolysis. Because of its location on the surface of lipid droplets, PNPLA3 with decreased adiponutrin activity in subjects carrying C-allele at rs1010023 is suggested to downregulate the TG lipolysis [39, 40], which successively inhibits the oxidation and mobilization of fatty acids from the liver to peripheral adipose tissues [41]. The accumulation of TG-rich lipid droplets resultantly induces hepatic steatosis with diagnostic criteria of over 5% [30].

Table 5: Association tests of *PNPLA3* rs1010023 with insulin sensitivity glucose and metabolism.

Indexes	Normal group			HS group			CHB group			CHB+HS group		
	TC/CC	TT	*P*	TC/CC	TT	*P*	TC/CC	TT	*P*	TC/CC	TT	*P*
Insulin (pmol/L)	7.32 ± 2.02	8.09 ± 1.24	0.248	3 29.51 ± 17.86	39.25 ± 28.27	0.346	7.07 ± 2.96	8.95 ± 1.49	0.249	25.57 ± 17.55	37.23 ± 27.85	0.226
HOMA-IR	1.04 ± 0.25	1.14 ± 0.47	0.475	5.65 ± 3.26	11.15 ± 7.29	0.045	1.24 ± 0.55	1.41 ± 0.30	0.578	4.98 ± 3.14	9.98 ± 6.64	0.031
FBG (mmol/L)	3.09 ± 0.55	3.79 ± 1.25	0.074	4.27 ± 0.82	5.52 ± 1.11	0.003	4.07 ± 0.45	4.21 ± 0.43	0.309	4.81 ± 0.92	5.86 ± 2.11	0.017
Hb1Ac (%L)	4.92 ± 0.80	5.42 ± 1.01	0.128	5.41 ± 1.33	5.99 ± 1.26	0.234	5.04 ± 1.10	5.31 ± 0.96	0.420	5.54 ± 1.23	6.34 ± 1.64	0.108

CHB: chronic hepatitis B; HS: hepatic steatosis; HOMA-IR: homeostasis model assessment index; FBG: fasting blood glucose.

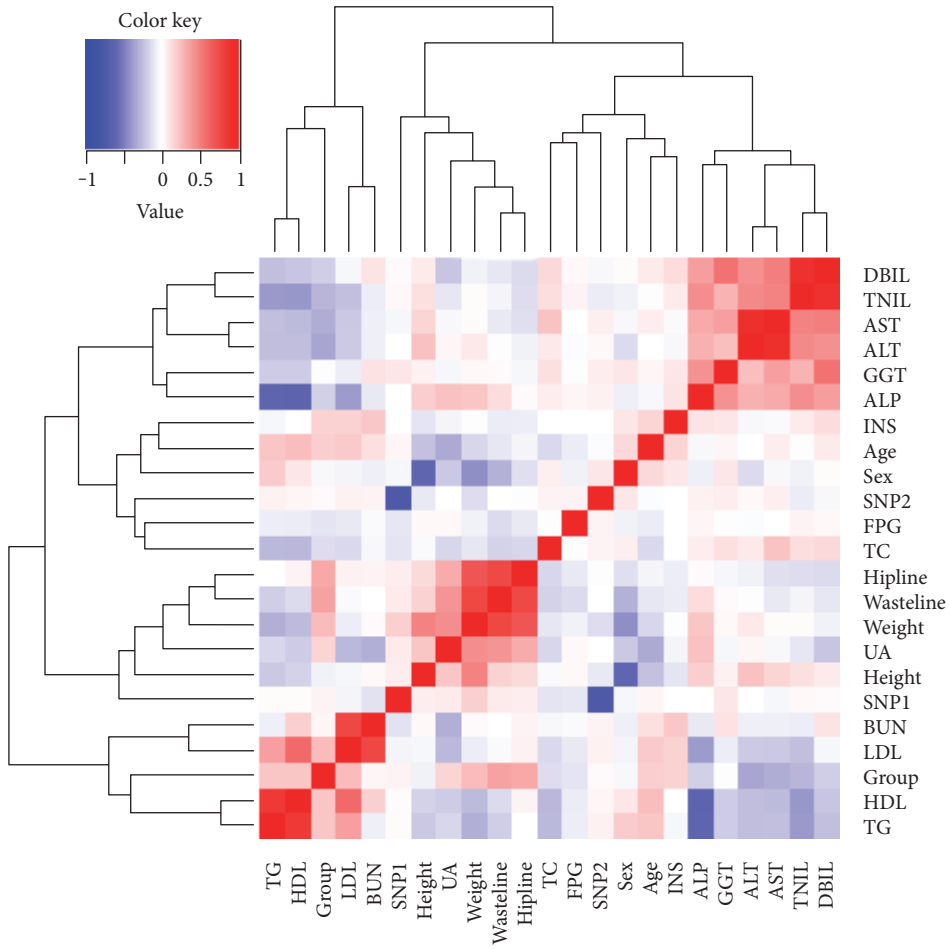

Figure 1: Association results are shown for the *PNPLA3* SNPs and clinical phenotypes. ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; DBIL: direct bilirubin; FBG: fasting blood glucose; GGT: gamma-glutamyltransferase; HDL: high-density lipoprotein; INS: insulin; LDL: low-density lipoprotein; SNP1: *PNPLA3* rs738409; SNP2: *PNPLA3* rs1010023; TBIL: total bilirubin; TC: total cholesterol; TG: triglyceride; UA: uric acid.

To take deep insight into the role of *PNPLA3* rs1010023 within pathological progression, the degree of hepatocyte steatosis was assessed according to the SAF criteria. As a result, no significant association could be observed between *PNPLA3* rs1010023 and the severity of hepatic steatosis (S1 or >S1). Thus, *PNPLA3* rs1010023 is indicated to underlie the occurrence of liver steatosis in chronic hepatitis B patients. Interestingly, the percentage of C-allele at *PNPLA3* rs1010023 was similar between the normal and chronic hepatitis B groups, regardless of age and gender adjustment, suggesting the host metabolism rather than viral infection to be responsible for hepatic steatosis in Chinese chronic hepatitis B patients.

It has been demonstrated that the intracellular TG of hepatocytes undergoes lipolysis, and follows by re-esterification so as to incorporate into VLDL particle within the endoplasmic reticulum [35]. Therefore, reduction of PNPLA3-based lipolysis may minimize the VLDL formation, and subsequently the outward transport of hepatic TG. When compared to those with TT genotype, there was indeed a significant decrease of fasting TG level in subjects carrying CC and TC genotypes at rs1010023, no matter in the groups of normal, HS, and CHB+HS. Similarly, decreased TG level characterizes the NAFLD patients with T-allele at *PNPLA3* rs139051 [24]. These findings shed light on a paradoxical dissociation between hepatosteatosis susceptibility and improved serum TG on the basis of rs1010023, and perhaps other SNPs, of *PNPLA3*.

Resulting from its disturbing effect on TG metabolism, *PNPLA3* rs1010023 plays an important role in the hepatic and peripheral lipid distribution. A theoretic scenario is proposed that C-allele-dependent enzymatic loss of PNPLA3 hampers the TG transportation from hepatocytic lipid droplets to adipose tissues, with clinical features of liver steatosis and lowered serum level of TG, and then protects subjects from progressive obesity [35, 36, 39–41]. As evaluated by BMI, both HS and CHB+HS patients carrying CC and TC genotype of *PNPLA3* rs1010023 showed much less sensitive to obesity in comparison to those with TT genotype.

Recent studies have verified the prominent impact of obesity (BM ≥71.3th–85th percentile) on IR in various age and ethnic groups [42–45]. When assessed at a tissue-specific

Table 6: Association tests of *PNPLA3* rs738409 with insulin sensitivity glucose and metabolism.

Indexes	Normal group			HS group			CHB group			CHB+HS group		
	GG/GC	CC	*P*	GG/GC	CC	*P*	GG/GC	CC	*P*	GG/GC	CC	*P*
Insulin (pmol/L)	7.47 ± 1.89	7.64 ± 1.94	0.897	33.32 ± 18.05	37.76 ± 23.85	0.829	7.36 ± 2.50	8.38 ± 1.64	0.243	28.65 ± 21.13	32.92 ± 25.40	0.645
HOMA-IR	1.23 ± 0.52	1.03 ± 0.35	0.273	7.63 ± 5.73	8.96 ± 5.35	0.696	1.20 ± 0.46	1.46 ± 0.36	0.459	6.32 ± 4.20	8.36 ± 6.04	0.373
FBG (mmol/L)	3.66 ± 1.18	3.23 ± 0.84	0.249	4.75 ± 0.69	5.08 ± 1.39	0.364	4.37 ± 0.48	3.93 ± 0.71	0.217	5.27 ± 0.96	5.52 ± 1.80	0.371
Hb1Ac (%L)	5.39 ± 0.98	4.80 ± 0.83	0.106	5.74 ± 1.13	5.21 ± 1.99	0.597	5.24 ± 0.95	5.09 ± 1.37	0.814	5.94 ± 1.20	5.74 ± 0.94	0.185

CHB: chronic hepatitis B; HS: hepatic steatosis; HOMA-IR: homeostasis model assessment index; FBG: fasting blood glucose.

level, subcutaneous adipose tissue in the nonalcoholic steatohepatitis patients exhibits IR, with much insulin (>6-fold) to cause less suppression of glycerol release (1/2-maxima level), even seriously than that of liver and skeleton muscle [46]. Intra-abdominal fat mass in polycystic ovary syndrome (PCOS) women shows a positive relation to the up-regulated serum level of fasting insulin, indicating the existence of IR [47]. Mechanically, excessive peripheral lipid, no matter the subcutaneous and intra-abdominal fat mass, promotes the release of free fatty acids (FFAs) [48]. Enlarged adipocytes are also integral to the increased secretion of proinflammatory chemokines and cytokines (i.e., MCP-1, TNF-α, IL-1, IL-6, and IL-8) [49–51]. Both FFAs and obesity-induced inflammatory response serve as critical stimulators of systemic IR. As a result, IR specific to peripheral lipid facilitates the dysregulation of glucose metabolism [46, 47, 52]. Our experiments confirmed that rs1010023 C-allele carriers in both HS and CHB+HS groups were protected from IR and hyperglycemia, which featured the TT genotype carriers with significantly elevated levels of HOMA-IR and FBG. Thus, inverse correlation between peripheral lipid and insulin sensitivity may exhibit the mechanisms underlying the improvement of glycolipid metabolism [53]. Moreover, other risk SNPs (e.g., *PNPLA3* rs139051) for NAFLD have recently been revealed to associate with reduced levels of BMI and IR [28, 54].

Except for the results obtained from normal and CHB +HS groups, another noticeable observation of the present study lied in that *PNPLA3* rs1010023 did not associate with the levels of TG, insulin, FBG, and HOMA-IR in chronic hepatitis B patients. The protective role of HBV infection in glycolipid metabolism and related diseases, which are characterized by lower prevalence of NAFLD, hypertriglyceridemia, MetS [3, 6, 55], and IR rate [56], are likely to counteract the effect of PNPLA3 polymorphism. *PNPLA3* rs738409, also known as I148M, has been well established to act as the genetic basis of NAFLD [17–28, 39, 41, 57]. With respect to the association of polymorphisms, *PNPLA3* rs1010023 and rs738409 shared the susceptibility to hepatic steatosis in our experiments. But they differed from each other in aspects of obesity, IR, and glucose metabolism, respectively. In contrast to the obesity risk for T-allele at rs1010023, there was no statistical difference in BMI between subjects with C- and G-allele at rs738409 [57]. Furthermore, similar glycolipid indexes (TG, insulin, FBG, and Hb1Ac) and HOMA-IR characterized the patients with CC-, CG-, and GG-genotypes of rs738409 in both HS and CHB+HS groups [57]. Limited effect of *PNPLA3* rs738409 on the redistribution of total fat mass could be responsible for these presentations.

Some limitations of the study should be considered. By reason of its biopsy-proven, steatosis-predisposing characteristics in parallel to that of other SNPs, *PNPLA3* rs1010023 is supposed to function in a loss-of-function pattern. Nevertheless, mechanic study would highlight the precise role of *PNPLA3* rs1010023 during TG metabolism. Second, comparison of rs1010023 and SNPs other than rs738409 may provide us with preferable understanding of *PNPLA3* polymorphisms related to hepatic steatosis and glycolipid metabolism.

5. Conclusions

PNPLA3 rs1010023 predisposes chronic hepatitis B patients to hepatic steatosis in the Chinese Han population. Contrastively, *PNPLA3* rs1010023 protects them from glucose dysregulation by attenuating the insulin resistance, probably on the basis of BMI reduction.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qin Pan, Mei-Mei Chen, and Rui-Nan Zhang contributed equally to this paper.

Acknowledgments

The work is supported by the State Key Development Program for Basic Research of China (no. 2012CB517501); National Natural Science Foundation of China (nos. 81070322, 81270491, and 81470840); 100 Talents Program (no. XBR2011007h); and Program of the Shanghai Committee of Science and Technology (nos. 09140903500 and 13ZR14267).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 7125760, 6 pages
https://doi.org/10.1155/2017/7125760

Research Article

The Relationship between Cardiovascular Autonomic Dysfunction and Ocular Abnormality in Chinese T2DM

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Received 5 October 2016; Revised 14 February 2017; Accepted 26 March 2017; Published 1 June 2017

Academic Editor: Ruozhi Zhao

Objective. This study aims to explore the relationship between autonomic nerve dysfunction—assessed by cardiovascular autonomic neuropathy risk score (CAN-RS)—and ocular abnormality in Chinese type 2 diabetes mellitus (T2DM). *Method*. This is a cross-sectional study. A total of 335 subjects with T2DM were enrolled. The state of visual acuity, the lens, the vitreous, and the fundus were tested by professional ophthalmic instruments. The electrochemical skin conductance (ESC) of the hands and feet was measured by SUDOSCAN, from which a cardiovascular autonomic neuropathy risk score (CAN-RS) was calculated. Receiver operating characteristic (ROC) curves were drawn to evaluate the feasibility and accuracy of CAN-RS in diabetic oculopathy screening. *Results*. Abnormalities of the lens, vitreous, and fundus accounted for 7.8%, 5.1%, and 9.9%, respectively, in this study. The means of hands and feet ESC were higher than 60 μS, and CAN-RS was $33.1 \pm 14.8\%$. In logistic regression analysis, CAN-RS was positively associated with lens (OR = 1.055, $P < 0.001$) and vitreous (OR = 1.044, $P < 0.01$) abnormality. The area under ROC to detect lens and vitreous abnormality was 0.713 and 0.725, respectively. *Conclusion*. CAN-RS, a cardiac autonomic nerve dysfunction index calculated by SUDOSCAN, may be a promising index for lens and vitreous abnormality screening in T2DM patients. Further studies are needed to confirm the conclusion.

1. Introduction

Diabetic oculopathy is one of common complications in diabetics. Nearly all of the oculopathies can occur in diabetics, including retinopathy, uveitis, cataract, vitreous opacity, glaucoma, and optic neuropathy [1]. The Chinese Diabetes Committee reported that the blindness rate in diabetics was 25-fold higher than that in nondiabetics. So it is important to detect and diagnose diabetic oculopathy early.

Autonomic neuropathy and microangiopathy usually develop in parallel in diabetic patients. Many studies have demonstrated that retinopathy is related to cardiac autonomic neuropathy (CAN) [2–4]. Diabetic retinopathy (DR) may be a strong predictor for CAN [5, 6]. Evidence has shown that the close association between CAN and retinopathy likely stems from changes in the vasomotor control of the small vessels [7]. In these studies, the diagnostic method for CAN mainly refers to cardiovascular autonomic reflex tests (CARTs) [8], which are cumbersome, time consuming, and require strict cooperation. As for DR, the principal tools are funduscope and fluorescein angiography, which require considerable professional skill and time. Under these conditions, it is difficult to screen non- or poorly compliant patients in daily clinical practice, especially in a resource-poor medical environment. It is supposed that there may be other relatively simple substitutes for CARTs such as heart rate variability, postural blood pressure changes, baroreflex sensitivity, and cardiac radionuclide imaging [9] as well as the exercise-related heart rate changes [10, 11], and the relationship between these alternative methods and diabetic oculopathy should be verified further.

Damage to small nerve fibers may develop in the early course of diabetes and can be assessed by sudomotor function testing [10]. SUDOSCAN (Impeto Medical, France) is a recently developed sudomotor function test of the electrochemical skin conductance (ESC) of the hands and feet, which has been used widely in early diagnosis of symmetrical diabetic neuropathy [12, 13]. SUDOSCAN can also be used

for the efficient screening of CAN by means of its proprietary cardiovascular autonomic neuropathy risk score (CAN-RS) [13–15], which is derived from ESC, HbA1c, age, and BMI. Many studies have been published which investigate the relationship between CAN-RS and diseases such as metabolic syndrome [16] and arterial stiffness [17], but its association with diabetic oculopathy remains unexplored. The previous studies usually focus mainly on DR in diabetic patients; however, other types of ocular abnormality are rarely investigated.

This study aims to examine the relationship between CAN-RS and diabetic oculopathy (including fundus lesion and other eye abnormalities) and to further explore whether CAN-RS can be used to screen for diabetic oculopathy.

2. Materials and Methods

2.1. Subject. Type 2 diabetes patients above 18 years of age were enrolled from the outpatient population in the People's Liberation Army (PLA) Diabetes Diagnosis & Treatment Center. Exclusion criteria included patients with tumors, thyroid disease, immunological diseases, and autonomic nervous function disorders with pathogenies other than diabetes. Subjects with autonomic nerve function asymmetry and oculopathy caused by other pathogenesis were also excluded as well as subjects who were unable to cooperate with the inspectors, such as those with diabetic retinal hemorrhage, serious visual impairment, critical disease, and dementia. The study protocol was supported by The 306th Hospital of People's Liberation Army and followed the guidelines of the Declaration of Helsinki. All the participants completed informed consent forms before the study.

2.2. Methods. Participant demographic characteristics, history of chronic disease, diabetes duration, clinical characteristics, and current medications were collected via a questionnaire. Anthropometric measurements including height and weight were carried out by a nurse, and BMI was calculated. Patient blood pressure was measured by an electronic sphygmomanometer (OMRON, Japan). Venous blood samples were collected after 8 hours overnight fasting for biochemical examination, including glucose, glycated hemoglobin (HbA1c), total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), fasting blood glucose (FBG), and fasting insulin (FINS). Postprandial blood glucose and postprandial insulin (PINS) were tested 2 hours after eating snacks.

2.2.1. Eye Examination. All study participants received systematic eye examinations in the ophthalmology clinic of The 306th Hospital of People's Liberation Army. Examination included visual acuity and lens, vitreous body, and fundus exam.

(i) Visual acuity examination: international standard visual acuity chart with light was applied at a distance of 5 meters. Both eyes were checked. We registered uncorrected visual acuity (UCVA) if the subjects did not wear glasses; if they wore glasses regularly, we tested corrected visual acuity (CVA). According to the World Health Organization criteria, blindness is defined as CVA in any one eye is no more than 0.05 [18]. Results were recorded as 0 = nonblind, 1 = blindness.

(ii) The scanning laser ophthalmoscope (England, panoramic 200) was administered by a trained clinical practitioner to check the condition of the fundus. Image analysis was performed by a professional ophthalmologist. The results were recorded as 0 or 1 (0 = normal, 1 = abnormal). If it fails to distinguish the site of turbidity or bleeding in the refractive media, slit lamp and direct ophthalmoscope were applied.

(iii) Slit lamp exam was performed to check for abnormality of the iris and lens. The iris results were recorded as 0 = normal, 1 = posterior synechia, and 2 = neovascularization. The lens results were registered as 0 = normal, 1 = abnormal.

(iv) Direct ophthalmoscope was used to assess the abnormality of the vitreous. Results were recorded as 0 = normal, 1 = opacity or synchysis, 2 = bleeding, and 3 = organization.

2.2.2. Measurement of Sudomotor Function. Sudomotor nerves, the smallest autonomic sympathetic nerves in the human peripheral nervous system, are long, thin, and unmyelinated C-fibers. These have been shown to be susceptible to damage early in the course of diabetes [13, 19]. SUDOSCAN measures skin conductance based on an electrochemical principle; specifically, the ability of sweat glands to release chloride ions activated by an electrical stimulus, which is a surrogate measure of sudomotor function.

Subjects place their feet and hands on electrode plates (nickel-plated stainless steel sensors). The device applies low, incrementally increasing DC current (<4 V) to the skin of the soles and palms. The current on the electrodes attracts chloride ions in the sweat glands by reverse iontophoresis. At such low voltages, the stratum corneum acts as an insulator, permitting chloride ions to pass solely through the sweat duct [20]; this ensures that only sweat gland function is measured. A time/ampere curve is drawn to calculate ESC (μS). When the C-fibers are damaged, ESC values decrease with the reduction of sweat gland function. An ESC $\geq 60\,\mu$S denotes no sweat function impairment, ESC 40–60 μS represents moderate damage, and ESC $\leq 40\,\mu$S represents severe damage. In addition, a cardiovascular autonomic neuropathy risk score (CAN-RS) was calculated automatically by integrating age, gender, HbA1c value, and ESC to assess the risk of cardiovascular autonomic dysfunction. CAN-RS $\geq 25\%$ represents risk of CAN.

2.2.3. Statistical Analysis. Statistical analysis was performed by SPSS version 18.0. Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as n (%). Student's t-test was used to compare the mean difference between binary variables. Conditional

Table 1: Demographic and clinical characteristics of the patients with T2DM.

	Whole population
N	335
Male, *n* (%)	198 (59.1)
Age (years)	54.7 ± 11.8
Duration of diabetes (months)	83.0 ± 73.8
BMI (kg/m^2)	26.0 ± 3.5
SBP (mmHg)	127.3 ± 13.5
DBP (mmHg)	77.2 ± 8.1
BPwl (mmHg)	9.56 ± 4.5
HbA1c (%)	8.1 ± 1.9
TG (mmol/L)	1.8 ± 1.5
Total cholesterol (mmol/L)	4.8 ± 1.4
HDL-C (mmol/L)	1.5 ± 0.4
LDL-C (mmol/L)	2.6 ± 0.9
CRP (mg/L)	1.1 ± 2.6
FBG (mmol/L)	14.7 ± 20.4
PBG (mmol/L)	13.3 ± 9.0
FINS (uIU/mL)	14.7 ± 20.4
PINS (uIU/mL)	51.7 ± 49.4
HESC (μS)	74.1 ± 10.4
FESC (μS)	75.7 ± 13.0
CAN-RS	33.1 ± 14.8

Data are mean ± standard deviation for continuous variables and *n* (%) for categorical variables. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; BPwl: lying to standing blood pressure difference; HbA1c: glycated hemoglobin; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein; FBG: fasting blood glucose; FINS: fasting insulin; PBG: postprandial blood glucose; PINS: postprandial insulin; HESC: hands electrochemical skin conductance; FESC: feet electrochemical skin conductance; CAN-RS: cardiovascular autonomic neuropathy risk score.

logistic regression analysis was conducted to investigate the correlative factors in diabetic oculopathy, and ROC was drawn to evaluate the accuracy of CAN-RS in screening diabetic oculopathy. A two-tailed P value of <0.05 was considered significant.

3. Results

3.1. Demographic Information. Subjects' demographic and clinical characteristics are presented in Table 1. Among the 335 patients, males constituted 59.1%, ages ranged from 18 to 83 years old, and the duration of diabetes was 83.0 ± 73.8 months. The proportion of lens abnormality reached up to 7.8%, and its major clinical manifestation was transparency reduction. The vitreous abnormalities were opacity and synchysis; these accounted for 5.1%. The major abnormal manifestation in the fundus was background diabetic retinopathy (9.9%) which presented as small bleeding dot, hemorrhage blot, hard exudates, and cotton-wool patches.

3.2. The Relationship between Autonomic Nerve Function and Ocular Abnormality. Table 2 presents comparisons of the means of CAN-RS, HESC, and FESC between the different ocular abnormalities. The value of CAN-RS was much higher in the lens abnormality group, as well as the vitreous abnormality group, than that in normal ocular patients with T2DM. The differences were statistically significant. For patients with fundus abnormality, the value of CAN-RS was higher than that in normal subjects, but there was no statistically significant difference. The value of HESC and FESC had no statistical difference among the lens, vitreous, and fundus.

3.3. Association between CAN-RS Value and Ocular Abnormality. To further investigate the relationship between CAN-RS and ocular abnormality, we conducted conditional logistic regression analysis. The following factors were incorporated into the logistic regression model step by step: CAN-RS, BMI, CRP, total cholesterol, TG, LDL-C, HDL-C, FBG, FINS, postprandial blood glucose, and PINS. The results are listed in Table 3. CAN-RS was positively associated with lens abnormality (OR = 1.055, $P < 0.001$) and vitreous abnormality (OR = 1.044, $P < 0.01$), but not related significantly with fundus abnormality.

LDL-C and PINS could be significant factors that influence fundus abnormality. The risk of abnormal fundus can increase 1.5 times when the LDL-C rose 1 mmol/L, while an increase of 1 unit of PINS led to 1.3% lower risk of fundus abnormality.

3.4. The Feasibility and Accuracy of CAN-RS in Screening Ocular Abnormality. Participants were categorized using eye examination as the gold standard. Receiver operating characteristic (ROC) curves of CAN-RS were drawn to investigate its feasibility and accuracy in screening for abnormalities of the lens, vitreous, and fundus (seen in Table 4). The optimum cut-off point of CAN-RS for lens abnormality was 37.5%, with a sensitivity of 77% and a specificity of 66%. The area under curve (AUC) was 0.713 (seen in Figure 1). The AUC for detecting vitreous abnormality was 0.725 with a sensitivity of 72% and a specificity of 69% (seen in Figure 2), while the screening efficiency for fundus abnormality was somewhat low with an AUC of 0.537.

4. Discussion

The American Association of Clinical Endocrinologists (AACE) recommended sudomotor function as a marker to diagnose early peripheral autonomic neuropathy [19]. SUDOSCAN is a simple, noninvasive method [21–23], and various studies have validated its efficacy in both detecting diabetic peripheral neuropathy (DPN) and CAN screening [12, 15, 21]. In this study, we have demonstrated that ESC of the hands and feet were correlated with CAN-RS (HESC, $r = -0.342$; FESC, $r = -0.496$, $P < 0.001$), and also correlated with resting to standing blood pressure difference (HESC, $r = -0.192$, $P < 0.001$; FESC, $r = -0.135$, $P = 0.014$) in CARTs (these results are not shown in Section 3). Based on this correlation, CAN-RS was applied to investigate the relationship between CAN and diabetic oculopathy.

As for CAN-RS, some investigations have released it as an effective assessment of CAN [14, 15, 24]. A large cross-

Table 2: Distribution of autonomic nervous function in normal and abnormal lens, vitreous, and fundus.

	Lens		P	Vitreous		P	Fundus		P
	0	1		0	1		0	1	
CAN-RS	32.3 ± 14.8	43.3 ± 11.6	<0.01*	32.6 ± 14.7	42.0 ± 14.8	0.01*	32.7 ± 14.6	36.5 ± 16.9	0.17
HESC	73.9 ± 10.5	77.3 ± 7.9	0.11	74.1 ± 10.4	74.1 ± 10.1	0.99	73.9 ± 10.5	76.2 ± 9.3	0.21
FESC	76.2 ± 13.0	71.1 ± 13.3	0.06	75.8 ± 13.1	74.3 ± 12.4	0.64	75.6 ± 13.4	77.5 ± 9.2	0.44

Data are mean ± standard deviation. P values are Student's t-test across the three groups. 0 = normal, 1 = abnormal. CAN-RS: cardiovascular autonomic neuropathy risk score; HESC: hands electrochemical skin conductance; FESC: feet electrochemical skin conductance. *$P < 0.05$.

Table 3: Logistic regression analysis of risk factors associated with different ocular abnormalities.

Dependent variable	Equation variables	β	Wald	P	OR	95% CI	
						Upper limit	Lower limit
Lens abnormality	CAN-RS	0.054	12.678	<0.001	1.055	1.025	1.087
	PINS	−0.015	4.064	0.044	0.985	0.972	1.000
Vitreous abnormality	CAN-RS	0.043	6.301	0.012	1.044	1.010	1.080
Fundus abnormality	LDL-C	0.434	3.852	0.050	1.543	1.001	2.379
	PINS	−0.013	4.389	0.036	0.987	0.976	0.999

Data are correlation coefficient (β), Wald value, P for trend, odds ratios, and 95% confidence interval. CAN-RS: cardiovascular autonomic neuropathy risk score; LDL-C: low-density lipoprotein cholesterol; PINS: postprandial insulin.

Table 4: ROC curve of the CAN-RS for abnormality in the lens, vitreous, and fundus.

	AUC	Sensitivity	Specificity	Cut-off value	95% CI of ACU	
					Lower	Upper
Lens abnormality	0.713	0.77	0.66	37.5	0.632	0.819
Vitreous abnormality	0.725	0.72	0.69	39.5	0.577	0.850
Fundus abnormality	0.537	0.34	0.82	46.5	0.457	0.689

sectional study involving 4109 people, suggesting that CAN-RS was associated with arterial stiffness independent of traditional risk factors and glucose tolerance [17]. Zhu et al. showed that there was a link between CAN-RS and the incidence and composition of metabolic syndrome [16]. Beyond this, there have been other studies which have indicated that CAN-RS is an effective indicator of CAN and could potentially take the place of the traditional method (CARTs) [13, 14].

For diabetic oculopathy, researchers still focus mainly on diabetic retinopathy (DR). This study expanded its scope into lens and vitreous lesions in addition to abnormalities of the fundus. Our findings suggest that CAN-RS is associated with lens and vitreous abnormality, which may be explained by their pathogenic mechanisms like endothelial dysfunction [25] and polyol pathway [26]. Eranki et al. [27] measured the sensitivity and specificity of CAN-RS for screening at least one microangiopathy (DPN, diabetic nephropathy, and DR) in diabetics; CAN-RS had a sensitivity of 82% and a specificity of 61%, but for retinopathy specifically, the sensitivity was 74% and the specificity was 63%, similar to our report. However, there is no significant correlation between CAN-RS and fundus abnormality in this study (not correlated with orthostatic blood pressure difference too), which was inconsistent with the previous studies. The reason for this discrepancy may lie in the indirect link between CAN and fundus lesions, the difference in enrolled patient population, or the different experimental methods. Significant symptoms of CAN or DPN did not occur in our patients, and fundus lesions were also mild in this study. Further investigation is required to confirm whether CAN-RS is associated with severe fundus lesions like proliferative retinopathy. In addition, a large cross-sectional study recruiting 1736 type 2 diabetic subjects suggested that insulin therapy was an independent risk factor for severity of DR [28]. Since PINS was a protective factor for the lens and fundus in our study, one might suppose that intensive insulin therapy might improve ocular lesions and postpone the onset of cataracts and retinopathy.

The novelty of this research lies in that it took CAN-RS as a new indicator of CAN, instead of traditional methods, and investigated the relationship between CAN and diabetic ocular abnormalities. In addition, this study expanded the scope of diabetic oculopathy research, not limited to fundus lesions. The main finding revealed that CAN-RS correlated with lens and vitreous abnormality, but not with fundus lesions. The data also demonstrated CAN-RS estimated by SUDOSCAN may be a promising index for lens and vitreous abnormality screening in T2DM. However, a prospective study is needed to further confirm whether CAN-RS can be effectively used to screen diabetic ocular abnormality in clinical practice—our research was only an academic exploration.

Our study had some limitations. First, although the SUDOSCAN test has been proven to be a reliable method

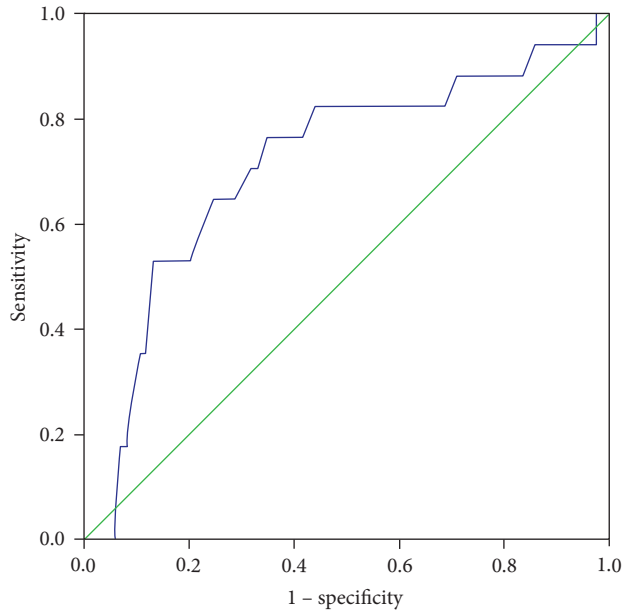

Figure 1: ROC curve for lens abnormality.

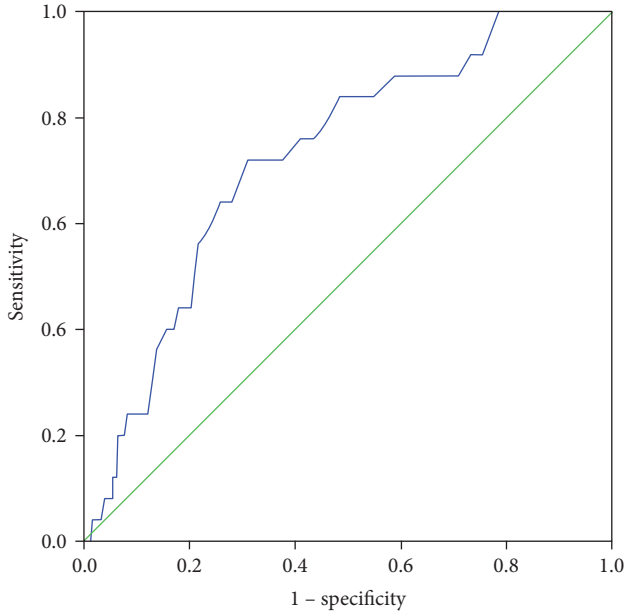

Figure 2: ROC curve for vitreous abnormality.

to assess cardiac autonomic function, and orthostatic blood pressure difference was performed, complete CARTs should be performed. Second, the study sample size was relatively small, and the subjects who completed a variety of eye examinations had few and mild compliances. Therefore, there are some problems and biases in the representation of the general population. Third, for safety, patients were not allowed to stop insulin treatment, hypoglycemic agents, or antihypertensive drugs. We did not assess the influence of drug treatment on the results. Finally, this was a cross-sectional study, and future longitudinal research is needed to validate its conclusions.

In conclusion, our findings suggest that cardiac autonomic nerve dysfunction, measured by CAN-RS, may be correlated with lens and vitreous abnormality in patients with T2DM. Though longitudinal studies are required, CAN-RS could play a promising role in lens and vitreous abnormality screening.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study is supported by grants from the National Natural Science Foundation of China (213300104), Beijing Municipal Science & Technology Commission (Project: Application of Clinical Characteristics in Capital), and National Science and Technology Major Project (2011ZX09307-001-08). The authors would like to thank Mr. Yongen Gu for his guidance of analyzing the data.

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 9328347, 10 pages
https://doi.org/10.1155/2017/9328347

Review Article

Mesenchymal Stem Cells Improve Healing of Diabetic Foot Ulcer

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Received 24 September 2016; Accepted 28 February 2017; Published 12 March 2017

Academic Editor: Ruozhi Zhao

Mesenchymal stem cells (MSCs), an ideal cell source for regenerative therapy with no ethical issues, play an important role in diabetic foot ulcer (DFU). Growing evidence has demonstrated that MSCs transplantation can accelerate wound closure, ameliorate clinical parameters, and avoid amputation. In this review, we clarify the mechanism of preclinical studies, as well as safety and efficacy of clinical trials in the treatment of DFU. Bone marrow-derived mesenchymal stem cells (BM-MSCs), compared with MSCs derived from other tissues, may be a suitable cell type that can provide easy, effective, and cost-efficient transplantation to treat DFU and protect patients from amputation.

1. Introduction

In recent years, with the rapid economic growth and the change of diet structure, the incidence of diabetes mellitus (DM) increased gradually [1, 2]. According to epidemiological surveys, diabetes had spread to 422 million people worldwide by 2014 [3]. And the number of patients with DM may be more than 360 million in 2030 [4]. In addition, huge economic burden from treatment and care of DM is laid on the patients and society [5]. In the US, the cost on diagnosis of DM in 2012 was $245 billion, with a 41% increase compared with the expenditure in 2007 [6].

There is an alarming increase in the macro- and microvascular complications secondary to DM, in which DFU is one of the most common complications. Statistical data has demonstrated that more than a quarter of patients suffered from DFU [7]. According to the International Working Group on Diabetic Foot, risk of DFU increases with increasing age, long history of DM, and high HbA1c [8]. DFU is a complex and severe clinical problem that can lead to subsequent limb amputation. The amputation rate of DM was 19.03% in China in 2015 [9]. At present, patients with DFU are still bearing a high risk of amputation and high costs of treatment and care [10]. In summary, DFU is one of the leading factors that threaten human health and aggravate economic burden [11].

Diverse sources and the potential of self-renewing and multidifferentiation are main characteristics of stem cells, which make stem cell therapy a new alternative to repair and regenerate tissues. Nowadays, a growing number of diseases can be improved via wide applications of stem cell transplantation, such as congenital cataract [12], diabetic retinopathy and keratopathy [13], myocardial infarction [14], ocular surface burns [15, 16], serious skin burns [17, 18], Parkinson's disease [19], Huntington's disease [20], and especially DFU [21]. Accumulating evidence has pointed out that mesenchymal stem cells (MSCs) may enhance wound healing [22–24] and be served as a cell source for many tissue engineering applications including bone regeneration [25], cartilage regeneration [26–28], myocardial regeneration [29], neurogenesis [30, 31], inflammatory bowel diseases [32], and DFU [33, 34]. MSCs exist in many tissues, for example, bone marrow [35, 36], umbilical cord [37, 38], placenta [39, 40], adipose tissue [36, 41–43], gingiva [44, 45], oral mucosa [46], amniotic fluid [47], and brain [48]. However, the appropriate cell type and selection between autologous or allogeneic MSCs are yet to be discussed. Therefore, the present article reviews the roles of autologous or allogeneic MSCs derived from different tissues in wound healing of DFU.

2. BM-MSCs and DFU

2.1. Intrinsic Property. Bone marrow is one of the most common tissues from which MSCs can be acquired. BM-MSCs have no immunologic restriction and do not stimulate

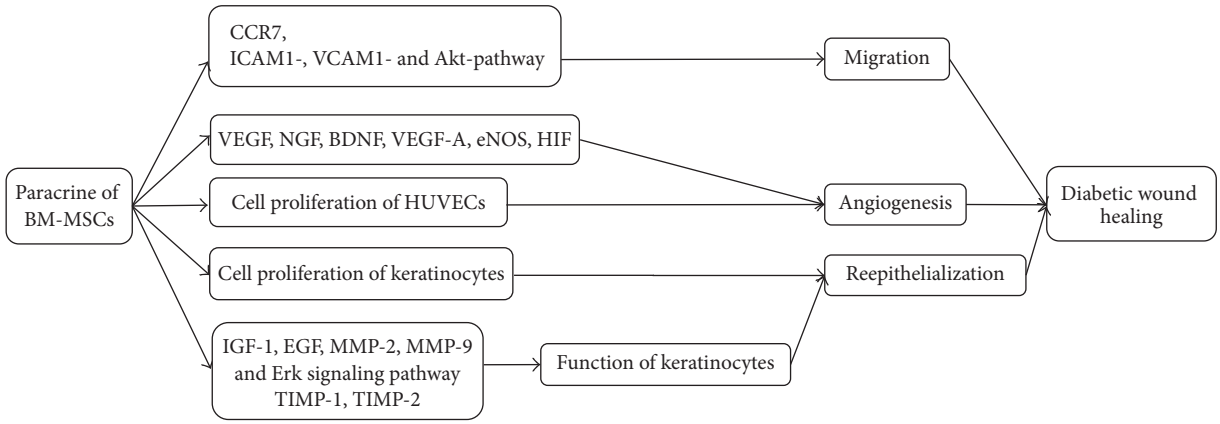

Figure 1: Mechanism of BM-MSCs for treatment of DFU. BM-MSCs can migrate and adhere via CCR7, ICAM1-, VCAM1-, and Akt-dependent mechanism and enhance angiogenesis through increasing VEGF, NGF, BDNF, VEGF-A, eNOS, and HIF. Cell proliferation of HUVECs and keratinocytes plays significant role in angiogenesis and reepithelialization, respectively. Keratinocyte function is improved by regulating IGF-1, EGF, MMP-2, MMP-9, TIMP-1, TIMP-2, and Erk signaling pathway. CCR7: C-C chemokine receptor type 7, ICAM1: intercellular adhesion molecule 1, VCAM1: vascular adhesion molecule 1, VEGF: vascular endothelial growth factor, NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, VEGF-A: vascular endothelial growth factor A, eNOS: endothelial nitric oxide synthase, HIF: hypoxia inducible factor, IGF-1: insulin-like growth factor 1, EGF: epidermal growth factor, MMP-2: matrix metalloproteinase-2, MMP-9: matrix metalloproteinase-9, TIMP-1: tissue inhibitor of metalloproteinase-1, and TIMP-2: tissue inhibitor of metalloproteinase-2.

alloreactivity because they have capability of escaping lysis by cytotoxic T-cell and natural killer (NK) cells, reducing the formation of cytotoxic lymphocytes [49], and suppressing T-cell-derived interferon-gamma (IFN-γ), as well as proliferation of T-cell and NK cells induced by cellular or humoral stimuli in vitro [50, 51]. Thus, BM-MSCs transplantation is a safe way for DFU, and intramuscular transplantation has been proved to have the best efficacy [22]. However, the number and differentiated potential of BM-MSCs decline with aging [52].

2.2. Mechanism

2.2.1. Paracrine. BM-MSCs can enhance the migration, angiogenesis, and reepithelialization via paracrine to accelerate wound repair.

Allogeneic BM-MSCs can migrate and home to the wound area [22] through expressing C-C chemokine receptor type 7 (CCR7) [53] and adhere to endothelial cells (ECs) via intercellular adhesion molecule 1- (ICAM1-), vascular adhesion molecule 1- (VCAM1-), and Akt-dependent mechanism [54].

Wan and colleagues found that allogeneic BM-MSCs could promote angiogenesis and thicken granulation tissue by increasing the expression of vascular endothelial growth factor (VEGF) in diabetic rats [22]. O'Loughlin and colleagues indicated that allogeneic BM-MSCs seeded in a collagen scaffold could improve wound healing by augmenting angiogenesis in diabetic rabbit ear ulcer model [55]. In the study of diabetic mice, neurotrophin-3- (NT-3-) stimulated human BM-MSCs in the biological tissue material expressed VEGF, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and other vascular endothelial factors to upregulate angiogenesis and thicken granulation tissue [56]. Coincidentally, the conditioned medium of heme oxygenase-1- (HO-1-) overexpressing human BM-MSCs promoted the proliferation and migration of human umbilical vein endothelial cells (HUVECs) in vitro. Therefore, the complex of HO-1 -overexpressing human BM-MSCs and collagen biomaterials also could promote angiogenesis and stimulate wound cicatrization in the mice of diabetic ischemic ulcer [57].

In addition, allogeneic BM-MSCs prestimulated with EGF stimulate the neovascularization through the modulation of vascular endothelial growth factor A (VEGF-A), endothelial nitric oxide synthase (eNOS), hypoxia inducible factor (HIF), and VEGF/VEGF receptor pathways in diabetic mice, thereby enhancing the recovery of blood flow [54].

Reepithelialization in the wound is a consequence of cell proliferation and modification of keratinocyte functions. Allogeneic BM-MSCs shortened the duration of wound healing in diabetic foot ulcerations on the plantar skin of rats via the improvement of keratinocytes which had been mentioned in vitro [33]. It has been found that BM-MSCs isolated from rats not only promote human keratinocytes (HKCs) to produce cytokine including matrix metalloproteinase-2 (MMP-2), epidermal growth factor (EGF), and insulin-like growth factor 1 (IGF-1) [33], but also enhance the migration and proliferation of rat keratinocytes by upregulating MMP-2 and matrix metalloproteinase-9 (MMP-9) and downregulating tissue inhibitor of metalloproteinase-1 (TIMP-1) and tissue inhibitor of metalloproteinase-2 (TIMP-2), as well as triggering Erk signaling pathway [58] (Figure 1).

2.2.2. Mobilization of Autologous Stem Cells. Iwamoto and colleagues demonstrated that autologous stem cells mobilized from bone marrow by systemic injections of granulocyte colony-stimulating factor (GCSF) improved wound

bed preparation and accelerated healing in mice [59]; albeit the presence of BM-MSCs in mobilized stem cells was not identified in this study, they were shown to be mobilized by GCSF in previous study [60]. In Tatsumi et al. study, GCSF might promote bone marrow-derived stem cells to mobilize and migrate to the wound site and improve granulation tissue to enhance epithelialization, rather than exerting a direct effect on epithelialization of wounds in both mice and human without diabetes. However, GCSF was unable to enhance epidermal migration from the wound margins in db/db diabetic mice with tail wounds.

The reasons are likely that diabetic microenvironment including hyperglycemia and persistence of inflammation may have an influence on population and function of endogenous stem cell. Compared to wild-type mice, db/db diabetic mice possessed fewer MSCs, of which viability, homing capacity, and therapeutic capacity were impaired [61]. Clinical trials showed that the number of MSCs was decreased and the phenotype of MSCs was altered in patients with diabetic foot syndrome [62]. Based on minimal criteria to define human MSCs [63], MSCs are defined as positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface markers. However, $CD45^-$, $CD29^+$, and $CD90^+$ MSCs were increased in subjects with diabetic foot syndrome [62]. Moreover, migration process was compromised as a result of less expression of adhesion molecules, such as ICAM1 and VCAM1 [54].

2.3. The Safety and Efficacy of Clinical Trials. In clinical trials, autologous transplantation of BM-MSCs can significantly ameliorate clinical parameters including decrease in wound size and increase in pain-free walking distance and maintain normal liver and renal function following intervention [64]. Leg perfusion is also sufficiently improved to minimize major amputations [65, 66]. It has been discovered that autologous biograft in combination with BM-MSCs decreases wound size and increases dermal vascularity and thickness in patients with DFU [67].

At 6 weeks after intramuscular injection of autologous BM-MSCs, the ulcer healing rate of T2DM patients with bilateral critical limb ischemia (CLI) and foot ulcer increased significantly. After 24 weeks of follow-up, painless walking time, limb perfusion, ankle-brachial index (ABI), transcutaneous oxygen pressure (TcO_2), and magnetic resonance angiography (MRA) analysis were also improved significantly [34] (Table 1).

3. Umbilical Cord Blood-Derived Mesenchymal Stem Cells (UCB-MSCs) and DFU

3.1. Intrinsic Property. UCB-MSCs have a similar morphology, cell surface antigens, and the potential of differentiation into BM-MSCs and umbilical cord-derived mesenchymal stem cells (UC-MSCs) [42, 52, 68]. Additionally, UCB-MSCs have several advantages, such as short doubling time [69], long viable time, and anti-inflammatory activity [42]. Thus, UCB-MSCs are considered as convenience and abundance seed cells for regenerative medicine.

3.2. Mechanism

3.2.1. Paracrine. Animal studies have indicated the ability of human UCB-MSCs to prevent or cure DFU via angiogenesis and the expression of nerve growth factor (NGF) in femoral nerve innervated gastrocnemius of diabetic foot ulceration rats [70]. In vitro, human UCB-MSCs might have capacity for diabetic wound healing by producing VEGF and basic fibroblast growth factor (bFGF) [71].

3.3. The Safety and Efficacy of Clinical Trials. It has been reported that transplantation of allogeneic UCB-MSCs injected into the quadriceps thigh muscles of individuals with DFU improves clinical profiles. All patients following allogeneic UCB-MSCs transplantation have decreased blood glucose, insulin dosage, levels of C-reactive protein (CRP), and tumor necrosis factor α (TNF-α), as well as increased VEGF and the ratios of $CD4^+CD25$ (hi) $FoxP3^+$Treg/Th17 and $CD4^+CD25$ (hi) $FoxP3^+$Treg/Th1 cells. Moreover, the ratio of Treg/Th17 also had a correlation with the levels of VEGF and interleukin-6 (IL-6) detected in the plasma of patients [72].

However, a phase I study on patients with CLI indicated that intramuscular injection of allogeneic UCB-MSCs improved symptoms or clinical parameters with some side effects. Adverse events including whole body urticaria, diarrhea, oral ulceration, and elevation of serum creatinine level were observed in three patients; however, all conditions were resolved in short order [73].

Up to now, the application of UCB-MSCs for DFU is little. We consider that the extraction of UCB-MSCs involved in privacy and ethic may be a concern; meanwhile the cost in preservation of umbilical cord blood is very high (Table 1).

4. MSCs Derived from Other Tissues

Up to date, preclinical studies on adipose-derived mesenchymal stem cells (AMSCs), umbilical cord-derived mesenchymal stem cells (UC-MSCs), placenta-derived mesenchymal stem cells (PMSCs), and human amniotic fluid-derived stem cells (AF-MSCs) for diabetic wound healing have been reported, but no clinical trials have been reported. However, human gingiva-derived mesenchymal stem cells (GMSCs) are only investigated in excisional wound model, and the data are quite limited.

4.1. AMSCs and Diabetic Wound Healing

4.1.1. Intrinsic Property. Adipose tissue derived from the mesenchyme is widely distributed and easily isolated. AMSCs have high colony frequency and represent an attractive alternative source of pluripotent cells, whose characteristics are similar to BM-MSCs [42, 74].

4.1.2. Mechanism. In diabetic rats with dorsal full-thickness skin wound, allogeneic AMSCs injected subcutaneously in

Table 1: Clinical trials of BM-MSCs and UCB-MSCs.

First author	Publication year	Cellular type	Object	Delivery method	Duration of observation	Clinical parameters
Dash	2009	Autologous BM-MSCs	24 patients with nonhealing ulcers of the lower limb (diabetic foot ulcers and Buerger disease)	Autologous cultured BM-derived MSCs along with standard wound dressing	12 weeks	Decrease in wound size, increase in pain-free walking distance, maintain normal liver and renal function, improve leg perfusion sufficiently
Amann	2009	Autologous BM-MSCs	51 patients with impending major amputation due to severe critical limb ischemia	Intramuscular transplantation	6 months	Improve leg perfusion sufficiently to reduce major amputations and permit durable limb salvage, reduce analgesics consumption, increase in pain-free walking distance
Vojtassak	2006	Autologous biograft composed of autologous skin fibroblasts on biodegradable collagen membrane (Coladerm) in combination with autologous BM-MSCs	Patients with diabetic foot	Directly to the wound and injected into the edges of the wound, finally covered with prepared autologous biograft, received two additional treatments with cultured MSC on days 7 and 17	29 days	Decrease in wound size and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed
Lu	2011	Autologous BM-MSCs	41 type 2 diabetic patients with bilateral critical limb ischemia and foot ulcer	Intramuscular injection	24 weeks	Increase in pain-free walking distance, improve leg perfusion, ankle-brachial index (ABI), transcutaneous oxygen pressure (TcO_2), magnetic resonance angiography (MRA) analysis
Procházka	2010	Autologous BM-MSCs	96 patients with critical limb ischemia and foot ulcer	Inject into the ischemic limb along the posterior and anterior tibial artery	120 days	79% limb salvage in patients
Li	2013	Allogeneic UCB-MSCs	15 diabetic patients with foot disease	10 mL is injected intramuscularly into impaired lower limbs and 2 mL is delivered into the basilar portions of foot ulcers and the surrounding subcutaneous tissues	12 weeks	Weakness, numbness, pain, cold feeling, or intermittent limp, skin temperature, ABI, and transcutaneous oxygen pressure (TcO_2) are improved

BM-MSCs: bone marrow-derived mesenchymal stem cells and UCB-MSCs: umbilical cord blood-derived mesenchymal stem cells.

the wound margin stimulated neoangiogenesis and increased tissue regeneration through paracrine and autocrine mechanisms [75]. The data showed that allogeneic AMSCs migrated to the wound margin and increased angiogenesis via the activation of endothelial activity and neoangiogenic capacities by increasing VEGF and von Willebrand factor (vWF). Simultaneously, as a proliferating cell nuclear antigen, Ki-67, was up-regulated to promote cellular proliferation. The proinflammatory reaction was reduced through the expression of EGF, VEGF, and prolyl 4-hydroxylase (rPH). Consistent with this notion, allogeneic AMSCs were harvested from the inguinal fat of normal rats secreted large amounts of several angiogenic growth factors including VEGF, hepatocyte growth factor (HGF), transforming growth factor beta 1 (TGF-β1), IGF-1, EGF, and keratinocyte growth factor (KGF) in vitro. In vivo, the transplantation of AMSCs sheets was created using cell-sheet technology accelerated wound healing and vascularization in full-thickness skin defects in Zucker diabetic fatty rats [76].

Additionally, direct injection of ASCs obtained from nondiabetic patients into full-thickness wound of diabetic mice model significantly increased the rate of wound closure [77]. In another study on diabetic mice, the new findings that silk fibroin patches cellularized with human adipose-derived MSCs (Ad-MSCs-SF) and silk fibroin patches decellularized with human adipose-derived MSCs (D-Ad-MSCs-SF) patches improved tissue regeneration and reduced the wound area through releasing angiogenic factors and collagen deposition stimulating molecules [78]. A decrease in the risk of transferring genetically mutated cells and the possibility of stimulating the immune system were the advantage of D-Ad-MSCs-SF patches, and decellularized patches could be prepared and stored for an extended period.

4.2. UC-MSCs and Diabetic Wound Healing

4.2.1. Intrinsic Properties. UC-MSCs are generally considered to be rich, safe, of short doubling time, and easy to collect [52]. Compared to BM-MSCs, it has been well documented that UC-MSCs have similar characteristics involving fibroblastic morphology, typical immunophenotypic markers, and multiple differentiation potential to BM-MSCs [79–82]. In addition, the trait of UC-MSCs has lower immunogenicity [83, 84].

4.2.2. Mechanism. In the study on DFU rats with UC-MSCs delivered through the left femoral artery, researchers found that UC-MSCs could specifically localize to the targeted area by detecting the expression of human leukocyte antigen type-I (HLA-1), a marker to track UC-MSCs in vivo.

Besides, UC-MSCs significantly reduced the size of foot ulcers and promoted epithelialization of ulcerated tissue via release of cytokeratin 19 from keratinocytes and formation of extracellular matrix [21]. In other studies of DFU rats, the data showed that administration of UC-MSCs contributed to improvement of vascular density [85, 86] and repair of wound and sensory functions [87] by the expression of VEGF, keratinocyte growth factor (KGF), platelet derived growth factor (PDGF), and brain-derived growth factor (BDGF).

4.3. PMSCs and Diabetic Wound Healing

4.3.1. Intrinsic Property. Placental tissue is readily available and can isolate a large number of MSCs for clinical application [88]. What is more, the morphology, size, surface phenotype, and immunosuppressive characteristics of PMSCs are similar to BM-MSCs, and the proliferation capability is better [39]. The best efficacy delivery is intraperitoneal injection [89].

4.3.2. Mechanism. In the research of diabetic Goto-Kakizaki (GK) rats, the experimental group showed that implanted PMSCs gathered to the wound tissue and differentiated into endothelial-like cells. Additionally, it has been found that PMSCs participate in angiogenesis in wound bed through secreting some proangiogenic molecules, such as VEGF, bFGF, and IGF-1, transforming growth factor-β (TGF-β) and hepatocyte growth factor (HGF) [90].

4.4. AF-MSCs and Diabetic Wound Healing.

Large numbers of human AF-MSCs can be easily harvested from as little as 2 mL of amniotic fluid [91]. Human AF-MSCs remain stable and show high proliferative capacity, multilineage differentiation potential, immunomodulatory activity, and lack of significant immunogenicity [92].

The transplantation of human AF-MSCs has been shown to accelerate wound healing by secreting factors [93] to stimulate proliferation and migration of dermal fibroblasts. In full-thickness excisional wound of diabetic NOD/SCID mice, human AF-MSCs significantly accelerated wound closure through increasing the angiogenic factors, IGF-1, EGF, and interleukin-8 (IL-8), as well as enhancing reepithelialization by expressing keratinocyte-specific proteins and cytokeratin in the wound area [94]. Additionally, in a model of mouse with excisional wound, human AF-MSCs significantly enhanced wound healing via the TGF-beta/SMAD2 pathway [95], while human AF-MSCs accelerated wound closure through TGF-β/SMAD2 and PI3K/Akt pathways under the condition of hypoxia [96].

4.5. GMSCs and Wound Healing.

Human GMSCs are homogenous, not tumorigenic [97], and easy to be isolated [98] and display stable phenotype. The most significant advantage of human GMSCs is without any ethical problems in clinical application [99]. Moreover, human GMSCs show a greater capacity of proliferation and migration than AMSCs [100] and BM-MSCs without growth factors [99].

In a murine excisional full-thickness skin wound model, systemic infusion of human GMSCs mitigated local inflammation mediated via suppression of inflammatory cells infiltration, production of IL-6 and TNF-α, and increasing expression of interleukin-10 (IL-10) [101]. This mechanism also existed in the hypoxic environment [102]. In addition, human GMSCs have elicited M2 polarization of macrophages, which may contribute to rapid reepithelialization, improvement of angiogenesis, and tissue remodeling of skin wound [101].

5. Are Autologous or Allogeneic MSCs More Appropriate?

It has been shown that autologous BM-MSCs are a major source and have obvious efficacy in cell therapy for patients suffering from DFU. Most recently, a study on the feasibility of autologous stem cell therapy in diabetic patients showed that AMSCs isolated from distal limbs of diabetic patients with critical ischemia was not satisfactory as an autologous AMSC source because of its improper phenotype and function [103]. In line with above evidence, the initial viability of the mouse MSCs extracted from the bone marrow of diabetic mice was poor in a normal glucose environment in vitro, but the expansion of that was subsequently improved [61].

Although allogeneic MSCs have had potent immunosuppressive properties, evidence also suggests that they elicit potential as a new therapeutic strategy for the treatment of DFU in animal models. Moreover, allogeneic UCB-MSCs have been successfully used to treat patients with DFU. With increasing number of clinical trials of allogeneic MSCs for acute and chronic diseases [104–107], a comprehensive understanding of the difference in immunological profile is essential.

Hence, the potential for autologous or allogeneic MSCs to be used to improve diabetic wound healing appears particularly promising. However, so far preclinical and clinical data are quite limited and further studies need to be explored for the feasibility of autologous and allogeneic MSCs therapy of DFU.

6. The Further Treatment for DFU

Recent studies showed that a transgenic *L. sericata larvae* could secrete platelet derived growth factor-BB (PDGF-BB), a dimeric peptide growth factor that could bind to the platelet derived growth factor (PDGF) receptor and stimulate cell proliferation and survival, and, hence, promote wound healing. It may be a cost-effective manner for nonhealing wounds, especially for patients with DFU [108] and be employed in regenerative medicine strategies to enhance tissue repair.

7. Conclusion

A variety of clinical applications need large number of functionally competent MSCs with stable phenotype to achieve successful results.

From the above, the morphology, size, and surface phenotype of MSCs derived from different tissues have no significant difference. Besides BM-MSCs, others possess rich source and greater proliferation capability and can be easily isolated. In addition, UC-MSCs and human AF-MSCs have lower immunogenicity, while AMSCs and human GMSCs pose fewer ethical problems. Although BM-MSCs have some limitations, they are firstly discovered and deeply studied in many clinical trials with satisfactory clinical efficacy. This paper supports the potential of BM-MSCs for treatment of DFU, and it may be the optimal cell type for safe and feasible transplantation of DFU.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This paper was funded by Scientific Foundation of the Science Technology Department of Jilin Province (3D515N603428) and Scientific Foundation of the Ministry of Education, Science and Technology Development Center (3M213BR43428).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 4845108, 8 pages
https://doi.org/10.1155/2017/4845108

Research Article

The Association between Leisure-Time Physical Activity and Risk of Undetected Prediabetes

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Received 16 October 2016; Revised 21 January 2017; Accepted 19 February 2017; Published 6 March 2017

Academic Editor: Amy L. Hui

Aims. The purpose of the study was to assess the effects of leisure-time physical activity on undetected prediabetes. *Methods*. Data from the National Health and Nutrition Examination Survey 2007–2012 were used in our analyses. Logistic regression was conducted to estimate the odds ratios (ORs) with 95% confidence intervals (CIs) of prediabetes associated with leisure-time physical activity. *Results*. A total of 8204 subjects were eligible for our analyses. For all subjects, high level of total leisure-time physical activity (OR = 0.78, 95% CI: 0.66, 0.94) and low level of vigorous leisure-time physical activity (OR = 0.72, 95% CI: 0.58, 0.90) were inversely associated with the risk of prediabetes in multivariate-adjusted model. For subjects under 45 years of age, high level of total leisure-time physical activity (OR = 0.78, 95% CI: 0.61, 0.99) and low (OR = 0.61, 95% CI: 0.45, 0.83) and high (OR = 0.72, 95% CI: 0.53, 1.00) level of vigorous leisure-time physical activity were associated with a decreased risk of prediabetes. In the 45 to 65 age group, only high level of total leisure-time physical activity (OR = 0.73, 95% CI: 0.57, 0.95) had protective effect on prediabetes. *Conclusions*. Leisure-time physical activity may be associated with a decreased risk of prediabetes.

1. Introduction

Prediabetes refers to the condition in which blood glucose concentration is higher than normal but not high enough for a diagnosis of diabetes and indicates an increased risk for the future development of diabetes and associated complications [1–3]. In 2012, the estimated percentage and number of US adults with prediabetes were 37% and 86 million, respectively [4]. Results from the Health Survey for England revealed that the prevalence of prediabetes increased from 11.6% to 35.3% during 2003–2011 in England [5]. A cross-sectional survey in a nationally representative sample of Chinese adults showed that the incidence of prediabetes was 50.1%, which represented up to 493.4 million patients with prediabetes in China in 2010 [6]. Even with a high prevalence, the awareness rate of prediabetes was very low. Research from the National Health and Nutrition Examination Survey (NHANES) showed that the estimated awareness rate of prediabetes was less than 14% in the US during 2005–2010 [7]. Hence, it is very important to pay attention to and prevent prediabetes considering the significant public health impact.

Physical activity is beneficial to the prevention of many chronic diseases [8]. A meta-analysis based on prospective studies found that various types of physical activity were beneficial to the prevention of diabetes, and the risk of diabetes decreased by 15% for 20 MET-hours/week increment of leisure-time physical activity [9]. Furthermore, several randomized controlled trials reported that physical activity could improve insulin sensitivity and glucose tolerance and then delay the onset of diabetes in subjects with prediabetes [10–13]. Leisure-time physical activity (sports, exercise and recreational activity, etc.) has more advantages than work-related physical activity. It allows a more flexible schedule than work-related physical activity and cannot cause strain associated with vigorous-intensity work. Therefore, leisure-time physical activity may be a good choice for the prevention of chronic diseases. But there are few studies about the association between leisure-time physical activity and the risk

of prediabetes. Thus, we performed analyses on a subsample of NHANES 2007–2012 to assess the association between leisure-time physical activity and the risk of prediabetes.

2. Methods

2.1. Study Population. Data from NHANES 2007–2012 were used in this study. With a complex, multistage probability design, NHANES examines a nationally representative sample of the US civilian noninstitutionalized population of all ages. NHANES participants undergo an at-home health interview and a clinic examination in a specially designed and equipped mobile examination center (MEC). The clinical examination lasts 3-4 hours and all data collection methods are standardized to minimize site-specific bias. All participants provided informed consent for both the at-home interview and MEC examination [14]. The response rates of interviewed sample were 78.4%, 79.4%, and 72.6% for 2007-2008, 2009-2010, and 2011-2012, respectively. And the response rates of examined sample were 75.4%, 77.3%, and 69.5% for 2007-2008, 2009-2010, and 2011-2012, respectively [15]. A total of 30,442 individuals participated in the NHANES during 2007–2012. We excluded individuals with hemoglobin A1c (HbA1c) ≥ 6.5% (≥48 mmol/mol) (1376) or self-reported diabetes or receiving diabetes treatment (1241) and individuals with self-reported prediabetes (419) or self-reported borderline diabetes (164). We further excluded the participants with a history of congestive heart failure, coronary heart disease, angina pectoris, heart attack, emphysema, and chronic bronchitis. Besides, participants without data about body mass index (BMI), race, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, physical activity, and measurements of blood pressure (BP) and HbA1c were also excluded. Finally, 8204 subjects between 20 and 65 years old were included in our analyses.

2.2. Prediabetes Assessment. Prediabetes was defined according to HbA1c criteria of American Diabetes Association (ADA) [1]. In 2015, ADA recommended that the A1c test should be performed using a method that was certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trail (DCCT) reference assay [1]. By reviewing the NHANES laboratory and participant HbA1c data, the NGSP group concluded that the NHANES laboratories met the NGSP criteria for bias and precision [16]. And participants with an HbA1c level of 5.7–6.4% (39–46 mmol/mol) were classified as having prediabetes [1].

2.3. Physical Activity Assessment. Each NHANES participant completed a physical activity questionnaire which was based on the Global Physical Activity Questionnaire (GPAQ) [17]. The physical activity questionnaire included questions related to daily activities and leisure-time activities. The activity information was collected before the physical examination in the home using the Computer-Assisted Personal Interviewing (CAPI) (interviewer administered) system [17]. Vigorous-intensity activities are activities that require hard physical effort and cause large increases in breathing or heart rate, and moderate-intensity activities are activities that require moderate physical effort and cause small increases in breathing or heart rate [17]. Work-related physical activity refers to paid or unpaid work, studying or training, household chores, and yard work. Leisure-time physical activity refers to sports, fitness, and recreational activities. The suggested metabolic equivalent (MET) scores for vigorous work-related physical activity, moderate work-related physical activity, walking or bicycling for transportation, vigorous leisure-time physical activity, and moderate leisure-time physical activity were 8.0, 4.0, 4.0, 8.0, and 4.0, respectively [17]. The average number of hours per week spent in each activity was multiplied by the suggested MET scores to get an estimate of MET-hours per week. MET-hours per week of vigorous and moderate work-related physical activity were summed to obtain an estimate of total work-related physical activity. MET-hours per week of vigorous and moderate leisure-time physical activity were summed to obtain an estimate of total leisure-time physical activity.

2.4. Covariates. Demographic information included age, gender, race (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race), and educational level (less than 9th grade, 9th–11th grade, high school graduate/GED or equivalent, some college or AA degree, and college graduate or above). Other covariates included BMI, BP, smoking status (smoking at least 100 cigarettes in life or not), alcohol consumption (having at least 12 alcohol drinks/year or not), dietary pattern (excellent healthy, very good healthy, good healthy, fair healthy, and poor healthy), and daily total energy intake. Educational level was divided into <high school, high school, and >high school. Weight (kg) and height (cm) were measured according to standard procedures and BMI was calculated as weight in kilograms divided by height in meters squared [18]. The BMI was divided into <25.0, 25.0 to <30.0 and ≥30.0 kg/m^2. After resting quietly in a seated position for 5 minutes and determining the maximum inflation level, three consecutive BP readings were obtained [19]. The average value of three measurements was calculated to identify hypertension. Participants were identified as hypertensive if the mean systolic BP was ≥140 mmHg or diastolic BP was ≥90 mmHg or receiving antihypertensive medicine [20]. All NHANES participants were eligible for two 24-hour dietary recall interviews. The first dietary recall interview was collected in person in the MEC and the second interview was collected by telephone 3 to 10 days later [21]. Daily total energy intake was calculated as the mean of the total energy intake in the first and second interview.

2.5. Statistical Analysis. t-tests were used to compare the mean levels of continuous variables between subjects with and without prediabetes if the variables conformed to the normal distribution. Nonparametric tests were used to compare the averages of continuous variables between subjects with and without prediabetes if the variables were nonnormal data. Chi-square tests were used to compare the prevalences of categorical variables between subjects with and without

prediabetes. The MET-hours per week of each leisure-time physical activity were divided into tertiles based on the distribution in the study sample. The tertiles of each leisure-time physical activity represented low, moderate, and high level of leisure-time physical activity, respectively, with 0 MET-hours per week as the referent group. Logistic regression analyses were conducted to assess the associations between total, vigorous, and moderate leisure-time physical activity and the risk of prediabetes, respectively. Crude odds ratios (ORs), age- and gender-adjusted ORs, and multivariate-adjusted ORs (age, gender, BMI, race, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation) were calculated from logistic regression analyses with 95% confidence intervals (CIs). Then, the above-mentioned logistic regression analyses were conducted separately by age (<45 and ≥45) to assess the protective effects of total, vigorous, and moderate leisure-time physical activity on prediabetes in different age groups.

All statistical analyses were performed with STATA version 12.0 (Stata Corporation, College Station, TX, USA). All reported probabilities (P values) were two-sided with $P < 0.05$ considered statistically significant.

3. Results

Characteristics of subjects by prediabetes status were shown in Table 1. Of the 8204 subjects without diabetes and self-reported prediabetes, 1914 (23.33%) were identified as having undetected prediabetes based on HbA1c criteria. The percentage of undetected prediabetes was significantly higher in the 45- to 65-year group. Subjects with prediabetes tended to be male and non-Hispanic Black. Subjects with prediabetes were significantly more likely to be obese and hypertensive than controls. Compared with normal subjects, those with prediabetes received a lower level of education. The proportion of subjects who smoked at least 100 cigarettes in life was higher in the group with prediabetes. The proportion of subjects who had at least 12 alcohol drinks per year was lower in the group with prediabetes. The proportion of subjects who had excellent and very good healthy dietary pattern was significantly lower in the group with prediabetes. The daily total energy intake in subjects with prediabetes was significantly lower than that in controls. Total, vigorous, and moderate leisure-time physical activity were significantly lower in subjects with prediabetes. However, no statistically significant differences were found in terms of total work-related physical activity and walking or bicycling for transportation between subjects with and without prediabetes.

The ORs (95% CIs) of prediabetes according to category of leisure-time physical activity for all subjects were shown in Table 2. The crude ORs of prediabetes indicated that any level of total, vigorous, and moderate leisure-time physical activity was associated with a decreased risk of prediabetes (OR = 0.46–0.77). After adjustment for age and gender, the results were consistent with the crude ORs (OR = 0.61–0.80). Additional adjustment for BMI, race, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation, high level of total leisure-time physical activity (OR = 0.78, 95% CI: 0.66, 0.94), and low level of vigorous leisure-time physical activity (OR = 0.72, 95% CI: 0.58, 0.90) were inversely associated with the risk of prediabetes, respectively. However, the protective effect of moderate leisure-time physical activity on prediabetes was no longer statistically significant after adjustment for more covariates.

The associations between leisure-time physical activity and the risk of prediabetes in different age groups were shown in Table 3. For subjects younger than 45 years of age, any level of total and vigorous leisure-time physical activity was associated with a decreased risk of prediabetes in unadjusted model and age- and gender-adjusted model. Compared with no moderate leisure-time physical activity, the low level of moderate leisure-time physical activity was inversely associated with the risk of prediabetes in unadjusted model and age- and gender-adjusted model. Additional adjustment for BMI, race, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation and high level of total leisure-time physical activity were inversely associated with the risk of prediabetes (OR = 0.78, 95% CI: 0.61, 0.99). In multivariate-adjusted model, the ORs (95% CIs) of prediabetes were 0.61 (0.45, 0.83), 0.76 (0.56, 1.03), and 0.72 (0.53, 1.00) for the lowest through the highest tertile of vigorous leisure-time physical activity. However, the inverse association between low level of moderate leisure-time physical activity and the risk of prediabetes was not statistically significant in multivariate-adjusted model.

In the 45- to 65-year group, any level of total, vigorous, and moderate leisure-time physical activity was associated with a decreased risk of prediabetes in unadjusted model and age- and gender-adjusted model. Additional adjustment for BMI, race, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation, however, largely attenuated the protective effects of vigorous and moderate leisure-time physical activity on prediabetes. The inverse association between high level of total leisure-time physical activity and prediabetes was slightly attenuated but remained significant in the multivariate-adjusted model (OR = 0.73, 95% CI: 0.57, 0.95). But the inverse associations of low and moderate level of total leisure-time physical activity with prediabetes were not statistically significant in multivariate-adjusted model.

4. Discussion

Our study used a large national database to explore the associations between leisure-time physical activity and the risk of undetected prediabetes. Our analyses included 8204 nonpregnant adults without diabetes and self-reported prediabetes. Among those seemingly healthy subjects, 1914 (23.33%) were identified as prediabetics. For all subjects, total, vigorous, and moderate leisure-time physical activity were associated with a decreased risk of prediabetes in unadjusted model and age- and gender-adjusted model. What is more,

Table 1: Characteristics of participants by prediabetes status, NHANES 2007–2012, ages 20–65 y.

	Prediabetes	Control	*P* value
Number of subjects (%)	1914 (23.33)	6290 (76.67)	
Gender (%)			0.007
Male	54.02	50.48	
Age (year)	47.64 ± 11.99	38.42 ± 12.57	<0.001
Age group (%)			<0.001
20–44	36.94	67.77	
45–65	63.06	32.23	
BMI (kg/m^2)	30.58 ± 7.08	27.58 ± 6.06	<0.001
BMI group (%)			<0.001
<25 kg/m^2	19.85	37.07	
25 to <30 kg/m^2	34.85	34.44	
≥30 kg/m^2	45.30	28.49	
Hypertension (%)	18.34	9.33	<0.001
Daily total energy intake (kcal)	2141.34 ± 892.91	2203.72 ± 913.18	0.003
Smoke at least 100 cigarettes in life (%)	46.50	40.67	<0.001
Have at least 12 alcohol drinks/year (%)	74.45	78.08	0.001
Dietary pattern (%)			0.025
Excellent healthy	6.90	8.19	
Very good healthy	18.55	20.49	
Good healthy	42.63	42.37	
Fair healthy	25.97	24.01	
Poor healthy	5.96	4.94	
Educational level (%)			<0.001
<high school	29.05	20.52	
High school	25.76	21.70	
>high school	45.19	57.77	
Race (%)			<0.001
Mexican American	19.70	17.01	
Other Hispanic	11.60	10.68	
Non-Hispanic White	30.51	46.30	
Non-Hispanic Black	30.51	17.11	
Other race	7.68	8.90	
Level of physical activity (MET-hours/week)			
Total leisure-time physical activity	11.66 ± 24.94	16.99 ± 30.04	<0.001
Moderate leisure-time physical activity	5.65 ± 12.72	6.52 ± 13.65	<0.001
Vigorous leisure-time physical activity	6.01 ± 19.34	10.47 ± 24.41	<0.001
Total work-related physical activity	48.20 ± 103.72	47.67 ± 97.48	0.223
Walking or bicycling for transportation	8.01 ± 23.49	7.63 ± 22.82	0.288

Data are means ± SD unless indicated otherwise.
BMI, body mass index; MET, metabolic equivalent.

the protective effects of high level of total leisure-time physical activity and low level of vigorous leisure-time physical activity on prediabetes were still statistically significant in multivariate-adjusted model. To further assess the effects of leisure-time physical activity on prediabetes in different age groups, we conducted logistic regression in under 45-year and 45- to 65-year group, respectively. And we found that the protective effect of vigorous leisure-time physical activity on prediabetes was more pronounced in under 45-year group.

As our research, some other studies had suggested that physical activity might reduce the risk of prediabetes. A population-based survey conducted in a predominantly rural Demographic Surveillance Site in eastern Uganda reported that person who met the WHO minimum recommended physical activity level had a significantly lower likelihood of abnormal glucose regulation [22]. Another study based on the NHANES 2003–2006 assessed the relationship between objectively measured physical activity and prediabetes [23].

Table 2: Odds ratios of prediabetes according to category of leisure-time physical activity, NHANES 2007–2012, ages 20–65 y.

	Case	Participant	Crude		Model 1		Model 2	
			OR	95% CI	OR	95% CI	OR	95% CI
Total leisure-time physical activity (MET-hours/week)								
None (reference)	1065	3793	1.00		1.00		1.00	
Low (≤12)	357	1700	0.68	0.59, 0.78	0.72	0.63, 0.83	0.88	0.76, 1.03
Moderate (>12 to 28)	258	1249	0.67	0.57, 0.78	0.75	0.64, 0.88	0.96	0.81, 1.14
High (>28)	234	1462	0.49	0.42, 0.57	0.64	0.54, 0.75	0.78	0.66, 0.94
P-trend				<0.001		<0.001		0.016
Vigorous leisure-time physical activity (MET-hours/week)								
None (reference)	1564	5882	1.00		1.00		1.00	
Low (≤16)	126	881	0.46	0.38, 0.56	0.61	0.49, 0.75	0.72	0.58, 0.90
Moderate (>16 to 32)	120	733	0.54	0.44, 0.66	0.69	0.55, 0.85	0.81	0.65, 1.02
High (>32)	104	708	0.48	0.38, 0.59	0.67	0.53, 0.84	0.82	0.64, 1.04
P-trend				<0.001		<0.001		0.010
Moderate leisure-time physical activity (MET-hours/week)								
None (reference)	1199	4635	1.00		1.00		1.00	
Low (≤6)	220	1209	0.64	0.54, 0.75	0.70	0.59, 0.83	0.91	0.76, 1.08
Moderate (>6 to 14)	255	1230	0.75	0.64, 0.87	0.78	0.66, 0.91	1.02	0.86, 1.21
High (>14)	240	1130	0.77	0.66, 0.90	0.80	0.68, 0.94	0.99	0.83, 1.18
P-trend				<0.001		<0.001		0.949

OR, odds ratio; CI, confidence interval; MET, metabolic equivalent.
Model 1 adjusted for age and gender.
Model 2 adjusted for age, gender, race, BMI, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation.

The results indicated that, compared with subjects within the lowest tertile, those within the highest tertile were 0.77 times as likely to have prediabetes when controlling for BMI alone [23]. However, the inverse association became nonstatistically significant after controlling for more covariates [23]. But very few studies have looked at the association between specific type of physical activity and the risk of prediabetes. Compared to work-related physical activity, leisure-time physical activity has more advantages, such as being more flexible, entertaining, and relaxing. Therefore, we speculated that leisure-time physical activity may play a key role in the prevention of prediabetes. It is necessary to conduct this research to explore the association between leisure-time physical activity and the risk of prediabetes.

There are several mechanisms underlying the association between physical activity and the risk of prediabetes. First, physical activity can improve energy balance and prevent obesity [24], which is a major risk factor independently related to prediabetes [22, 25]. Second, physical activity can reduce blood glucose and increase insulin sensitivity in people with and without diabetes directly [26–28]. And vigorous physical activity has greater effects on the improvements in insulin sensitivity and reductions in blood glucose than nonvigorous physical activity [28]. This supports our result that the protective effect of vigorous leisure-time physical activity on prediabetes is more pronounced than moderate leisure-time physical activity. Third, glucose transporter 4 (GLUT4) is the predominant glucose transporter isoform expressed in skeletal muscle. The process of glucose uptake into the contracting skeletal muscles is regulated by the translocation of GLUT4 to the plasma membrance and transverse tubules [29]. Long-term physical activity can increase the expression of GLUT4 and the concentration of mitochondrial enzyme and transform fiber type in skeletal muscle, thus reducing the risk of glucose abnormality [30, 31].

People with prediabetes are at high risk, not only to develop diabetes, but also to suffer from adverse cardiovascular (CV) events (myocardial infarction, stroke, and CV death) later in life [2]. Therefore, from clinical perspective, we suggested that people should take more leisure-time physical activity to prevent the occurrence of prediabetes. We hope that increased leisure-time physical activity can effectively increase participants' quality of life.

There are several strengths in our study. The main strength is the large and representative sample included, increasing the statistical power of the study to detect the decrease in risk of prediabetes. Second, we excluded subjects with self-reported prediabetes, who might actively participate in leisure-time physical activity based on the recommendations of healthcare workers. This made the observed associations between leisure-time physical activity and the risk of prediabetes more actual and credible. Third, the protective effects of total leisure-time physical activity and vigorous leisure-time physical activity on prediabetes were still statistically significant after adjustment for total work-related physical activity and walking or bicycling for transportation, increasing the authenticity of the observed associations. Fourth, the anthropometric measurements were collected by trained health technicians through standard procedures, further increasing the accuracy of the results.

Table 3: Odds ratios of prediabetes according to category of leisure-time physical activity, stratified by age, NHANES 2007–2012, ages 20–65 y.

	Case	Participant	Crude		Model 1		Model 2	
			OR	95% CI	OR	95% CI	OR	95% CI
20–44 y								
Total leisure-time physical activity (MET-hours/week)								
None (reference)	352	2062	1.00		1.00		1.00	
Low	130	1037	0.70	0.56, 0.86	0.71	0.57, 0.89	0.88	0.69, 1.11
Moderate	100	789	0.71	0.56, 0.90	0.73	0.57, 0.93	0.90	0.70, 1.17
High	125	1082	0.63	0.51, 0.79	0.66	0.53, 0.83	0.78	0.61, 0.99
P-trend				<0.001		<0.001		0.050
Vigorous leisure-time physical activity (MET-hours/week)								
None (reference)	524	3227	1.00		1.00		1.00	
Low	60	660	0.52	0.39, 0.68	0.53	0.40, 0.71	0.61	0.45, 0.83
Moderate	64	529	0.71	0.54, 0.94	0.71	0.54, 0.95	0.76	0.56, 1.03
High	59	554	0.61	0.46, 0.82	0.65	0.49, 0.87	0.72	0.53, 1.00
P-trend				<0.001		<0.001		0.007
Moderate leisure-time physical activity (MET-hours/week)								
None (reference)	416	2698	1.00		1.00		1.00	
Low	86	804	0.66	0.51, 0.84	0.66	0.51, 0.85	0.90	0.69, 1.18
Moderate	104	760	0.87	0.69, 1.10	0.91	0.72, 1.16	1.21	0.93, 1.56
High	101	708	0.91	0.72, 1.15	0.92	0.73, 1.17	1.14	0.88, 1.48
P-trend				0.195		0.305		0.171
45–65 y								
Total leisure-time physical activity (MET-hours/week)								
None (reference)	713	1731	1.00		1.00		1.00	
Low	227	663	0.74	0.62, 0.90	0.73	0.61, 0.88	0.88	0.72, 1.07
Moderate	158	460	0.75	0.60, 0.93	0.76	0.61, 0.94	0.98	0.78, 1.23
High	109	380	0.57	0.45, 0.73	0.60	0.47, 0.76	0.73	0.57, 0.95
P-trend				<0.001		<0.001		0.047
Vigorous leisure-time physical activity (MET-hours/week)								
None (reference)	1040	2665	1.00		1.00		1.00	
Low	66	221	0.66	0.49, 0.89	0.70	0.52, 0.95	0.86	0.62, 1.18
Moderate	56	204	0.59	0.43, 0.81	0.63	0.46, 0.87	0.79	0.57, 1.12
High	45	154	0.64	0.45, 0.92	0.67	0.47, 0.96	0.86	0.58, 1.26
P-trend				<0.001		<0.001		0.135
Moderate leisure-time physical activity (MET-hours/week)								
None (reference)	783	1937	1.00		1.00		1.00	
Low	134	405	0.73	0.58, 0.91	0.74	0.59, 0.93	0.91	0.72, 1.16
Moderate	151	470	0.70	0.56, 0.86	0.69	0.55, 0.85	0.88	0.70, 1.10
High	139	422	0.72	0.58, 0.90	0.71	0.56, 0.89	0.87	0.69, 1.11
P-trend				<0.001		<0.001		0.150

OR, odds ratio; CI, confidence interval; MET, metabolic equivalent.
Model 1 adjusted for age and gender.
Model 2 adjusted for age, gender, race, BMI, educational level, smoking status, alcohol consumption, dietary pattern, daily total energy intake, hypertension, total work-related physical activity, and walking or bicycling for transportation.

However, some limitations in our study have to be mentioned. First, the data we used in our analyses was from a cross-sectional study, which limited the establishment of the causal association between leisure-time physical activity and the risk of prediabetes. Second, we only adopted the HbA1c level as the diagnostic criteria for prediabetes on account of the large number of missing values about fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT) data. We might miss some subjects with prediabetes though the HbA1c test had several advantages over FPG and OGTT, including greater convenience, greater preanalytical stability, and less day-to-day perturbations during stress and illness [1]. Third, the information on physical activity in our analyses was obtained by self-report, which might be subject to recall bias.

5. Conclusion

Our study indicates that leisure-time physical activity may decrease the risk of prediabetes. Large cohort studies are needed to verify the potential causal association between leisure-time physical activity and the risk of prediabetes. It is very important to improve the awareness of prediabetes and prevent the onset of prediabetes for the prevention and postponement of diabetes.

Competing Interests

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

Authors' Contributions

Jia Wang and Dongfeng Zhang designed the study. Jia Wang and Yili Wu conducted the statistical analyses. All authors participated in drafting the manuscript and approved the final version.

Acknowledgments

The authors thank the US Centers for Disease Control and Prevention for making the datasets of NHANES available on their website. This study was supported by the grants from the Natural Science Foundation of Shandong Province (BS2013SF002), the Natural Science Foundation of Qingdao City (J10LF23), and Entrepreneurial Innovation Talents Project of Qingdao City (13-CX-3).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 5625371, 7 pages
https://doi.org/10.1155/2017/5625371

Review Article

A Different Perspective for Management of Diabetes Mellitus: Controlling Viral Liver Diseases

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Received 1 December 2016; Revised 21 January 2017; Accepted 30 January 2017; Published 2 March 2017

Academic Editor: Ruozhi Zhao

Knowing how to prevent and treat diabetes mellitus (DM) earlier is essential to improving outcomes. Through participating in synthesis and catabolism of glycogen, the liver helps to regulate glucose homeostasis. Viral related liver diseases are associated with glycometabolism disorders, which means effective management of viral liver diseases may be a therapeutic strategy for DM. The present article reviews the correlation between DM and liver diseases to give an update of the management of DM rooted by viral liver diseases.

1. Introduction

Insulin deficiency and/or insulin resistance, which causes glycometabolism disorders, leads to the symptoms of diabetes mellitus (DM). DM is an increasingly recognized global health concern. By 2030, the prevalence of diabetes among adults is expected to rise from 6.4% to 7.7% worldwide. During the next decade, the number of adults with diabetes is expected to rise by 20% in developed nations and 69% in developing nations [1, 2]. Long-term complications of DM include micro- and macrovascular damage, which include dysfunction of eyes, kidneys, nerves, gastrointestinal tracts, hearts, and blood vessels. Serious illness or stress can result in acute metabolic disorders, such as diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar status. Recently, some studies suggested that diabetes heightens patients' susceptibility to several cancers, such as colorectal, pancreatic, liver, and kidney cancers [3]. Thus, DM and its long-term complications deteriorate the health of residents and cause many deaths, ultimately expending national resources. Diabetes prevention and early management, as well as proper treatment of DM-related complications, are especially important factors.

As is known so far, several pathogenic processes and different factors are involved in the development of diabetes, and the interactions among factors are complicated. Genetic, environmental, and immune factors all contribute to the development of DM. Obesity, hypertension, alcohol consumption, and tobacco smoking are all risk factors of DM. Meanwhile, insulin resistance and β-cell dysfunction are common mechanisms of DM, and glucose and lipid toxicities are important mechanisms of the development of DM [1, 2].

Glycometabolism disorder is the key point of the development of DM, and the regulation of glucose homeostasis is particularly important for maintaining normal glucose metabolism. The liver is an important organ that can regulate glucose homeostasis, by means of the synthesis and catabolism of glycogen. Once chronic liver damage onsets, glycometabolism disorders may follow. It is well documented that patients with liver diseases, such as hepatitis B, hepatitis C, nonalcoholic fatty liver disease (NAFLD), fatty liver, cirrhosis, and hepatocellular carcinoma (HCC), have high comorbidity of type 2 diabetes mellitus (T2DM) [4, 5]. Many studies have proposed that liver-injury patients are likely to have a greater risk of DM [6, 7]. Cirrhosis complications may also cause DM, leading to hepatogenous diabetes (HD) [6–9]. In contrast, prevention or treatment of viral hepatitis leads to a definite improvement of insulin sensitivity [10–12]. This review illuminates the underlying relationship between DM and viral liver diseases, as well as the possible mechanisms.

2. DM and Hepatitis B

2.1. Epidemiological Characteristics. Custro et al. [13] had discovered a relation between HBV and T2DM more than a decade ago. The following studies also proved this relationship [14, 15].

A meta-analysis by Cai and coworkers demonstrated that, among 15 studies analyzed, 6 presented studies found a greater rate of DM risk in HBV-infected patients than in noninfected subjects, 7 did not support this association, and 2 showed weak correlations. In the Asia-Pacific area, the rate of DM in patients with HBV was higher than in patients uninfected with HBV [odds ratio (OR) 1.67] [16]. Hepatitis B surface (HBsAg) carriers had a three times higher rate (32.9%) of developing gestational diabetes compared with the regular population [17]. The HBV-related cirrhotic patients with good liver function had relatively normal insulin levels [18]. There was a higher incidence of T2DM for CHB patients, and HBeAg status and HBV DNA levels affected this incidence [19]. A 10-year follow-up study suggested that HBV did not elevate the chances of developing T2DM, since asymptomatic HBV carriers did not have higher risks of developing T2DM than controls. The researchers proposed that, instead of the virus, perhaps HBV-related parenchymal liver deficiencies led to greater rates of DM [15, 20, 21].

2.2. Correlation and Mutual Influence. Papatheodoridis et al. found that DM risk was related to the severity of liver damage, the serum gammaglutamyl-transpeptidase (GGT) level, and the grade of fibrosis [20]. DM risk was not related to the etiology of liver diseases. Viral infection was not a risk factor of diabetes [15].

Patients with chronic hepatitis B (CHB) were more prone to developing hepatic steatosis than those without CHB [22]. Hepatic steatosis was prevalent among youthful males with ongoing CHB (51.2% of the patients), and triglyceride was the independent factor for hepatic steatosis, while steatosis and viral factors, including HBV DNA and hepatitis B e antigen (HBeAg) negative or positive, had no relationship. CHB was associated with IR [23]. Wang et al. also found that chronic HBV did not raise the likelihood of IR [24], while hepatitis patients of various etiologies had an increased incidence of diabetes. So their views were that the positive correlation of HBV and diabetes might be a result of HBV-induced liver damage instead of HBV [15]. Diabetes increases the risk of cirrhosis in CHB patients and promotes the progress of cirrhosis [25].

2.3. Mechanism. Many mechanisms may explain the relation of HBV and DM onset. The liver plays an important role in regulating glucose homeostasis. HBV-related liver damage and inflammation cause the disorder of glucose metabolism. Inflammation can reduce the effects of insulin on the liver, thus leading to liver dysfunction, which in turn induces IR [16, 26–29].

Serum insulin increases for patients with cirrhosis, since hepatic function disorders lead to abnormal insulin levels, which might decrease hepatic blood supply and inhibit insulin-stimulated glucose uptake [30]. The appearance of inducible nitric oxide synthase has been proved to exacerbate HBV-infection liver diseases. HBV is replicated in the extrahepatic tissues, such as the pancreas, which damages β-cells. HBsAg is detected in bile and pancreatic secretions [31], and the DNA of HBV is found in many extrahepatic tissues, such as the pancreas [32]. So accompanied by the injury of pancreatic β-cells, DM may occur after the disorder of glucose metabolism. Beyond that, IR may be involved in the development of hepatogenous diabetes. Ji et al. [33] claimed that pre-S2 protein downregulated insulin receptor genes, which results in IR [17]; then the level of serum soluble tumor necrosis factor receptors increases and participates in the regulation of gluconeogenesis [30].

Lin et al. found that immunization against hepatitis B could reduce the danger of developing diabetes by 33% (odds ratio 5 0.67, 95% confidence interval 0.52–0.84) [10]. In a cross-sectional study of 15,316 adult subjects, successful immunizations more positively correlated with a lower rate of diabetes (odd ratio (OR) 0.67, 95% CI: 0.52–0.84) [11].

3. DM and Hepatitis C

3.1. Epidemiological Characteristics. Since 1994, Allison et al. performed [34] epidemiological studies to claim the association between HCV, or HCV cirrhosis, and DM. It is generally considered that chronic HCV infection causes several extrahepatic complications, such as T2DM, IR, cognitive impairment, cardiovascular disorders (i.e., stroke, ischemic heart disease), and glomerulonephritis renal insufficiency [35, 36]. Viral eradication can change the clinical progression of patients with chronic hepatitis C (CHC) and T2DM [37–43]. More and more evidence shows the increased risk of T2DM in individuals with HCV infection [43, 44]. Much evidence shows that increasing severities of HCV are positively correlated with an increased risk of T2DM [43, 44]. The rate of DM in chronic HCV-seropositive populations in Europe, North America, and Asia ranged from 13 to 33% [45, 46]. Approximately 20–30% of CHC patients who had liver cirrhosis later developed diabetes. The incidence of DM was noticeably larger in people with HCV cirrhosis than from other etiologies, and HCV infection was detected prior to T2DM in most cases [46].

3.2. Correlation and Mutual Influence between the Two Diseases. Hammerstad et al. reported that age, gender, family history, genotype of HCV, therapeutic regimen, and virological response were factors of T2DM in HCV patients. For example, the danger of developing diabetes was higher in people who had a positive family history than in those with only one diabetic parent, while genotype 1 and sustained viral response (SVR) reduce the development of DM [47]. Zornitzki et al. claim that CHC patients with interferon (INF) treatment had a greater (10–18-fold) danger of developing type 1 diabetes mellitus (T1DM) than the common population, with a median onset age of 43 (range: 24–66 years) in Caucasians and 52 (range: 45–63 years) in Japanese. Most patients developed T1DM during the treatment. The

median time of onset was 4.2 months for Caucasians and 5.7 months for Japanese [48]. But in another study among the US population, the authors found that there was no distinction between the incidence of diabetes and prediabetes by HCV status, and HCV was not related to diabetes or to IR in people with normal glucose levels. In contrast, higher GGT and alanine aminotransferase (ALT) levels were related to diabetes independent of HCV infection [49].

With or without cirrhosis, DM reduces SVR [7]. In a different study, researchers claim that a mix of metformin, pegylated interferon, and ribavirin prolonged the SVR and increased insulin sensitivity only in women having CHC [47]. A few studies showed a significantly reduced incidence of T2DM among CHC patients with SVR [50, 51]. Arase et al. retrospectively analyzed a cohort of 2,842 patients with HCV who were treated with IFN monotherapy or both IFN and ribavirin. They found that the yearly rate of T2DM development in people with HCV was 0.8% to 1.0% and that SVR reduced the risk of T2DM onset by two-thirds in patients with HCV who accepted antiviral treatment [52]. Pavone et al. retrospectively evaluated 149 HCV-positive diabetics receiving direct antiviral drugs (DAA) and found that the subjects could gain a rapid reduction of fasting glucose (FG) levels [12]. Antiviral therapy, which eradicated HCV, decreased the rate of DM onset [53].

Imazeki et al. found that there was a greater rate of DM and IR in patients infected with HCV than in those infected with HBV [54]. Other studies did not validate the difference [21, 55, 56]. In addition to HCV, other risk factors that may lead to glucose abnormalities include age, gender, BMI, and cirrhosis [54].

3.3. Mechanisms. Through increasing oxidative stress, IR, and glucose intolerance, HCV results in hyperuricemia, arterial hypertension, and atherosclerosis, thus damaging the cardiovascular system [57]. HCV likely increases the risk of T2DM through elevating IR [58]. Studies have elucidated the mechanisms through which HCV impairs the insulin-signaling pathway in liver cells [59]. Specific steps include targeting the serine phosphorylation of insulin receptors (IRS), increasing levels of tumor necrosis factor-α (TNF-α), overexpressing inhibitors of cytokines (SOC-3), inducing SOC-7 [39], increasing reactive oxygen species 2 and other inflammatory cytokines3, and directly alternating insulin signaling by HCV4 and β-cell dysfunction [46].

4. DM and Viral Cirrhosis

4.1. Epidemiological Characteristics. Epidemiological data indicates that DM is associated with liver cirrhosis. Studies have found that many DM patients have a high rate of cirrhosis and advanced fibrosis [59]. Nearly 80% of people with liver cirrhosis also have problems metabolizing glucose, with 30% developing DM [60]. DM participates in the progress of liver diseases. Elkrief et al. retrospectively assessed the effect of DM on hospitalized patients with liver decompensation, liver transplantation, and mortality. They found that diabetes was an independent prognostic indicator which is unrelated to the model for end-stage liver disease (MELD) score. Diabetes significantly decreases transplantation-free survival [61].

Sporea et al. used Transient Elastography to evaluate stages of liver fibrosis in DM patients. They found that 18.8% of patients with DM had significant fibrosis and 13.8% had cirrhosis, rates which are significantly higher than in the common population [62].

Jepsen and colleagues found that 22% of the patients with cirrhosis and first-onset hepatic encephalopathy (HE) had diabetes. Child-Pugh class C patients with DM had higher risk for HE than those without diabetes [63].

4.2. Mechanisms. Inflammation and fibrosis are increased in DM, and DM elevates the hepatic complications and mortality hazard in patients with cirrhosis. The possible mechanism of liver damage is that adipokines increases mitochondrial oxidative stress which promotes inflammation and fibrosis [7]. In addition, inflamed subcutaneous and visceral adipose tissues produce proinflammatory factors such as tumor necrosis factor-α, leptin, interleukin 6, and adiponectin. Several of these factors signal stellate cells to produce more collagen, leading to a greater release of growth factors for connective tissue, which accumulates proteins in the extracellular matrix and results in fibrosis containing immuno-competence. This condition increases the danger of infections, including bacterial peritonitis, which may lead to high death rates [7, 64]. DM contributes to the activation of hepatic stellate cells, inflammation, apoptosis, angiogenesis, and hepatic sinusoidal capillarization, which progresses liver fibrosis and cirrhosis. Patients with cirrhosis have reduced liver insulin clearance and increased advanced glycation end-products, hypoxia, and hypoxia-inducible factors [63].

4.3. Correlation and Mutual Influence between the Two Diseases. DM treatment improves the survival rate of patients with liver cirrhosis. Metformin is related to a rise in survival rate and a reduction of liver complications [7]. The use of the sodium-glucose cotransporter-2 (SGLT2) inhibitors and incretin treatments, including oral inhibitors of dipeptidylpeptidase-4 (DPP-4) and injectable glucagon-like peptide-1 (GLP-1) receptor agonists, ameliorated diabetic conditions and reduced liver fibrosis and inflammation [60].

5. DM and HCC

5.1. Epidemiological Characteristics. The number of new onset HCC is 700,000 per year. HCC is the 3rd greatest cause of cancer-related deaths. Besides common risk factors, such as chronic viral hepatitis and liver cirrhosis, other hepatic diseases, including metabolic, autoimmune, and alcoholic liver diseases, may also elevate the risk of HCC [65, 66]. Studies of epidemiological data indicated that DM raises the danger of developing HCC. Factors included age, alcohol drinking, increased alkaline phosphatase levels, decreased serum triglyceride (TG) levels, increased GGT levels, increased aspartate aminotransferase-to-platelet ratio index (APRI) score, decreased platelet counts, and increased Fibrosis-4 (FIB-4) score. Multivariate Cox regression analysis

claimed that age > 65 years, low TG levels (<150 mg/dl), and high GGT levels (>40 IU/L) were independent risk factors for HCC [67].

5.2. Correlation and Mutual Influence between the Two Diseases. Hung et al. found that DM was associated with both the rate of HCC and the survival rate. DM was associated with HCC progress among IFN-based antiviral therapy treated CHC patients [67, 68], especially the ones who obtained SVR, whereas, 2 years after acquiring SVR, the danger of progression to HCC may reduce [68]. Accepting IFN-based CHC patients with baseline DM having overall poor prognosis and lower survival rate than the non-DM patients ($p < 0.001$), a further analysis found that DM could act as an independent prognostic factor for HCC among noncirrhosis patients and also increase the likelihood of HCC onset of them [67].

Yang et al. confirmed the follow-up time related incidence of DM as 34% (253/739) and the rate of DM patients developing HCC after a follow-up of 38 months was 9% (69/739) by a study of 739 patients. Diabetes increased the danger of patients with non-HCV cirrhosis developing HCC. In HCV cirrhosis patients with already a high risk of HCC, diabetes might not elevate the risk any further [4]. Researchers also found that DM was an important risk factor for HCC among the CHC patients with SVR. In comparison to patients without DM, patients with cirrhosis and DM had a sevenfold higher risk for development of HCC, and the HCC risk per year for them was 7.9% during a two-year follow-up after SVR. As time went on, the risk declined [69].

Systemic risks of HCC, such as hyperinsulinemia, obesity-related hypoxia, systemic inflammation, systemic influences of cytokines and adipokines, systemic immune dysregulation, systemic effects of the gut microbiome, autophagy, and local factors all contributed to HCC risk [70].

5.3. Mechanisms. Hyperinsulinemia, which can induce tumor cell growth and metastasis in T2DM, is believed to cause carcinogenesis through affecting the proliferating pathway. The state targets the pathway, after insulin receptors, through the effect of insulin-like growth factor IGF-1 and is directly involved in carcinogenesis by acting on cancer cells. Insulin decreases the expression of IGF binding protein-1, thus increasing the bioactive IGF-1. In contrast to insulin, IGF-1 has more potent mitogenic and antiapoptotic effects, promoting growth of preneoplastic and neoplastic cells. Adiponectin, largely expressed by adipokines, has anti-inflammatory and antitumor roles. Adipose tissues can produce different kinds of inflammatory cytokines, such as interleukin-6, plasminogen activator inhibitor-1, and monocyte chemoattractant protein, which may aid the progression of cancer. Higher leptin and lower adiponectin levels could also increase the risk of cancer in patients with obesity or T2DM. The lasting action of inflammatory cytokines would interrupt the normal capacity for intracellular antioxidants, making cells more susceptible to malignant changes [71].

Hyperinsulinemia is believed to be an independent risk factor for HCC, and it is reported that major dysregulation of insulin dependent pathways was common in patients with HCC. Signals from IGF-I and more so from IGF-II affect the progression of HCC. Aberrant mammalian targets of rapamycin (mTOR) signaling in HCC have been suggested to exist in tumors. Additionally, DM is associated with elevated serum estrogen levels, which could reduce HCC progression by suppressing chronic low-grade hepatic inflammation. Recently, studies showed that the AMPK-independent pathway (represented by the LKB1/AMPK/mTOR axis), miRNAs downstream of this biguanide, and their messenger RNAs were the key points of cell survival and proliferation [72].

Some studies claimed that the condition of DM tended to progress HCC development in patients, in both the presence or the absence of cirrhosis [72]. Cytokines are important for both the mechanisms of IR and the glucose disposal defects, as well as the development of liver diseases. Capone et al. reported that the T2DM-HCC patients had higher levels of *ADIPOQ*, β-nerve growth factor (β-NGF), chemokine ligand1 (CXCL1), CXCL12, hepatocyte growth factor (HGF), several interleukin (IL) members, and IFN-α and lower levels of leptin than T2DM or HCC patients. They also had higher levels of CXCL9, platelet endothelial cell adhesion mole-1 (PECAM-1), prolactin, and glucagon and lower levels of soluble vascular endothelial growth factor sVEGFR-1 and sVEGFR-2 than T2DM. These patients had similar levels of CXCL9, PECAM-1, prolactin, glucagon, sVEGFR-1, and sVEGFR-2. The serum levels of TP53 in HCC and T2DM patients were higher and had no correlation with CXCL1, interleukin-2 receptor-alpha (IL-2R alpha), PECAM-1, and prolactin, whereas there was an important correlation between tumor protein p53(TP53) and CXCL12 in HCC and in T2D-HCC patients [73].

6. DM and Liver Transplant

6.1. Epidemiological Characteristics. Over recent years, increasing success rates of surgery and immunosuppressive treatments have led to high survival rates after liver transplantation (LT). Nevertheless, many transplant complications still reduce the survival rate, such as the development of de novo malignancies, recurrence of underlying diseases, obesity, hypertension, new-onset diabetes, dyslipidemia, and cardiovascular diseases [74]. A common complication is new-onset diabetes after transplantation (NODAT), which has an incidence of roughly 30% [75]. The specific rate of NODAT depends on different diagnostic criteria, the duration of follow-up, and the study populations. The rate of NODAT in China is similar to that in Western countries [76].

6.2. Correlation and Mutual Influence between the Two Diseases. Existing preoperative cirrhotic complications, such as ascites, esophageal varices, and hepatic coma, were risk factors for post-LT NODAT. In Western countries, the three major risk factors of NODAT are HCV infection, obesity, and alcoholic cirrhosis. The major risk factor in China is viral hepatitis [74–76].

6.3. Mechanisms. The early recurrence of hepatitis C and immunosuppressive drugs after liver transplantation is

related to LT-NODA. Steroids increase insulin resistance and reduce β-cell secretion. Many recent studies show that intestinal microbiota took part in the regulation of carbohydrate metabolism and affected the pathogenesis of glucose metabolism disorders. It was noted that liver transplantation could affect intestinal microbiota through multiple factors, such as immunosuppression [74]. Glycaemic-controlled NODAT is also an independent risk factor of HCC recurrence. NODAT patients who received hypoglycaemic treatment had a worse prognosis and a higher HCC recurrence in comparison to those without treatment [76]. Actively intervening with these risk factors could decrease the occurrence rate of metabolic syndrome after liver transplantation and improve the patient's quality of life [77].

7. Summary

DM is constantly increasing around the world. Prevention and early treatment of DM improves the outcomes of DM patients. The relationships between DM and liver diseases are complex. The prevalence of DM in patients with liver diseases is higher than that in regular populations; the presence of diabetes is a predictor of worse outcomes in patients with liver diseases. In addition to healthy diet, regular physical activity, keeping a normal BMI, and avoiding smoking, the controlling of liver diseases, especially viral liver diseases, is also important to managing DM. Proper management of DM improves the outcomes of patients with liver diseases, HCC, and liver recipients.

Disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the paper.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

Yingying Zhao is major contributor to the review.

Acknowledgments

This study was supported in part by grants from the National Science and Technology Major Project of China (2014ZX10005001), the Beijing Municipal Science and Technology Project of Traditional Chinese Medicine (JJ2014-25), Collaborative Innovation Center of Infectious Diseases, and National Natural Science Foundation of China (no. 81201160).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 8289740, 6 pages
https://doi.org/10.1155/2017/8289740

Research Article

Electrochemical Skin Conductance May Be Used to Screen for Diabetic Cardiac Autonomic Neuropathy in a Chinese Population with Diabetes

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Received 8 September 2016; Revised 1 December 2016; Accepted 9 January 2017; Published 9 February 2017

Academic Editor: Chi Zhang

Aims. This study aimed to assess whether the electrochemical skin conductance (ESC) could be used to screen for diabetic cardiac autonomic neuropathy (DCAN) in a Chinese population with diabetes. *Methods*. We recruited 75 patients with type 2 diabetes mellitus (T2DM) and 45 controls without diabetes. DCAN was diagnosed by the cardiovascular autonomic reflex tests (CARTs) as gold standard. In all subjects ESCs of hands and feet were also detected by SUDOSCAN™ as a new screening method. The efficacy was assessed by receiver operating characteristic (ROC) curve analysis. *Results*. The ESCs of both hands and feet were significantly lower in T2DM patients with DCAN than those without DCAN (67.33 ± 15.37 versus 78.03 ± 13.73, $P = 0.002$, and 57.77 ± 20.99 versus 75.03 ± 11.41, $P < 0.001$). The ROC curve analysis showed the areas under the ROC curve were both 0.75 for ESCs of hands and feet in screening DCAN. And the optimal cut-off values of ESCs, sensitivities, and specificities were 76 μS, 76.7%, and 75.6% for hands and 75 μS, 80.0%, and 60.0% for feet, respectively. *Conclusions*. ESC measurement is a reliable and feasible method to screen DCAN in the Chinese population with diabetes before further diagnosis with CARTs.

1. Introduction

The prevalence of diabetes is increasing rapidly worldwide [1], especially in China [2]. 60% to 70% of patients with type 2 diabetes mellitus (T2DM) suffer from diabetic neuropathy, including diabetic cardiovascular autonomic neuropathy (DCAN), which can lead to increased risk of cardiovascular mortality [3]. The American Diabetes Association (ADA) has recommended that physicians screen for DCAN at the time of diagnosis for patients with T2DM and within 5 years of diagnosis for patients with type 1 diabetes mellitus (T1DM) [4]. Unfortunately, the significance of DCAN has generally been overlooked in current clinical diagnostic and treatment routines, and physicians have had no practical point-of-care tool available for the detection of subclinical DCAN [5]. The battery of cardiovascular autonomic reflex tests known as CARTs is widely accepted as the gold standard to screen for DCAN [6, 7]. However, CARTs have significant disadvantages [7] such as cumbersome administration, subjective criteria, time-consuming analysis, and weak repeatability, which make them ill-suited for annual DCAN screening. Sudomotor dysfunction, characterized by sweating deficiency due to loss of small sympathetic nerve fibers, has been shown to develop early in the course of diabetes. Electrochemical skin conductance (ESC) measured by the SUDOSCAN technology (Impeto Medical, Paris, France) is a new index to detect sudomotor dysfunction early and rapidly; it has been used in previous studies to screen for prediabetes and diabetes, as well as in the detection of diabetic microvascular complications and DCAN [8–14]. Since the assessment of sudomotor function has been proposed to evaluate autonomic disturbances [15], this study aimed to explore whether this new ESC index could reliably screen for

Table 1: Components and scoring system for the cardiovascular reflex tests [7, 16].

CARTs	Scores (values)		
	Normal (0 points)	Borderline (1 point)	Abnormal (2 points)
(A) HR response to deep breathing	⩾15	11–14	⩽10
(B) Valsalva ratio	⩾1.21	1.11–1.20	⩽1.10
(C) HR response to standing (30 : 15 ratio)	⩾1.04	1.01–1.03	⩽1.00
(D) Postural blood pressure change	⩽10	11–29	⩾30

CARTs, cardiovascular reflex tests.

DCAN in the Chinese population with diabetes and reduce the number of subjects tested with CARTs.

2. Materials and Methods

2.1. Study Population. A total of 75 patients with T2DM and 45 nondiabetic controls were recruited at Qilu Hospital of Shandong University from March to August 2014. All diabetes patients were inpatients. To match the age and sex of the two groups, we chose as controls similarly aged spouses or relatives of the T2DM patients or members of the hospital cleaning staff most of whom had never taken drugs for chronic disease before. We excluded those with diabetes, hypertension, coronary heart disease, and so forth. Diabetes mellitus was defined according to the 2013 ADA diagnostic criteria [4]. Study exclusion criteria were as follows: presence of (or history of) acute myocardial infarction, cerebral hemorrhage, severe hypertension, and implanted cardiac pacemaker; severe thyroid, hepatic, or renal disease; retinal proliferative lesions or retinal hemorrhage; taking any of the following medications within one month of study enrollment: digoxin, β-blockers, and antidepressants; long-term consumption of coffee, tea, alcohol, or other caffeinated drinks. The study was conducted in accordance with the principles of the Helsinki Declaration and approved by the Qilu Hospital research ethics committee. All subjects provided signed informed consent.

2.2. Data Collection and Clinical Evaluation. Basic information was collected from all subjects by professional physicians, including medical history, age, and gender. A clinical examination was administered to record height, weight, waist circumference, and blood pressure. After fasting for at least 8 hours, venous blood was collected from both the T2DM and control groups for measurement of fasting plasma glucose (FPG, by automatic biochemical analyzer 400, Toshiba, Japan, 3.9–6.1 mmol/L), fasting C-peptide (FC-P by immune chemiluminescence apparatus, BAYER CENTAUR, 0.81–3.85 ng/mL), fasting insulin (FINS, by immune chemiluminescence apparatus, BAYER CENTAUR, 5–10 μIU/mL), and glycated hemoglobin A1c (HbA1c, by high pressure liquid chromatograph, VARIANT II, Bio-Rad, 4–6%) in the endocrinology laboratory of Qilu Hospital.

Cardiovascular autonomic reflex tests were used as the gold standard clinical testing method [7]. The whole process was conducted by ECG according to Ewing et al. [16] and included heart rate (HR), response to deep breathing (the difference between the maximum and minimum heart rates during each deep expiration and inspiration at 6 breaths per minute), Valsalva maneuver (the ratio of the longest R-R interval shortly after Valsalva maneuver to the shortest R-R interval during Valsalva maneuver), heart rate response to standing (30 : 15 ratio, the ratio of the R-R intervals of the 30th beat to the 15th beat cycle after standing up unaided), and postural blood pressure change (the difference in systolic blood pressure change between lying down and standing up after 2 min). Diagnostic criteria and staging of DCAN are still being debated. The Toronto Diabetic Neuropathy Expert Group [7, 17] suggests that at least two abnormal HR tests are required for a definite or confirmed diagnosis of cardiovascular autonomic neuropathy. However, this grading system fails to consider the relative effect of each CART; therefore, in the present study, we selected the other recommended scoring system (Table 1) [16] and use the total score to define the severity of cardiovascular autonomic dysfunction. According to the scoring system a normal result scores 0 points, a borderline result 1 point, and an abnormal result 2 points. The total scores are calculated by adding individual points. Severity groups of DCAN were divided according to the total score: 0-1 point was defined as no-DCAN (no-DCAN group); 2 to 3 points denoted early-DCAN (early-DCAN group); and 4 to 8 points confirmed definite-DCAN (definite-DCAN group).

ESC was measured using the SUDOSCAN device (Impeto Medical, Paris, France). Participants were asked to place their bare hands and feet on stainless steel electrode plates. The device applies incremental low direct current (DC) voltage potential (less than 4 V) to the plates during a 2-minute testing period. Electrochemical skin conductance (ESC), derived from the sweat chloride ion current produced in response to the applied voltages, is automatically calculated by the equipment for each hand and foot. The test is painless, noninvasive, portable, and very simple to operate.

2.3. Statistical Analysis. The data are presented as mean ± Standard Deviation (SD). Independent sample t tests were used to compare two groups while multiple groups using one-way ANOVA (analysis of variance). Chi-square tests were used to compare categorical variables between groups. Receiver operating characteristic (ROC) curve was used to evaluate the sensitivity and specificity of the diagnostic evaluation methods. Significance was defined as a two-tailed $P < 0.05$. Statistical procedures were performed with the statistical package SPSS 17.0.

Table 2: Clinical features of T2DM patients and controls.

	T2DM ($n = 75$)	Controls ($n = 45$)	P value (<0.05)
Age (yrs)	55.55 ± 14.36	50.80 ± 12.48	0.068
Male (n, %)	53.33%	46.67%	0.572
BMI (kg/m^2)	26.55 ± 6.01	25.21 ± 3.34	0.171
SBP (mmHg)	132.17 ± 18.24	127.27 ± 17.64	0.151
DBP (mmHg)	79.28 ± 13.66	71.98 ± 10.21	0.001**
Waist circumference (cm)	93.80 ± 13.04	85.08 ± 10.18	<0.001**
HbA_{1C} (%)	8.89 ± 2.28	5.34 ± 0.36	<0.001**
FPG (mmol/L)	8.42 ± 2.91	5.50 ± 0.52	<0.001**
FC-P (ng/mL)	1.55 ± 0.89	1.23 ± 0.52	0.03*
FINS (uIU/mL)	14.02 ± 10.37	6.07 ± 2.68	<0.001**
HOMA-IR	4.87 ± 3.27	1.49 ± 0.68	<0.001**
CARTs total score	3.23 ± 1.67	2.04 ± 1.58	<0.001**
Mean hands ESC (μS)	73.75 ± 15.25	78.36 ± 9.74	0.046*
Mean feet ESC (μS)	68.13 ± 17.96	73.81 ± 9.34	0.025*

Data were mean ± SD for continuous variables and n (%) for categorical variables. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FC-P, fasting C-peptide; FINS, fasting insulin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; T2DM, type 2 diabetes mellitus; CARTs, cardiovascular reflex tests; ESC, electrochemical skin conductance; P values were for one-way ANOVA or Chi-square tests across the 2 groups. *$P < 0.05$. **$P < 0.001$.

3. Results

3.1. Clinical Features of T2DM Patients and Controls. A total of 75 patients with T2DM and 45 nondiabetic controls were included in the study. As shown in Table 2, waist circumference, diastolic blood pressure, and relevant indicators of glucose metabolism in the T2DM patients were significantly higher than in the controls, while age, BMI, and systolic blood pressure were not different between the two groups. The means of hands ESC and feet ESC, the indicators of sudomotor function, were significantly lower in T2DM patients than in controls (hands ESC, $P = 0.046$; feet ESC, $P = 0.025$). The total CARTs score was higher in T2DM patients than in controls. In 45 Chinese controls, the mean hands ESC was 78.36 ± 9.74 μS and the mean feet ESC was 73.36 ± 9.78 μS.

3.2. Ratio of DCAN in T2DM Patients and Controls. Based on CARTs total scores, 39.9% (30/75) of T2DM patients were diagnosed with DCAN as shown in Figure 1. It should be noted that 13.4% (6/45) of controls were also diagnosed with nondiabetic cardiac autonomic neuropathy. Certainly, however, the ratio of DCAN was significantly higher in T2DM patients than in controls ($P = 0.002$). Otherwise, the proportion of the no-DCAN group in controls was 48.9% (22/45), which was much higher than the 14.7% (11/75) found in T2DM groups.

3.3. Comparison of Electrochemical Skin Conductance (ESC) in DCAN and No-DCAN Subjects. As shown in Figure 2, both hands and feet ESC in T2DM patients with DCAN were significantly lower than in T2DM patients without DCAN (67.33 ± 15.37 versus 78.03 ± 13.73, $P = 0.002$, and 57.77 ± 20.99 versus 75.03 ± 11.41, $P < 0.001$). Using CARTs total score as the standard, the T2DM patients were divided into 30 cases of DCAN, 34 cases of early-DCAN, and 11 no-DCAN patients (Table 3). Compared with no-DCAN patients, patients with DCAN and early-DCAN demonstrated lower ESC, which is positively correlated with the severity.

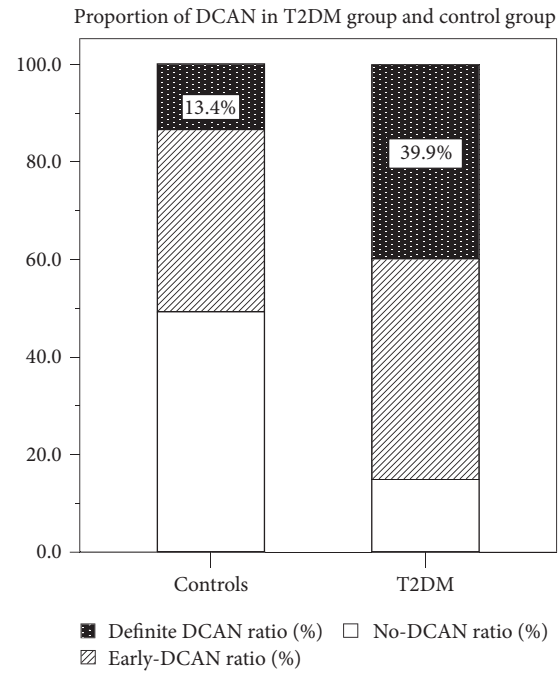

Figure 1: Proportion of confirmed diabetic cardiovascular autonomic neuropathy (definite-DCAN), early stage of diabetic cardiovascular autonomic neuropathy (early-DCAN), and no diabetic cardiovascular autonomic neuropathy (no-DCAN) in patients with type 2 diabetes mellitus (T2DM) and controls.

Table 3: Comparison of different indicators in different severity DCAN.

	T2DM				
	No-DCAN group (n = 11)	Early-DCAN group (n = 34)	Definite-DCAN group (n = 30)	F value	P value
Age (yrs)	48.00 ± 14.89	56.56 ± 10.82	57.17 ± 17.07	1.834	0.167
Duration of DM (yrs)	7.32 ± 9.35	9.81 ± 7.56	9.03 ± 8.57	0.383	0.683
BMI (kg/m^2)	26.30 ± 4.32	25.61 ± 4.27	27.70 ± 7.91	0.974	0.382
SBP (mmHg)	131.09 ± 15.12	133.35 ± 21.52	131.23 ± 15.51	0.127	0.881
DBP (mmHg)	75.00 ± 8.33	81.12 ± 15.46	78.77 ± 13.01	0.865	0.425
Waist circumference (cm)	92.36 ± 10.61	92.79 ± 12.37	95.47 ± 14.69	0.406	0.668
Resting Heart rate (bmp)	71.00 ± 7.48	72.62 ± 8.36	78.53 ± 10.40	0.707	0.497
HbA_{1C} (%)	9.05 ± 2.07	8.50 ± 2.21	9.28 ± 2.43	0.944	0.300
FPG (mmol/L)	7.58 ± 3.15	8.11 ± 2.98	8.34 ± 2.75	1.074	0.347
FC-P (ng/mL)	1.87 ± 0.61	1.38 ± 0.90	1.63 ± 0.94	1.510	0.228
FINS (uIU/mL)	12.43 ± 6.63	13.46 ± 4.57	15.25 ± 15.25	0.382	0.684
Mean hands ESC (μS)	79.73 ± 13.12[a*]	77.81 ± 14.11[c*]	67.33 ± 15.37	4.916	0.010[*]
Mean feet ESC (μS)	72.32 ± 17.64[b*]	75.91 ± 8.72[d**]	57.77 ± 20.99	10.707	<0.001[**]

Data were mean ± SD for continuous variables and n (%) for categorical variables. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FC-P, fasting C-peptide; FINS, fasting insulin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; T2DM, type 2 diabetes mellitus; HC, health controls; DCAN, diabetic cardiovascular autonomic neuropathy; no-DCAN, no diabetic cardiovascular autonomic neuropathy; ESC, electrochemical skin conductance; F values and P values were for one-way ANOVA across the 3 groups. [a]$P = 0.029$; [b]$P = 0.012$; [c]$P = 0.005$; [d]$P < 0.001$, compared with the diagnosed DCAN group. [*]$P < 0.05$. [**]$P < 0.001$.

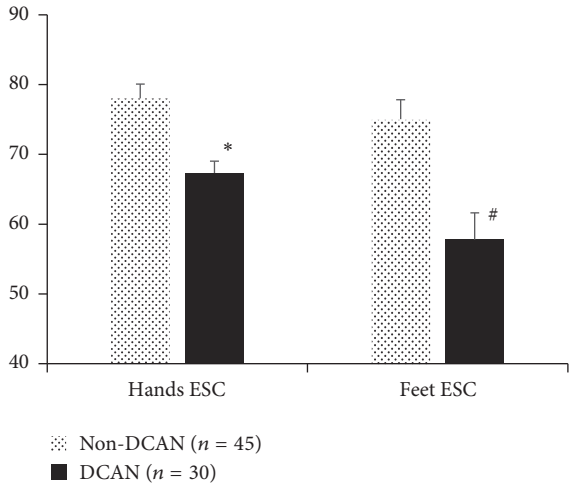

Figure 2: Feet and hands electrochemical skin conductance (ESC) in subjects with cardiovascular autonomic neuropathy (DCAN) and subjects without diabetic cardiovascular autonomic neuropathy (no-DCAN). Data are mean ± SD values. [*]$P = 0.002$. [#]$P < 0.001$.

3.4. Diagnostic Efficiency of ESC for Screening DCAN. Using CARTs total score as the standard, we evaluated the diagnostic efficiency of ESC for screening DCAN in patients with T2DM. The areas under the ROC curve (AUC) of mean hands ESC and mean feet ESC were 0.750 (95% CI: 0.631~0.869) and 0.747 (95% CI: 0.630~0.865) separately (Figure 3). The accuracy of ESC to screen for DCAN is shown in Table 4. Corresponding to the highest Youden index (feet ESC, 0.400;

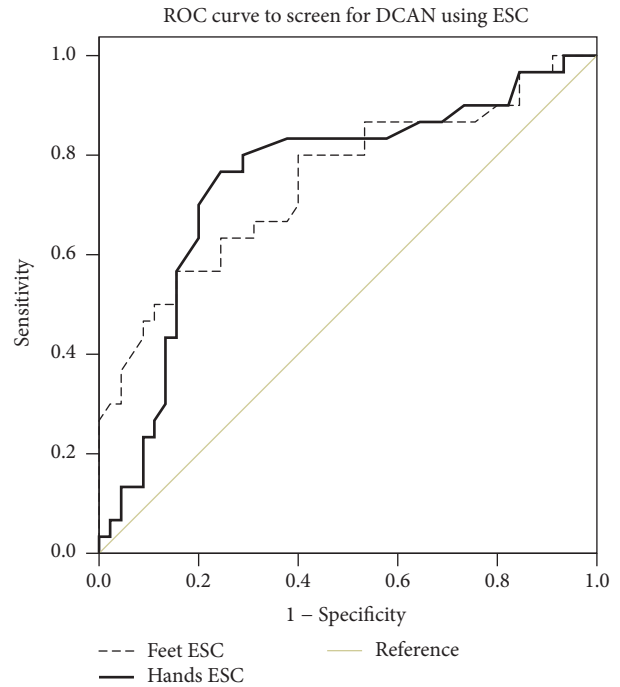

Figure 3: Receiver operating characteristic (ROC) curve of mean hands electrochemical skin conductance (ESC) and mean feet ESC to screen for diabetic cardiovascular autonomic neuropathy (DCAN) in diabetes group, using the cardiovascular autonomic reflex tests (CARTs) total score as the criteria to diagnose DCAN. The areas under the ROC curve (AUC) of mean hands ESC (black line) and feet ESC (dashed line) to predict DCAN were 0.750 and 0.747, respectively. P value < 0.01.

Table 4: Diagnostic efficiency of electrochemical skin conductance in the screening of diabetic cardiac autonomic neuropathy.

	Criterion*	Sensitivity (%)	Specificity (%)	+PV (%)	−PV (%)	TC (%)
Feet ESC	75.19 μS	80.0	60.0	57.1	81.8	68.0
Hands ESC	75.76 μS	76.7	75.6	67.6	82.9	76.0

ESC, electrochemical skin conductance; +PV, positive predictive value; −PV, negative predictive value; TC, total consistence rate. *Criterion corresponding to the highest Youden index (feet ESC, 0.400; hands ESC, 0.522).

hands ESC, 0.522), the optimal cut-off values of mean hands ESC and mean feet ESC were 75.76 μS and 75.19 μS, respectively. The sensitivity and specificity for optimal mean hands ESC cut-off value were 76.7% and 75.6%, respectively, while the sensitivity and specificity for optimal mean feet ESC cut-off value were 80.0% and 60.0%, respectively.

4. Discussions

DCAN is one of the common and chronic complications of diabetic neuropathy (DN). In the early phases it is characterized by an insidious onset, manifesting as resting tachycardia, exercise intolerance, and orthostatic hypotension [12]. In the late phase, a meta-analysis of DCAN and mortality showed that DCAN was strongly associated with higher mortality risk owing to acute painless myocardial infarction [18, 19]. Even so, current screening methods for DCAN are neglected, and only resting heart rate is monitored in regular clinical practice [20]. DCAN was detected in only 7% of T1DM and T2DM at the time of diagnosis. In this study, we explored how the assessment of sudomotor function by measuring hands and feet ESC could be a helpful and practicable tool to screen for DCAN in Chinese subjects in clinical practice.

Traditionally, CARTs are the gold standard tests for diagnosing cardiovascular autonomic neuropathy and are recommended not only by the American Diabetes Association but also by the Cardiovascular Autonomic Neuropathy Subcommittee of the Toronto Consensus Panel [7]. CARTs were put forward by Ewing et al. in 1985 [16] and comprise five tests. In many large clinical studies [3], three to five CARTs tests are used as diagnostic criteria. The sustained handgrip test is less commonly selected than others, for example. In some studies the Valsalva maneuver is excluded for reasons of patient safety [12]. On the subject of patient safety we excluded diabetes with retinal proliferative lesions or retinal hemorrhage during enrollment as a safety precaution. In our study we used four CARTs tests (Table 1) as the diagnostic standard for DCAN. Owing to a few disadvantages such as tediousness and time-consuming operation [12], CARTs are not usually performed in everyday clinical practice until later, more severe typical manifestations of dysautonomia arise such as orthostatic hypotension. Currently, screening rates for and awareness of DCAN are relatively low. Given DCAN's association with high risk of mortality discussed above, we think it is advisable to find an easier method to screen for DCAN. According to this study's screening results, the number of patients who need to be diagnosed by CARTs should be reduced significantly, especially in countries with increasingly large diabetic populations such as China.

Apart from quantitative sudomotor axon reflex testing (QSART), we have discussed how sudomotor function can also be measured by electrochemical skin conductance (ESC) using the SUDOSCAN device, which applies low amplitude voltages (less than 4 V) to the palms and soles and monitors the variability of the ionic flow (Cl^-) through sweat glands [8, 21]. A recent study of a healthy Chinese population (n = 120) [22] found mean hands ESC values of 61.2 ± 15.5 μS and mean feet ESC values of 69.1 ± 16.8 μS. In our study, both feet and hands ESC in controls were higher than those measured in the former study (78.36 ± 9.74 μS and 73.36 ± 9.78 μS, resp.). Sudomotor dysfunction has been observed in both prediabetes and diabetes and is closely linked to impaired epidermal C-nerve fibers, which are themselves associated with chronic high glucose [23]. We verified that both feet and hands ESC in diabetic subjects were lower than in controls (Table 2). Furthermore, ESC scores in those patients with DCAN were much lower than in those without DCAN (Figure 2). Mean hands ESC and mean feet ESC may be independent predictors of DCAN, as confirmed by ROC curve analysis (Figure 3). Similarly, Casellini et al. evaluated the relationship of hands and feet ESC to diabetic peripheral neuropathy including autonomic function, with a resultant AUC of 0.86 and 0.88, respectively [24]. Selvarajah et al. in the UK and Yajnik et al. in India [14, 25] have also conducted similar international studies.

Compared to the results of CARTs, the ESC report is quite easy to understand. Quantitative results and the lack of time-consuming and complex data analysis are both advantages of the ESC test for the clinical physician. At present, related research concerning the sensitivity and reliability of ESC for DCAN risk screening is very limited. Moreover, research data in Chinese populations are also scarce. We therefore recommend further exploration of the application of the ESC test in screening for DCAN.

Our study has a few limitations: (i) the sample size was relatively small and limited to the Shandong province of China, (ii) the subjects we studied underwent treatment with different types of antidiabetic drugs, meaning the results may not be free of the effect of therapeutic medicine, and (iii) subjects had no other evaluation of small fiber neuropathy.

In conclusion, ESC measurement is reliable and feasible to screen for DCAN among Chinese diabetic patients as a noninvasive, quantitative, and fast method especially in routine clinical practice and large-scale epidemiological surveys before further diagnosis with cardiovascular reflex tests. Further research is needed to confirm the above results and explore new applications of ESC measurement in the management of diabetic neuropathy.

Disclosure

All authors read and approved the final manuscript.

Competing Interests

The authors declare no conflict of interests.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (Grant no. 81370943), the National Health and Family Planning Commission Foundation (Grant no. 201502007), the Specific Foundation of Clinical Research of the Chinese Medical Association (13040520437, 12020170277, and 2014ZZCX02201), and Jinan Clinical Science and Technology Innovation Program (a newly established mode of diabetes prevention and treatment based on a remote wireless blood sugar management system).

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Hindawi
Journal of Diabetes Research
Volume 2017, Article ID 7602408, 11 pages
https://doi.org/10.1155/2017/7602408

Clinical Study

Rationale, Design, and Baseline Characteristics of Beijing Prediabetes Reversion Program: A Randomized Controlled Clinical Trial to Evaluate the Efficacy of Lifestyle Intervention and/or Pioglitazone in Reversion to Normal Glucose Tolerance in Prediabetes

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Received 24 October 2016; Accepted 28 November 2016; Published 12 January 2017

Academic Editor: Ruozhi Zhao

Background. Patients with prediabetes are at high risk for diabetes and cardiovascular disease (CVD). No study has explored whether intervention could revert prediabetes to normal glycemic status as the primary outcome. Beijing Prediabetes Reversion Program (BPRP) would evaluate whether intensive lifestyle modification and/or pioglitazone could revert prediabetic state to normoglycemia and improve the risk factors of CVD as well. *Methods*. BPRP is a randomized, multicenter, 2 × 2 factorial design study. Participants diagnosed as prediabetes were randomized into four groups (conventional/intensive lifestyle intervention and 30 mg pioglitazone/placebo) with a three-year follow-up. The primary endpoint was conversion into normal glucose tolerance. The trial would recruit 2000 participants (500 in each arm). *Results*. Between March 2007 and March 2011, 1945 participants were randomized. At baseline, the individuals were 53 ± 10 years old, with median BMI 26.0 (23.9, 28.2) kg/m^2 and HbA1c 5.8 (5.6, 6.1)%. 85% of the participants had IGT and 15% had IFG. Parameters relevant to glucose, lipids, blood pressure, lifestyle, and other metabolic markers were similar between conventional and intensive lifestyle intervention group at baseline. *Conclusion*. BPRP was the first study to determine if lifestyle modification and/or pioglitazone could revert prediabetic state to normoglycemia in Chinese population. Major baseline parameters were balanced between two lifestyle intervention groups. This trial is registered with www.chictr.org.cn: ChiCTR-PRC-06000005.

1. Introduction

About 6.9% of adults are estimated to have IGT (impaired glucose tolerance) globally, and the IGT prevalence is likely to be increased to 8% by 2035 [1]. The burden of prediabetes is significantly high in China. The national survey conducted during 2007-08 reported 15.5% prevalence of prediabetes in adult Chinese population [2]. Patients with prediabetes are at high risk for both diabetes and its complications, especially the cardiovascular disease (CVD) [3, 4]. Studies have shown that 1.5%–7.4% of individuals with prediabetes develop type 2 diabetes annually [4]. After 3–5 years of follow-up, 1/4 of

the patients with prediabetes would develop type 2 diabetes [4]. The rate of incidence of diabetes in the control arm of Da Qing study was 15.7 per 1000 person years, during 6 years of follow-up [5]. Patients with prediabetes were also at high risk of developing CVD [6].

Over the last two decades, studies in China and other countries had shown that lifestyle intervention, with or without therapeutic intervention, could decrease the risk of developing type 2 diabetes in patients with prediabetes [5, 7–13]. In Da Qing study, after 6 years of follow-up, compared with control group, relative risk of developing type 2 diabetes was reduced by 42% in intensive lifestyle intervention group [5]. Finnish diabetes prevention study (DPS) compared the efficacies of lifestyle intervention in preventing diabetes in patients with prediabetes [8]. The incidence of diabetes in intervention group was less than half of that in control group after two years of follow-up. Diabetes prevention program (DPP) also showed that intensive lifestyle intervention in patients with prediabetes could prevent the development of type 2 diabetes in 1 of 7 followed up over 3 years [7]. Apart from lifestyle intervention, use of antidiabetes drugs (ADDs) had shown the effectiveness in preventing diabetes in patients with prediabetes [7, 10–14]. As compared with placebo, medication intervention might reduce the relative risk of developing diabetes by 25–60% and might increase the possibility of conversion rate up to 70% [10, 11, 14–16].

Besides the prevention of diabetes, lifestyle intervention may also decrease the risk of CVD and mortality in prediabetes population. The 23-year follow-up data from the Da Qing study showed that the cumulative incidence of cardiovascular disease mortality was decreased from 19.6% to 11.9% in the lifestyle intervention group [17]. All-cause mortality was also decreased from 38.4% to 28.1% after an initial 6 years of lifestyle intervention [17]. Some studies also suggested that patients with prediabetes might also get some potential cardiovascular benefits from antidiabetic drugs since many surrogate markers were improved [18, 19].

Until now, three thiazolidinedione (TZD) drugs, including troglitazone, were used to prevent diabetes in IGT population. Troglitazone markedly reduced the incidence of diabetes during its limited period of use compared with all the other interventions in the DPP study [16]. Later on, rosiglitazone and pioglitazone were also tested to prevent diabetes in IGT population. Both of them showed a significant effect on reducing the risk of developing diabetes [4, 11, 12, 17]. However, no study have explored whether intervention could revert prediabetes to normal glycemic status as the primary outcome. The earlier studies were aimed at evaluating the efficacy of intervention on preventing progression to diabetes in individuals with impaired glucose tolerance (IGT), not prediabetes.

The purpose of Beijing Prediabetes Reversion Program (BPRP) was to examine whether lifestyle modification with or without pioglitazone could revert prediabetic state to normoglycemia over 3 years of follow-up in patients with prediabetes. Apart from the description of the study protocol of BPRP, the baseline characteristics of the randomized study subjects, by age groups and lifestyle intervention status, are also presented in this study.

2. Materials and Method

2.1. Study Design. Beijing Prediabetes Reversion Program (BPRP) is a prospective, multicenter, randomized, double blinded, and placebo controlled clinical trial, based on a 2×2 factorial design. Patients with prediabetes were randomized into four groups: conventional lifestyle intervention + placebo, conventional lifestyle intervention + pioglitazone hydrochloride 30 mg daily, intensive lifestyle intervention + placebo, and intensive lifestyle intervention + pioglitazone hydrochloride 30 mg daily. The study hypothesis was that intensive lifestyle intervention and/or pioglitazone 30 mg QD would increase the conversion rate of patients with prediabetes to normal glycemia, compared to conventional lifestyle intervention only.

Approval of protocol and consent forms by the local institutional review board was obtained at Peking University Health Science Center.

2.2. Trial Population. Individuals with high risk for diabetes were screened to confirm the glycemic state by oral glucose tolerance test (OGTT). High risk population included individuals with previously elevated fasting glucose level between 6.1 and 7.0 mmol/L or elevated 2-hour postprandial glucose level between 7.8 and 11.1 mmol/L. Our goal was to recruit 2000 participants form outpatient departments at 36 public hospitals in Beijing, China. Patients with prediabetes (confirmed by OGTT) were eligible for inclusion. Major inclusion criteria are listed as follows.

BPRP Study Major Inclusion Criteria

(i) Voluntarily participating in the trial and signing subject's informed consent form

(ii) Prediabetic patients

(iii) Both males and females

(iv) Not limited to ethnicity

(v) 25 years of age–70 years of age

(vi) $22\ \text{kg/m}^2 \leq \text{BMI} < 35\ \text{kg/m}^2$

Meanwhile, detailed inclusion and exclusion criteria are listed in Supplementary Table 1 (see Supplementary Material available online at https://doi.org/10.1155/2017/7602408). Informed consent was obtained before the individuals could participate in any screening procedures. Eligible participants were then randomized into one of the four arms of the study.

2.3. Randomization and Follow-Up. Randomization was undertaken by an independent statistician using a computer generated random sequence and was performed as block randomization with a 1 : 1 : 1 : 1 allocation ratio in four arms. Sealed envelopes were used for random allocations at the study sites. Both the participants and healthcare providers were blinded by the medication, while they were open to the lifestyle intervention.

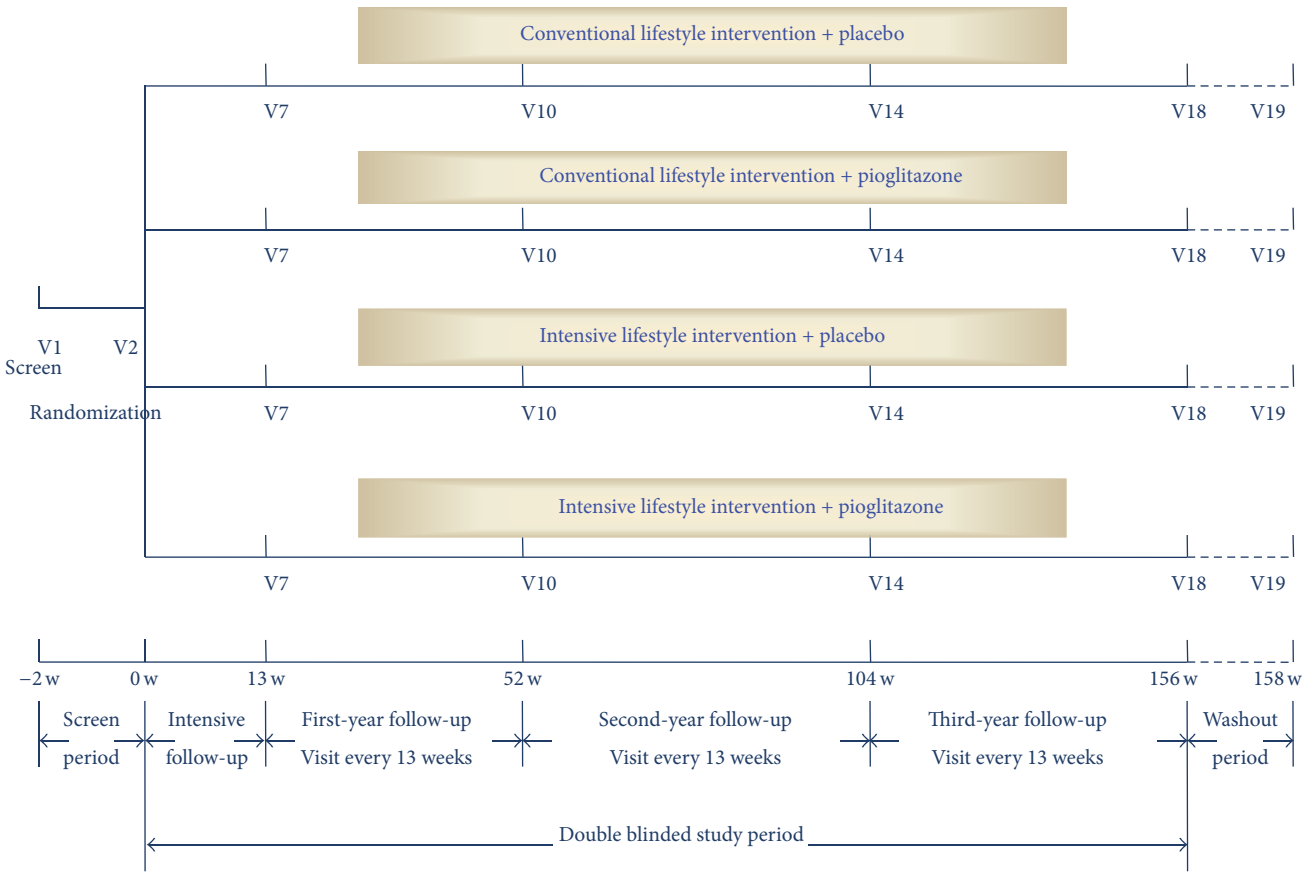

Figure 1: Study flow chart.

All the participants were recruited and followed up in the outpatient clinic in 36 study cites in Beijing. The follow-up of the study would last for three years with 19 scheduled visits. All participants would have an annual examination for glucose status (OGTT) during the follow-up period. Once the participants have been reverted to normal glucose state or have developed diabetes, defined by OGTT performed at annual examination, he or she would be terminated from the study (Figure 1).

For all four arms, study visits are scheduled for every 2-3 weeks during the first 13 weeks and every 13 weeks thereafter. Participants were required to complete a lifestyle diary comprising 3-day food records and average frequency of exercise per week for each visit.

In intensive lifestyle intervention group, participants would be educated at each visit after randomization, and the investigators would prescribe an individualized lifestyle prescription for them at each visit according to their body weight and lifestyle diary. A software program was developed to collect the lifestyle information from participants' lifestyle diary. Based on this information and the body weight at each visit, the software could calculate the compliance with lifestyle recommendations of each participant. The software would then generate a lifestyle prescription including the diet and exercise recommendations. Specific indicators of intensive lifestyle intervention are presented as follows.

Specific Indicators of Intensive Lifestyle Intervention

Exercise Principle

(i) Mainly whole-body aerobic endurance exercise, moderate intensity (3–6 MET), ≥30 min/day, 3–7 days/week, ≥150 min/week, and 180–300 min/week, was recommended.

(ii) Resistance exercise (resisted movement) as supplement was as follows: 40–50% of 1 repetition maximum (40–50% 1 RM), 3 sets of 8–10 exercises with 10–15 repetitions/set, and 2-3 days/week.

(iii) Moderate stretching exercise and flexibility exercise were as follows: ≥15 min/day and 3–7 days/week.

(iv) Energy consumption of exercise was as follows: total accumulative energy consumption ≥150 kcal/day, typically 150–300 kcal/day, and ≥750 kcal/week, typically 900–1500 kcal/week.

(v) Exercise was performed according to three stages, that is, adaption stage, consolidation stage, and maintenance stage.

Diet Principle

(i) Based on Harris-Benedict formula [16], according to the participant's specific condition, required calorie

was calculated, and rational diet plan was made for the participant.

The Goal of Weight Control

(i) For those with BMI ≥ 24 (kg/m^2), waist circumference ≥80 cm in females, or waist circumference ≥ 85 cm in males, weight should be reduced according to negative energy balance principle.

(ii) The goal of weight loss was 5–10% of the current weight.

(iii) Weight loss rate was 2–4 kg/month.

In conventional lifestyle intervention group, participants would receive the usual lifestyle modification advice at baseline and at annual visits, without any individualized counseling. They would not get a lifestyle evaluation and lifestyle related prescription. To avoid the contamination between groups, similar visit schedule was designed for all the groups.

Among participants who received pioglitazone, the dose of the medication (30 mg/day) remains the same throughout the follow-up period. The active pioglitazone and the matched placebo were manufactured by Beijing Taiyang Pharmaceutical Company. The supply chain of active medication and placebo was managed by the study investigators at the participating study centers.

Participants who were identified to have achieved normal glucose level at annual visit were asked to stop the medication and were invited for OGTT two weeks after the last visit. This procedure was followed for those participants who remained prediabetic during the course of 3 years of follow-up. Those who remained prediabetic or regressed back to normal glucose status were advised to seek usual care. Those who were found to have developed diabetes after 2 weeks of washout period were also advised to seek standard care for diabetes. All participants were advised to follow standard lifestyle management at the end of follow-up.

2.4. Primary and Secondary Outcomes. The primary aim of the study was to evaluate the proportions of participants regressing back to normal glucose level during follow-up. The normal glucose level was defined as FPG < 6.1 mmol/L and 2 hPG < 7.8 mmol/L during the OGTT. The secondary outcomes of the study were as follows: (1) incidence of type 2 diabetes, (2) time to achieving normal glucose level, (3) change in HbA1c, (4) change in body weight and waist circumference, (5) changes in blood pressure, LDL-cholesterol, HDL-cholesterol, and triglyceride, (6) changes in adiponectin, hsCRP, and insulin and C-peptide at fasting and after challenge, (7) change in urine albumin-creatinine ratio and serum creatinine, (8) composite of the incidence of at least one of the events—heart failure, nonfatal myocardial infarction, nonfatal stroke, or all-cause mortality, and (9) quality of life.

2.5. Study Measures. Details of study measurements are presented in Supplementary Table 2. At randomization and annual examinations, glucose tolerance status would be assessed by 75 g OGTT. OGTT was performed in the morning. All laboratory analyses are being conducted at the Peking University Peoples Hospital's central laboratory. Data on physical activity and diet habits would be collected from patients' diary. All lifestyle data are fed into the software to calculate the total calorie intake and physical activity level. HbA1c was measured by HPLC (Ultra2 HbA1c Detector; Primus Corporation, Duluth, GA, USA; normal range 4–6%, 20–42 mmol/mol). An immune-nephelometry method was used to measure the levels of LDL-cholesterol, HDL-cholesterol, and triacylglycerol (COBAS Integra 400 Plus System; Roche Diagnostics, Basel, Switzerland). Insulin and C-peptide were measured by an electrochemiluminescence immunoassay (Elecsys 2010 system; Roche Diagnostics). All the study drugs were withheld in the morning of testing.

At 13 weeks from randomization, ALT, AST, and serum creatinine would be measured to monitor side effects of pioglitazone and to rule out the participants who have had serious conditions which may not be suitable for the continuation of the study. Vital signs, body weight, waist circumference, and blood pressure would be recorded at each study visit. Urine HCG would also be tested at each study visit in order to avoid the use of pioglitazone during unexpected pregnancy in women within gestational age. Participants who were found pregnant during follow-up were terminated from the study.

2.6. Statistical Considerations

2.6.1. Power Analysis. BPRP would recruit 2000 participants (500 in each arm) and would be followed for a planned maximum follow-up of 3 years. This sample size is expected to provide approximately 90% power with 5% type 1 error to detect 10% relative increase in the rate of primary outcomes among participants assigned to intensive lifestyle intervention compared with conventional lifestyle intervention group under the following assumptions:

(1) 35.3% for the conventional lifestyle plus placebo, 44.3% for the conventional lifestyle plus pioglitazone, 45.3% for the intensive lifestyle plus placebo, and 54.3% for the intensive lifestyle plus pioglitazone.

(2) Participants would be recruited in half a year.

(3) 30% of the participants might be lost to follow-up during the whole study.

2.6.2. Analysis Approach. The primary and secondary outcomes of the study will be evaluated following the intention-to-treat approach, with additional supporting analyses based on the per-protocol population. A separate Statistical Analysis Plan is in place which details the analysis approaches.

2.6.3. Statistical Methods for Baseline Data Analysis. The study participants were randomized at baseline into four groups. However, the distributions of baseline study parameters are presented by the intensive and conventional lifestyle group and by different categories of age at randomization. Basic statistics were presented by number (%), mean (SD), or median (IQR) as appropriate. To evaluate the patterns of the distributions of glycemic parameters and body mass index by age groups, density plots were created. The distributions of

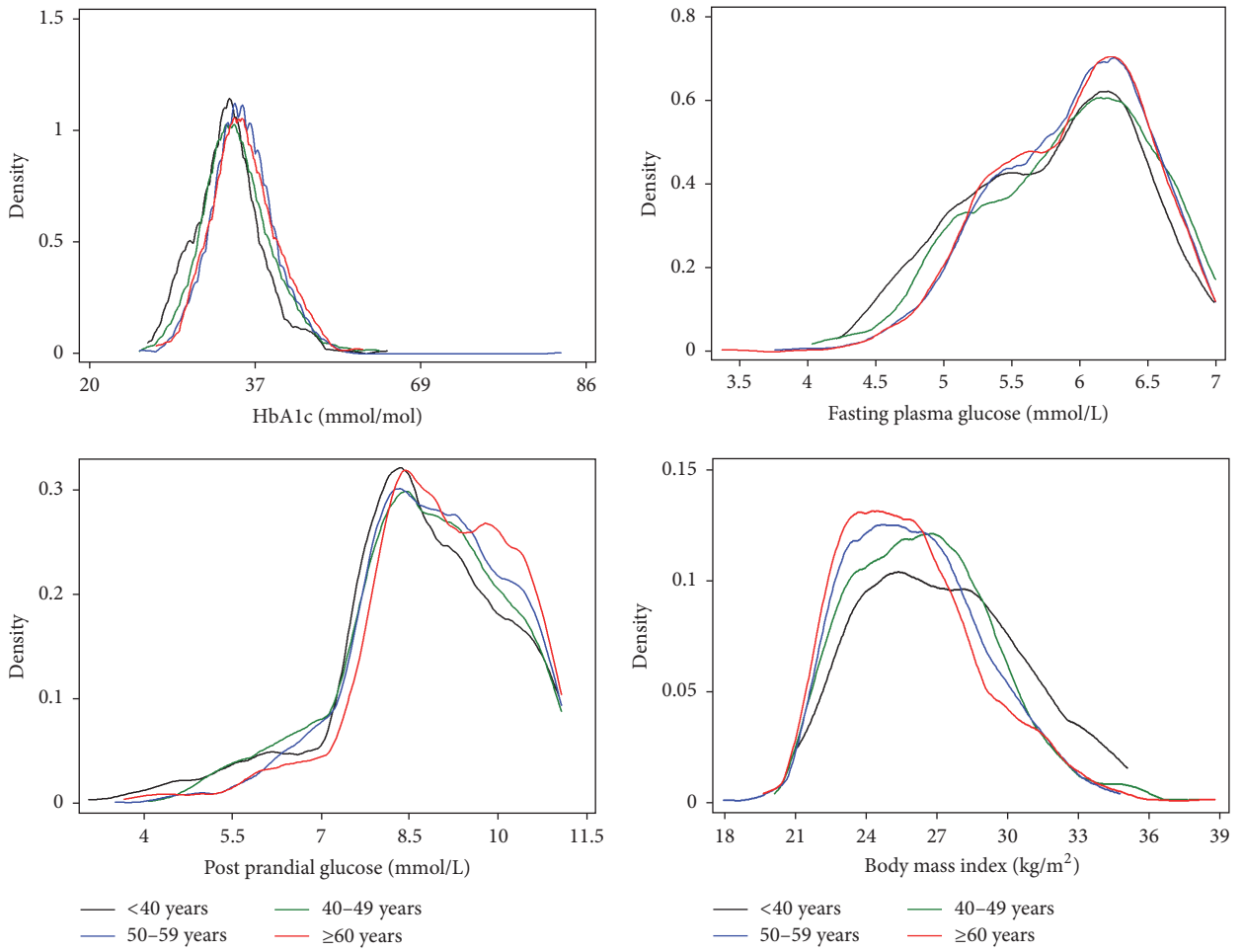

Figure 2: Density plots of HbA1c, fasting plasma glucose, postprandial glucose, and body mass index by age categories at randomization.

the study parameters were not compared between the groups for possible differences.

3. Results

3.1. Patient Recruitment. From March 2007 to March 2011, 4397 individuals were screened who met the screening criteria. Among these individuals 2034 (46.3%) were identified to have prediabetes. Following the inclusion and exclusion criteria, 1954 eligible patients were randomized to four groups in equal proportion in 36 participating study centers.

3.2. Baseline Characteristics. In the study cohort, 42% were male, with mean (SD) age 53 (10) years, median (IQR) BMI 26.0 (23.9, 28.2) kg/m^2, 49% were overweight and 12% were obese and 23% were current or ex-smokers (Table 1). Older patients were significantly less likely to be current or ex-smokers and obese, compared to patients below the age of 40 years (Table 2). Of all the participants, only 24% had low level physical activity.

The distributions of fasting and postprandial plasma glucose levels were similar between lifestyle intervention groups and across the age groups at randomization (Tables 1 and 2, Figure 2). With an average HbA1c level of 5.3% (34 mmol/mol) at baseline, about 7% patients had HbA1c ≥ 6.5% (48 mmol/mol). About 15% participants were identified with isolated IFG, while most of the subjects (85%) had IGT (54% had isolated IGT and 31% had IFG plus IGT). The distribution of metabolic and other risk factors were similar across age groups.

4. Discussion

BPRP is the first study to determine whether lifestyle modification and/or pioglitazone could revert prediabetic state back to normoglycemia in Chinese population and to explore the mechanism through which different interventions exert their effects on glucose metabolism and cardiovascular risk factors. Compared with previous diabetes prevention studies, our study has several unique features. First, while most of the earlier studies evaluated the efficacy of different interventions to prevent the development of diabetes in individuals with IGT, our study aims at evaluating the efficacy of intensive lifestyle intervention with or without TZD to regress back the prediabetic individuals to normoglycemic status [5, 10, 13, 20–23]. Only few studies have examined the effect of intervention(s) on conversion into normoglycemia in individuals with prediabetes [12]. However, the regression back

Table 1: Characteristics of participants by different lifestyle intervention at randomization.

	Conventional	Intensive	All
N	972 (50)	973 (50)	1945
Sex			
Male	450 (46)	374 (38)	824 (42)
Age (years)			
mean ± SD	52 ± 10	53 ± 10	53 ± 10
Occupation			
Professional/business	447 (47)	427 (45)	874 (46)
Workers	59 (6)	53 (6)	112 (6)
Retired	362 (38)	396 (42)	758 (40)
Jobless/other	83 (9)	76 (8)	159 (8)
Ethnicity			
Han	934 (97)	927 (96)	1861 (96)
Others	31 (3)	41 (4)	72 (4)
Education level			
Low	46 (5)	42 (4)	88 (5)
Middle	468 (49)	449 (47)	917 (48)
High	436 (46)	468 (49)	904 (47)
Smoking state			
Current smoker or past smoker	234 (24)	202 (21)	436 (23)
Body shape			
BMI (kg/m^2)	26 (24, 28)	26 (24, 28)	26 (24, 28)
Normal: BMI < 25	385 (40)	360 (37)	745 (38)
Overweight: 25 ≤ BMI < 30	464 (48)	495 (51)	959 (49)
Obese: BMI ≥ 30	123 (13)	118 (12)	241 (12)
Waist (cm)	89 (83, 96)	88 (82, 95)	89 (82, 95)
Blood pressure			
Systolic blood pressure (mmHg)	120 (111, 130)	120 (110, 130)	120 (110, 130)
Diastolic blood pressure (mmHg)	79 (70, 81)	77 (70, 80)	78 (70, 80)
Glucose level			
Fasting plasma glucose (mmol/L)	6.0 (5.5, 6.4)	6.0 (5.5, 6.4)	6.0 (5.5, 6.4)
2h plasma glucose (mmol/L)	8.8 (8.1, 9.8)	8.9 (8.1, 9.9)	8.9 (8.1, 9.9)
HbA1c (%)	5.8 (5.6, 6.0)	5.8 (5.5, 6.1)	5.8 (5.6, 6.1)
HbA1c (mmol/mol)	40 (38, 42)	40 (37, 43)	40 (38, 43)
HbA1c < 5.7% (39 mmol/mol)	331 (34)	327 (34)	658 (34)
5.7% (39 mmol/mol) ≤ HbA1c < 6.5% (48 mmol/mol)	572 (59)	572 (59)	1144 (59)
HbA1c ≥ 6.5% (48 mmol/mol)	69 (7)	74 (8)	143 (7)
IGT	821 (85)	833 (86)	1654 (85)
Isolated IGT	532 (55)	525 (54)	1057 (54)
IFG + IGT	289 (30)	308 (32)	597 (31)
Isolated IFG	151 (16)	140 (14)	291 (15)
Lipid level			
Total cholesterol (mmol/L)	4.9 (4.3, 5.5)	4.9 (4.3, 5.4)	4.9 (4.3, 5.5)
LDL-C (mmol/L)	3.2 (2.7, 3.7)	3.1 (2.6, 3.7)	3.2 (2.6, 3.7)
HDL-C (mmol/L)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)
Triglyceride (mmol/L)	1.5 (1.1, 2.1)	1.5 (1.1, 2.0)	1.5 (1.1, 2.1)
Liver function			
ALT (U/L)	21 (16, 31)	21 (15, 29)	21 (16, 30)
AST (U/L)	22 (18, 26)	21 (17, 26)	21 (18, 26)
Hemoglobin			
Hemoglobin (g/L)	143 (134, 153)	141 (132, 152)	142 (133, 153)

Table 1: Continued.

	Conventional	Intensive	All
HOMA			
HOMA-IR	2.4 (1.6, 3.5)	2.4 (1.6, 3.6)	2.4 (1.6, 3.5)
HOMA-beta	77. 5 (50.8, 113.1)	79.9 (52.3, 116.7)	78.5 (51.8, 114.7)
Cytokines			
CRP (μmol/L)	1.2 (0.7, 2.3)	1.2 (0.7, 2.4)	1.2 (0.7, 2.4)
Adiponectin	6.2 (4.2, 8.8)	6.2 (4.3, 9.0)	6.2 (4.3, 8.9)
SOD	6.8 (4.1, 10.3)	6.9 (4.3, 10.5)	6.9 (4.2, 10.4)
Amylin	7.7 (6.5, 9.5)	7.5 (6.4, 9.3)	7.6 (6.4, 9.4)
IL-6	2.3 (1.5, 4.2)	2.3 (1.5, 3.8)	2.3 (1.5, 4.0)
Urine ACR			
Urine albumin/Cr (mg/g)	7.4 (4.5, 15.2)	7.4 (4.4, 14.3)	7.4 (4.5, 14.7)
Diet			
Daily calories intake (kcal/d)	1521 (1242, 1874)	1554 (1248, 1920)	1535 (1243, 1899)
Proportion of total calories intake from carbohydrate (%)	60 (51, 69)	59 (50, 67)	60 (50, 68)
Proportion of total calories intake from protein (%)	14 (12, 16)	14 (12, 17)	14 (12, 17)
Proportion of total calories intake from fat (%)	23 (17, 30)	24 (18, 31)	24 (17, 30)
Physical activity			
Low level	232 (25)	224 (24)	456 (24)
Medium level	483 (52)	501 (53)	984 (53)
High level	223 (24)	213 (23)	436 (23)

Note. Estimates for continuous study parameters are presented by median (IQR), unless otherwise stated. Categorical study parameters are presented by number (percentage).

to normoglycemia was not the primary outcome of these studies.

In individuals with IGT, previous studies have showed that intensive lifestyle intervention can reduce the incidence of diabetes by 31%–58% [5, 15, 24]. The goal of lifestyle intervention, however, is difficult to achieve and maintain. Treatment of IGT with oral antidiabetic drugs, such as metformin, acarbose, or TZDs, has been shown to prevent or delay progression to diabetes in high risk individuals [10–12, 15] or prior gestational diabetes mellitus [25]. In addition, TZDs have shown greater efficacy in preventing IGT developing to diabetes, compared to that observed with acarbose or metformin. In IGT individuals receiving TZDs, the relative risk was reduced by 55–72% [11, 12, 25], compared to a risk reduction of 31% and 25% in IGT individuals receiving metformin [15] and acarbose [10], respectively.

Individuals with prediabetes receiving rosiglitazone were more likely to regress to normoglycemia compared with individuals receiving placebo [11]. After 5.7 years of median follow-up in DPP study, individuals who returned to normoglycemia at least once had a reduced risk of developing diabetes compared with individuals who consistently had prediabetes [26]. Increased β-cell function and insulin sensitivity may contribute to the reduced risk for diabetes in individuals who returned to normoglycemia during the intervention [26]. This suggests individuals who returned to normoglycemia may benefit more in terms of preventing diabetes. Studies aimed at evaluating the effect of intervention on conversion into normoglycemia in individuals with prediabetes and exploring the possible mechanisms involved in the conversion are needed.

Secondly, our prediabetic study population included both isolated elevated IFG and IGT population. Most of the earlier studies, including Da Qing study, DPP study, DPS study and ACT NOW trial, evaluated only the IGT population [5, 20, 21, 23]. The mechanism of isolated elevated fasting glucose level may be different with that of elevated postprandial glucose level. Only DREAM trial included individuals with IGT and with isolated IFG [22]. However, the primary outcome of this study was the incidence of diabetes during follow-up, and the efficacy of intensive lifestyle intervention was not evaluated with the intervention therapy (rosiglitazone and/or ramipril). Also, there is no data in Chinese population with isolated IFG. Our study would provide new insight into the possible efficacy of combination of lifestyle intervention and TZD in individuals with isolated IFG.

Thirdly, with a baseline BMI of 26 kg/m^2, our study offered an excellent opportunity to evaluate the possible efficacy of intensive lifestyle intervention with or without intervention with ADD in normal weight and overweight individuals. The Da Qing study and the Indian Prevention Program [5, 13] had similar BMI in the study population. However, the primary outcomes and the interventions in these studies were different. Other studies, primarily based on Caucasians from Europe and America, show a higher BMI level at baseline (around 30 kg/m^2) [12, 20, 22, 23]. However, our participants were not that obese as Caucasians in most previous diabetes prevention studies. So the goal we have set for intensive lifestyle intervention group might be a little bit difficult to achieve. But this may also provide us with an opportunity to find a proper goal of lifestyle intervention among normal weight population with prediabetes in the future.

Lastly, as there has been rapid development in the Chinese society and its lifestyle over the last decade, the general population is receiving more and more information from

Table 2: Characteristics of participants by different age groups at randomization.

	<40 years old	40–49 y	50–59 y	≥60 y
Total				
N	237 (12)	462 (234)	802 (41)	444 (23)
Sex				
Male	138 (58)	238 (52)	272 (34)	176 (40)
Occupation				
Professional/business	185 (80)	330 (73)	313 (40)	46 (11)
Workers	17 (7)	35 (8)	46 (6)	14 (3)
Retired	0 (0)	37 (8)	367 (47)	354 (82)
Jobless/others	30 (13)	48 (11)	62 (8)	19 (4)
Ethnicity				
Han	226 (96)	446 (97)	775 (97)	414 (94)
Others	9 (4)	16 (4)	22 (3)	25 (6)
Education level				
Low	1 (0)	12 (3)	37 (5)	38 (9)
Middle	63 (27)	194 (43)	454 (58)	206 (48)
High	170 (73)	250 (55)	299 (38)	185 (43)
Smoking state				
Smoker (current or past)	76 (32)	143 (31)	145 (18)	72 (16)
Body shape				
BMI (kg/m^2)	27 (25, 30)	26 (24, 28)	26 (24, 28)	25 (24, 28)
Normal: BMI < 25	73 (31)	164 (36)	316 (39)	192 (43)
Overweight: 25 ≤ BMI < 30	111 (47)	246 (53)	398 (50)	204 (46)
Obese: BMI ≥ 30	53 (22)	52 (11)	88 (11)	48 (11)
Waist (cm)	90 (84, 98)	90 (83, 96)	88 (82, 94)	88 (83, 95)
Blood pressure				
Systolic blood pressure (mmHg)	118 (110, 123)	120 (110, 126)	120 (112, 130)	125 (119, 133)
Diastolic blood pressure (mmHg)	76 (70, 80)	78 (70, 81)	79 (70, 81)	78 (70, 80)
Glucose level				
Fasting plasma glucose (mmol/L)	5.9 (5.3, 6.3)	6.0 (5.4, 6.4)	6.0 (5.5, 6.4)	6.0 (5.5, 6.3)
2 h plasma glucose (mmol/L)	8.7 (7.9, 9.6)	8.8 (8.0, 9.7)	9.0 (8.1, 9.9)	9.0 (8.3, 10.0)
HbA1c (%)	5.7 (5.4, 5.9)	5.7 (5.5, 6.0)	5.8 (5.6, 6.1)	5.8 (5.6, 6.1)
HbA1c (mmol/mol)	39 (36, 41)	39 (37, 42)	40 (38, 43)	40 (38, 43)
HbA1c < 5.7% (39 mmol/mol)	114 (48)	180 (39)	237 (30)	127 (29)
5.7% (39 mmol/mol) ≤ HbA1c < 6.5% (48 mmol/mol)	112 (47)	248 (54)	507 (63)	277 (62)
HbA1c ≥ 6.5% (48 mmol/mol)	11 (5)	34 (7)	58 (7)	40 (9)
IGT	198 (84)	379 (82)	680 (85)	397 (89)
Isolated IGT	142 (60)	253 (55)	425 (53)	237 (53)
IFG + IGT	56 (24)	126 (27)	255 (32)	160 (36)
Isolated IFG	39 (17)	83 (18)	122 (15)	47 (11)
Lipid level				
Total cholesterol (mmol/L)	4.6 (4.0, 5.2)	4.8 (4.2, 5.3)	5.0 (4.3, 5.7)	4.9 (4.3, 5.5)
LDL-C (mmol/L)	2.9 (2.5, 3.4)	3.1 (2.6, 3.6)	3.3 (2.7, 3.8)	3.2 (2.7, 3.7)
HDL-C (mmol/L)	1.1 (1.0, 1.3)	1.2 (1.0, 1.3)	1.2 (1.0, 1.4)	1.3 (1.1, 1.5)
Triglyceride (mmol/L)	1.7 (1.2, 2.5)	1.5 (1.1, 2.1)	1.5 (1.1, 2.1)	1.4 (1.1, 1.9)
Liver function				
ALT (U/L)	26 (18, 43)	22 (16, 32)	21 (16, 29)	18 (14, 24)
AST (U/L)	22 (18, 28)	20 (17, 26)	22 (18, 26)	21 (18, 25)

Table 2: Continued.

	<40 years old	40–49 y	50–59 y	≥60 y
Hemoglobin				
Hemoglobin (g/L)	148 (135, 161)	146 (134, 157)	140 (133, 150)	140 (131, 148)
HOMA				
HOMA-IR	3.2 (1.8, 4.2)	2.5 (1.5, 3.4)	2.4 (1.7, 3.5)	2.3 (1.5, 3.3)
HOMA-beta	103.8 (66.0, 157.1)	80.1 (51.9, 115.8)	75.4 (51.5, 109.9)	73.4 (48.7, 104.1)
Cytokines				
CRP (μmol/L)	1.1 (0.6, 2.3)	1.1 (0.7, 2.4)	1.2 (0.7, 2.4)	1.3 (0.7, 2.4)
Adiponectin	5.2 (3.7, 7.0)	5.6 (3.7, 8.2)	6.3 (4.6, 9.3)	6.9 (4.7, 9.5)
SOD	6.5 (3.7, 9.7)	7.0 (4.3, 10.4)	6.9 (4.1, 10.8)	6.7 (4.5, 10.0)
Amylin	7.7 (6.6, 9.4)	7.6 (6.5, 9.5)	7.5 (6.4, 9.3)	7.5 (6.3, 9.7)
IL-6	2.2 (1.5, 4.2)	2.0 (1.5, 3.4)	2.4 (1.5, 4.1)	2.5 (1.6, 4.3)
Urine ACR				
Urine albumin/Cr (mg/g)	6.4 (4.1, 13.5)	7.4 (4.3, 14.3)	7.4 (4.5, 14.5)	7.7 (4.8, 16.9)
Diet				
Daily calories intake (kcal/d)	1619 (1259, 2032)	1557 (1230, 1946)	1512 (1254, 1841)	1515 (1244, 1893)
Proportion of total calories intake from carbohydrate (%)	58 (50, 66)	59 (49, 68)	59 (50, 68)	61 (53, 68)
Proportion of total calories intake from protein (%)	14 (12, 17)	15 (12, 17)	14 (12, 17)	14 (12, 16)
Proportion of total calories intake from fat (%)	25 (19, 31)	24 (17, 31)	24 (18, 30)	23 (16, 29)
Physical activity				
Low level	95 (42)	133 (30)	168 (22)	60 (14)
Medium level	108 (47)	241 (54)	411 (53)	224 (53)
High level	25 (11)	75 (17)	195 (25)	141 (33)

Note. Estimates for continuous study parameters are presented by median (IQR), unless otherwise stated. Categorical study parameters are presented by number (percentage).

media on how to prevent diabetes. Therefore, in such an era of information explosion, it remains unknown whether individuals receiving intensive lifestyle intervention would at all show any significant benefit over those in the control group. Our study would provide necessary information to answer this question. To maximize the potential benefits from intensive lifestyle intervention, individualized lifestyle education and computerized prescription would be given to the participants in intensive lifestyle treatment group. This individualized education is based on the characteristics of each patient, considered with their body weight, diet habit, and exercise preference.

In summary, BPRP addresses the dramatically increasing population of prediabetes in China which is a major public health problem. A possible positive effect of the intensive lifestyle intervention on conversion rate from prediabetes into normoglycemia would provide a simple and powerful public health message. On the other hand, a finding that this intervention had no effect or was detrimental would be equally important and would indicate that efforts to improve diabetes care should be directed elsewhere.

5. Conclusion

BPRP was the first study to determine if lifestyle modification and/or pioglitazone could revert prediabetic state to normoglycemia in Chinese population. Major baseline parameters were balanced between two lifestyle intervention groups. In addition, with a baseline BMI of 26 kg/m^2, our study also offers an excellent opportunity to evaluate the possible efficacy of intensive lifestyle intervention with or without intervention with antidiabetic drug in normal weight and overweight individuals. Our study addresses the dramatically increasing population of prediabetes in China which is a major public health problem. This randomized clinical trial would provide the evidence of whether intensive lifestyle intervention and/or pioglitazone might convert prediabetes back into normoglycemia and would also quantify the benefits of the conversion into normoglycemia in different intervention groups.

Abbreviations

ACR: Albumin/creatinine
ADD: Antidiabetes drug
BMI: Body mass index
BPRP: Beijing Prediabetes Reversion Program
CRP: C-reactive protein
CVD: Cardiovascular disease
DPP: Diabetes prevention program
DPS: Finnish diabetes prevention study
HbA1c: Hemoglobin A1c
HDL-C: HDL-cholesterol
HOMA-beta: Homeostatic model assessment for beta cell function

HOMA-IR: Homeostatic model assessment for insulin resistance
IFG: Impaired fasting glucose
IGT: Impaired glucose tolerance
IL-6: Interleukin-6
LDL-C: LDL-cholesterol
OGTT: Oral glucose tolerance test
PG: Plasma glucose
SOD: Superoxide dismutase
TC: Total cholesterol
TG: Triglyceride
TZD: Thiazolidinedione.

Ethical Approval

Approval of protocol and consent forms by the local institutional review board was obtained at Peking University Health Science Center.

Consent

Informed consent was obtained before the individuals could participate in any screening procedures.

Disclosure

Infrastructure research support from the Australian Government's National Collaborative Research Infrastructure Strategy (NCRIS) initiative through Therapeutic Innovation Australia had no role in the design of this study and would not have any role during its execution, analyses, interpretation of the data, or decision to submit results. Beijing Taiyang Pharmaceutical Company has given all the medication support in this trial, without participating in study design, drug choice, execution, data analysis, and reporting.

Competing Interests

Sanjoy K. Paul has acted as a consultant and speaker for Novartis, GI Dynamics, and Amylin Pharmaceuticals LLC. He has received grants in support of investigator and investigator initiated clinical studies from Merck, Novo Nordisk, Hospira, AstraZeneca, Amylin Pharmaceuticals, and Pfizer. All the other authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

Linong Ji, Hongyuan Wang, Yingying Luo, Xianghai Zhou, Cuiqing Chang, and Wei Chen were responsible for the conceptualization and design of the Trial. Yingying Luo, Xianghai Zhou, Xiaohui Guo, and Jinkui Yang were involved in data acquisition. Sanjoy K. Paul and Hongyuan Wang performed the analysis and interpretation of the data. Yingying Luo, Sanjoy K. Paul, Xianghai Zhou, and Linong Ji drafted the manuscript. Cuiqing Chang, Wei Chen, Xiaohui Guo, Jinkui Yang, and Hongyuan Wang revised the manuscript for critical intellectual content. All authors approved the final the final version of the manuscript. Yingying Luo, Sanjoy K. Paul, Hongyuan Wang, and Linong Ji had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Yingying Luo and Sanjoy K. Paul contributed equally to this work and should be considered co-first authors.

Acknowledgments

Funding support was received from the Beijing Municipal Science & Technology Commission (Grant nos. D0905003040131, D131100005313008, and D121100004412002). QIMR Berghofer gratefully acknowledges infrastructure research support from the Australian Government's National Collaborative Research Infrastructure Strategy (NCRIS) initiative through Therapeutic Innovation Australia. The authors thank Beijing Taiyang Pharmaceutical Company to have given all the medication support in this trial. They thank all the participants for participating in this study. The authors also acknowledge all the principle investigators in the following study sites for their contribution to this trial: Beijing Tongren Hospital: Jingkui Yang; Beijing Hospital: Lixin Guo; The Military General Hospital of Beijing PLA: Xiaofeng Lv; Peking University First Hospital: Xiaohui Guo; Peking Union Medical Colledge Hospital: Hongding Xiang; Fuxing Hospital: Xiaoming Zhuang; Beijing No. 6 Hospital: Shangnong Wang; The Second Artillery General Hospital of Chinese People's Liberation Army: Quanmin Li; Beijing Zhanlanlu Hospital: Shuling Chang; Peking University Third Hospital: Tianpei Hong; Chinese PLA General Hospital: Juming Lu; The 304th Hospital: Shinan Yin; The 309th Hospital of Chinese People's Liberation Army: Yan Zhang; Navy General Hospital: Qiyu Guo; Air Force General Hospital: Xiaohong Guan; Beijing Haidian Hospital: Wei Huang; China-Japan Friendship Hospital: Guangwei Li; The 306th Hospital of PLA: Zhangrong Xu; Beijing Chaoyang Hospital: Yuan Xu; Beijing Chuiyangliu Hospital: Cuiping Liu; China Meitan General Hospital: Hongmei Li; Civil Aviation General Hospital: Dingqiong Peng; Xuanwu Hospital: Li Wang; Beijing Tiantan Hospital: Lirong Zhong; Aerospace 731 Hospital: Yuming Liu; Beijing Electric Power Hospital: Dongmei Ni; The Luhe Teaching Hospital of the Capital Medical University: Dong Zhao; People's Hospital of Beijing Daxing District: Changchun Xue; Beijing Pinggu Hospital: Yufeng Li; Daxing Hospital of Tranditional Chinese Medicine: Li Ma; Beijing Chaoyang Hospital (Jingxi Campus): Shan Gao; Beijing Hepingli Hospital: Yaping Liu; Peking University Shougang Hospital: Xiaoping Lu; Nanyuan Hospital: Kaijie Yang; Beijiao Hospital: Z. Jing; Nanfaxin Hospital: Rongmin Zhang.

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Hindawi Publishing Corporation
Journal of Diabetes Research
Volume 2016, Article ID 7592402, 7 pages
http://dx.doi.org/10.1155/2016/7592402

Research Article

Assessment of the Relationship between Diabetic Retinopathy and Nailfold Capillaries in Type 2 Diabetics with a Noninvasive Method: Nailfold Videocapillaroscopy

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Received 24 August 2016; Accepted 22 November 2016

Academic Editor: Xinghai Yao

Background and Objectives. Nailfold capillaroscopy is an easy and noninvasive technique used to investigate dermal microvasculature. Traditional investigations of vascularity do not detect changes until they are well-established in type 2 diabetics. The objective of the current study was to evaluate nailfold capillaries in type 2 diabetes mellitus patients and to determine the association of retinopathy with changes in the nailfold capillaries. *Materials and Methods*. Capillaroscopic findings by nailfold capillaroscopy and fundoscopic examinations were assessed in 216 patients with type 2 diabetes mellitus and 101 healthy controls included in this prospective study. *Results*. Retinopathy was detected in 43.05% of diabetic patients ($n = 93$). Capillaroscopic findings including tortuosity ($p < 0.001$), bushy capillary ($p < 0.001$), neoformation ($p < 0.001$), bizarre capillary ($p < 0.001$), microhemorrhage ($p = 0.001$), capillary ectasia ($p = 0.002$), and aneurysm ($p = 0.004$) were significantly higher in diabetic group than control group. In logistic regression analysis, only tortuosity was shown significant (OR, 2.16; $p = 0.036$). There was also a significant relation between diabetes duration and most of the capillaroscopic findings. *Conclusion*. Capillaroscopic changes were found to be correlated with diabetic retinopathy, in particular with longer disease duration in our study. Capillaroscopic imaging could be a useful new technique for assessment of diabetic microvascular changes.

1. Introduction

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies [1]. Diabetic vascular complications are the most common cause of mortality and morbidity worldwide, with numbers of affected individuals steadily increasing [2]. Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and remains a major cause of preventable blindness [3]. Microaneurysms, leukocyte adhesion, and apoptosis of vascular and neuronal cells are the early changes of DR. Capillary degeneration and development of acellular capillaries cause a reduction in capillary perfusion and hypoxia. Consequently, capillary neovascularization occurs and these findings are characteristic of proliferative DR [3]. The majority of patients with DR have no symptoms until an advanced stage and may rapidly deteriorate. Impairment of the mechanical structure of the vessel wall and vascular endothelial function causes vascular dysfunction and this also fuels the pathogenesis of vascular disease in type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) [4]. Unfortunately, traditional methods do not detect these complications until they are well-established.

Nailfold videocapillaroscopy (NVC) is an easy, noninvasive, safe, and useful diagnostic tool to evaluate the microvascular structure of nailfold. NVC is used to assess disturbances in the skin capillaries of patients with autoimmune connective tissue disorders, especially in systemic sclerosis.

NVC enables more accurate measurement and it is possible to store and analyze capillary data [5].

On the basis of this knowledge, the aim of the current study was to assess the nailfold capillaries to evaluate diabetic microvascular involvement, to determine any correlation between nailfold capillaroscopic findings and retinopathy and to search whether these changes have a relationship with duration of diabetes or not in patients with T2DM.

2. Materials and Methods

2.1. Patients. The study included 216 patients with T2DM and 101 healthy controls in the Internal Medicine Outpatient Clinic of University of Health Sciences Antalya Training and Research Hospital. Exclusion criteria were a history of ocular and retinal disease, Raynaud phenomenon, collagen tissue disease, drug usage affecting fibrinolysis metabolism (such as glucocorticoids and oral contraceptives), smoking, and occupation with a risk of microtrauma (e.g., farmer and gardener). All the patients were examined by an ophthalmologist for DR and by a rheumatologist for capillary assessment with the NVC device. Approval for the study was granted by the Local Ethics Committee and informed consent was obtained from all patients.

2.2. Ocular Examination. All subjects underwent a complete ophthalmic examination including best-corrected visual acuity, slip-lamp biomicroscopy, and dilated fundus examination. DR was confirmed by fundus photography (FFA Visucam NM/FA, Carl Zeiss, Germany) and optical coherence tomography imaging (Cirrus HD-OCT Model 5000, Carl Zeiss Meditec Inc., Dublin, CA, USA) and classified according to the ETDRS (Early Treatment of Diabetic Retinopathy Study, September 1, 2006) guidelines.

2.3. Capillaroscopic Assessment. After 20 minutes resting at a room temperature of 20–24°C, immersion oil was applied on the nailfold of all participants for better visualization. Capillaroscopy was applied to 8 fingers (excluding the thumbs) of all participants at ×200 magnification with a capillaroscopy device (Videocap, DS MediGroup, Milan, Italy) by a rheumatologist blinded to patient's condition and 4 images (1 × 1 mm in size) from the middle of the nailfold in each finger were evaluated [6]. A total of 32 images were obtained and recorded on the videocapillaroscopy device for each patient.

The nailfold capillary system was assessed for capillary distribution, density, and morphology according to the Maricq criteria modified by Bergman et al. [7]. In the normal nailfold, the distal capillary rows in the dermal papillae have a parallel course to the nail surface and can be seen in their whole length. These distal capillary rows appear as red and hairpin-shaped in healthy individuals [8]. However, characteristic capillaroscopy findings in patients with rheumatic disease are enlarged capillaries, giant capillaries, neoangiogenesis, capillary loss, and/or avascular areas [9].

Abnormal capillaroscopic findings were defined as follows: (1) tortuosity: 2 or more cross capillaries, each over 1 mm in length; (2) neoangiogenesis: tortuous, bush-like capillaries with marked heterogeneity in size, (a) as the presence of extremely tortuous, bushy, branching, ramified and coiled capillaries, (b) ≥4 capillaries within a single dermal papilla, and (c) thin and branching interconnected capillaries originating from a single loop; (3) microhemorrhage: 2 or more punctate bleeds around a single capillary in at least 2 fingers (separate or confluent microhemorrhage areas); (4) extravasation: leakage of capillary content; (5) avascular area: loss of at least 2 consecutive capillaries or ≤6 capillaries over each 1 mm length; (6) bizarre capillary: capillaries with abnormal appearance but not resembling other defined abnormal capillaries (clover leaf, musical note G, etc.); (7) ectatic capillaries: capillary wall diameter between 0.02 and 0.05 micrometers (regular or irregular); (8) megacapillary: capillary wall diameter >0.05 micrometers (Figure 1) [5, 8].

2.4. Statistical Analysis. Descriptive statistics are presented as frequency (n), percentage (%), median (minimum–maximum), and mean ± standard deviation (SD). Fisher's exact test and Pearson's chi-square test were used to assess relationships between categorical variables. Conformity to normality of distribution was tested using the Shapiro-Wilks test in groups of sample size ≤ 50 and with the Kolmogorov-Smirnov test in groups of sample size > 50. The difference between two groups was tested using the Mann–Whitney U test and Student's t-test where appropriate. One-way analysis of variance (one-way ANOVA) with Tukey's HSD post hoc test was used to compare differences between three groups with normal distribution, while the Kruskal-Wallis with Bonferroni-Dunn post hoc test was used for nonnormally distributed data. The Spearman correlation test was applied to test relationships of ordinal or quantitative variables with nonnormal distribution and the Pearson's correlation test to evaluate continuous variables with normal distribution. A value of $p < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS version 22.0.

3. Results

3.1. Baseline Characteristics. The age was comparable between patients with DR group ($n = 93$; 48 female, 45 male, 62 with proliferative DR, 31 with nonproliferative DR), patients without DR group ($n = 123$; 78 female, 45 male), and healthy control group ($n = 101$; 47 female, 54 male) [60.89 ± 8.281 versus 58.92 ± 8.506 versus 59.41 ± 11.867 years, $p = 0.316$]. Median disease duration of DR positive group was significantly higher than DR negative and control group [14 (0–40) versus 4 (0–25) versus 0 (0) years; $p < 0.001$]. HbA1c levels of patients with DR were higher than patients without DR group [8.7% (5.4–14.6) versus 7.2% (5.0–12.3); $p < 0.001$]. 68.8% of DR positive and 56.9% of DR negative patients have hypertension history and medication ($p = 0.074$) and 22.6% of patients with DR and 35.8% of patients without DR have antilipid treatments ($p = 0.036$) (Table 1).

3.2. Frequencies and Comparison of Capillaroscopic Findings of Patients with DR, Patients without DR, and Healthy Control Group. The frequencies of tortuosity [75 (80.6%) versus 71 (57.7%) versus 6 (5.9%); $p < 0.001$], bushy capillary [31 (33.3%)

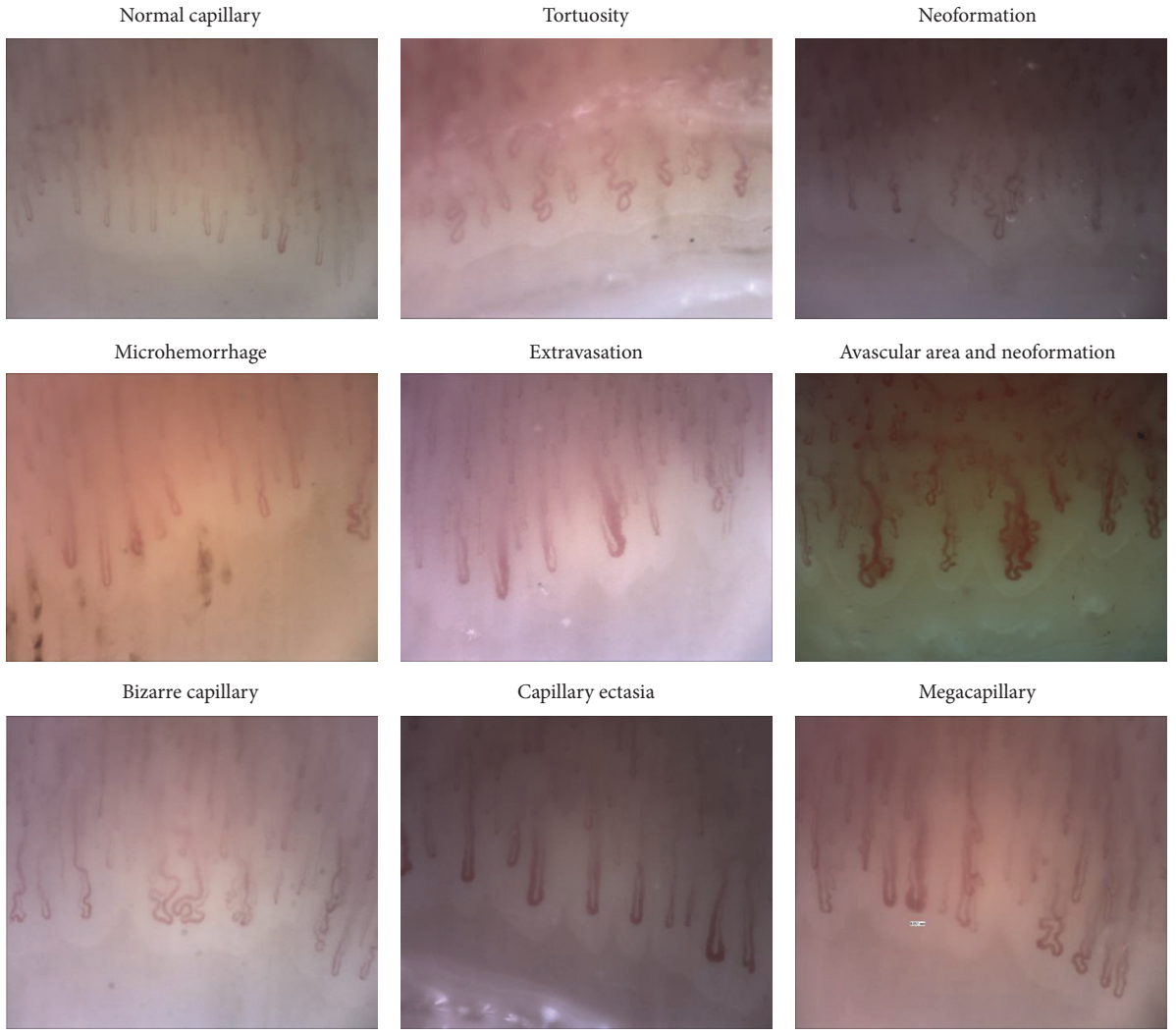

Figure 1: Normal and pathological videocapillaroscopy findings.

versus 15 (12.2%) versus 0 (0%); $p < 0.001$], neoformation [29 (31.2%) versus 15 (12.2%) versus 0 (0%); $p < 0.001$], bizarre capillary [36 (38.7%) versus 37 (30.1%) versus 7 (6.9%); $p < 0.001$], microhemorrhage [11 (11.8%) versus 5 (4.1%) versus 0 (0%); $p = 0.001$], capillary ectasia [11 (11.8%) versus 7 (5.7%) versus 0 (0%); $p = 0.002$], and aneurysm [10 (10.8%) versus 7 (5.7%) versus 0 (0%); $p = 0.004$] were found significantly increased in diabetics than healthy controls when patients with DR and without DR were compared with healthy controls (Table 1).

3.3. Correlation of Capillaroscopic Findings with the Severity of DR. Tortuosity [50 (80.6%) versus 25 (80.6%) versus 71 (57.7%); $p = 0.002$], bushy capillary [25 (40.3%) versus 6 (19.4%) vs. 15 (12.2%); $p < 0.001$], neoformation [22 (35.5%) versus 7 (22.6%) versus 15 (12.2%); $p = 0.001$], and capillary ectasia [10 (16.1%) versus 1 (3.2%) versus 7 (5.7%); $p = 0.029$] were significantly higher in patients with proliferative DR than nonproliferative DR and patients without DR (Table 2).

3.4. Comparison of Median Diabetes Years of Patients with and without Significant Capillaroscopic Findings. Median (min–max) diabetes years of tortuosity [10 (0–40) versus 3 (0–23) years; $p < 0.001$], bushy capillary [12.50 (0–40) versus 5 (0–40) years; $p < 0.001$], aneurysm [14 (2–33) versus 6 (0–40) years; $p = 0.001$], neoformation [12 (0–40) versus 5.50 (0–40) years; $p = 0.002$], and bizarre capillary [10 (0–40) versus 5.50 (0–33) years; $p = 0.049$] were significantly higher in diabetic patients who have these findings than patients lacking these findings (Table 3).

3.5. Comparison of Median Diabetes Years of Significantly Increased Capillaroscopic Findings between Patients with DR and without DR. Tortuosity [14 (0–40) versus 5.5 (0–25) years; $p < 0.001$], aneurysm [18 (10–33) versus 9 (2–14) years; $p = 0.003$], bizarre capillary [14.5 (0–40) versus 6.5 (0–25) years; $p < 0.001$], and microhemorrhage [16 (8–33) versus 5 (0–9) years; $p = 0.003$] have significantly longer median diabetes years in patients with DR than without DR (Table 3).

Table 1: Demographical characteristics and frequencies of capillaroscopic findings of patients with DR, patients without DR, and healthy controls.

	Diabetic patients (n = 216)		Controls (n = 101)	p
	DR (+) (n = 93)	DR (−) (n = 123)		
Mean age (SD)	60.89 (8.21)	58.92 (8.506)	59.41 (11.867)	0.316
Male gender, n	45 (36.6%)	45 (48.4%)	54 (53.5)	**0.033**
Hypertension, n	64 (68.8%)	70 (56.9%)	0	0.074
Hyperlipidemia, n	21 (22.6%)	44 (35.8%)	0	**0.036**
HbA1c, % (min–max)	8.7 (5.4–14.6)	7.2 (5.0–12.3)	0	**<0.001**
Diabetes years, median (min–max)	14 (0–40)	4 (0–25)	0	**<0.001**
Tortuosity, n	75 (80.6%)	71 (57.7%)	6 (5.9%)	**<0.001**
Bushy capillary, n	31 (33.3%)	15 (12.2%)	0 (0%)	**<0.001**
Neoformation, n	29 (31.2%)	15 (12.2%)	0 (0%)	**<0.001**
Bizarre capillary, n	36 (38.7%)	37 (30.1%)	7 (6.9%)	**<0.001**
Microhemorrhage, n	11 (11.8%)	5 (4.1%)	0 (0%)	**0.001**
Capillary ectasia, n	11 (11.8%)	7 (5.7%)	0 (0%)	**0.002**
Aneurysm, n	10 (10.8%)	7 (5.7%)	0 (0%)	**0.004**
Extravasation, n	6 (6.5%)	1 (0.8%)	0 (0%)	NA
Megacapillary, n	1 (1.1%)	0 (0%)	0 (0%)	NA
Meander capillary, n	4 (4.3%)	3 (2.4%)	0 (0%)	NA
Avascular area, n	3 (3.2%)	0 (0%)	0 (0%)	NA
Interstitial edema, n	1 (1.1%)	1 (0.8%)	0 (0%)	NA

NA: not applied; DR: diabetic retinopathy.

Table 2: Correlation of capillaroscopic findings with severity of DR.

	Proliferative DR (n = 62)	Nonproliferative DR (n = 31)	Patients without DR (n = 123)	p
Tortuosity, n	50 (80.6%)	25 (80.6%)	71 (57.7%)	**0.002**
Bushy capillary, n	25 (40.3%)	6 (19.4%)	15 (12.2%)	**<0.001**
Neoformation, n	22 (35.5%)	7 (22.6%)	15 (12.2%)	**0.001**
Capillary ectasia, n	10 (16.1%)	1 (3.2%)	7 (5.7%)	**0.029**
Bizarre capillary, n	25 (40.3%)	11 (35.5%)	37 (30.1%)	0.372
Microhemorrhage, n	9 (14.5%)	2 (6.5%)	5 (4.1%)	NA
Aneurysm, n	9 (14.5%)	1 (3.2%)	7 (5.7%)	NA
Extravasation, n	6 (9.7%)	0 (0%)	1 (0.8%)	NA
Megacapillary, n	1 (1.6%)	0 (0%)	0 (0%)	NA
Meander capillary, n	4 (6.5%)	0 (0%)	3 (2.4%)	NA
Avascular area, n	3 (4.8%)	0 (0%)	0 (0%)	NA
Interstitial edema, n	0 (0%)	1 (3.2%)	1 (0.8%)	NA

NA: not applied; DR: diabetic retinopathy.

3.6. Multivariate Logistic Regression Analysis of Significant Capillaroscopic Findings for DR Prediction. Tortuosity was significantly associated with DR [odds ratio 2.106, confidence interval 1.051 to 4.219; $p = 0.036$] (Table 4).

3.7. Estimates of Diagnostic Test for Significant Capillaroscopic Findings for DR Detection. The AUC (area under curve) values of tortuosity (0.615), bushy capillary (0.606), and neoformation (0.595) were lower than 80% with ROC (receiver operating characteristic) analysis (Table 5, Figure 2).

4. Discussion

DR is the leading cause of blindness and the goal is to detect clinically significant retinopathy before vision is threatened [10]. To identify individuals at risk of DR progression and early intervention can limit vision loss and reduce the costs associated with managing more advanced disease. NVC has been used for the analysis of microvascular structure especially in rheumatic disease and in some extrarheumatic diseases [5]. Any disease affecting the vascular structures may

Table 3: Comparison of median diabetes years of patients with and without significant capillaroscopic findings and comparison of median diabetes years of significant capillaroscopic findings patients with DR and without DR.

			Median (min–max) diabetes years		p^x	p^y
			Patients with DR	Patients without DR		
Tortuosity	+	10 (0–40)	14 (0–40)	5.5 (0–25)	**<0.001**	**<0.001**
	–	3 (0–23)				
Bushy capillary	+	12.50 (0–40)	14 (0–40)	8 (0–25)	**<0.001**	0.169
	–	5 (0–40)				
Aneurysm	+	14 (2–33)	18 (10–33)	9 (2–14)	**0.001**	**0.003**
	–	6 (0–40)				
Neoformation	+	12 (0–40)	14 (0–40)	8 (0–25)	**0.002**	0.143
	–	5.50 (0–40)				
Bizarre capillary	+	10 (0–40)	14.5 (0–40)	6.5 (0–25)	**0.049**	**<0.001**
	–	5.50 (0–33)				
Microhemorrhage	+	12.50 (0–33)	16 (8–33)	5 (0–9)	0.050	**0.003**
	–	7 (0–40)				
Capillary ectasia	+	10 (2–27)	10 (2–27)	8 (4–12)	0.203	0.273
	–	7 (0–40)				

(+): capillaroscopic finding is present; (−): capillaroscopic finding is absent. DR: diabetic retinopathy; p^x: comparison of median diabetes years of patients with versus without significant capillaroscopic finding; p^y: comparison of median diabetes years of significant capillaroscopic findings patients with DR versus without DR.

Table 4: Multivariate logistic regression analysis of significant capillaroscopic findings.

	p	OR (95% CI)
Tortuosity	**0.036**	2.106 (1.051–4.219)
Bushy capillary	0.270	2.754 (0.455–16.648)
Neoformation	0.919	1.098 (0.182–6.635)
Microhemorrhage	0.119	2.505 (0.790–7.941)
Bizarre capillary	0.803	1.086 (0.568–2.077)
Capillary ectasia	0.778	1.172 (0.388–3.545)
Aneurysm	0.592	1.359 (0.443–4.173)

Table 5: Estimates of diagnostic test for significant capillaroscopic findings for DR detection.

	AUC	95% CI
Tortuosity	0.615	0.540 to 0.689
Bushy capillary	0.606	0.528 to 0.683
Neoformation	0.595	0.517 to 0.673
Microhemorrhage	0.539	0.460 to 0.617
Bizarre capillary	0.543	0.465 to 0.621
Capillary ectasia	0.531	0.452 to 0.609
Aneurysm	0.525	0.447 to 0.604

AUC: area under curve; CI: confidence interval.

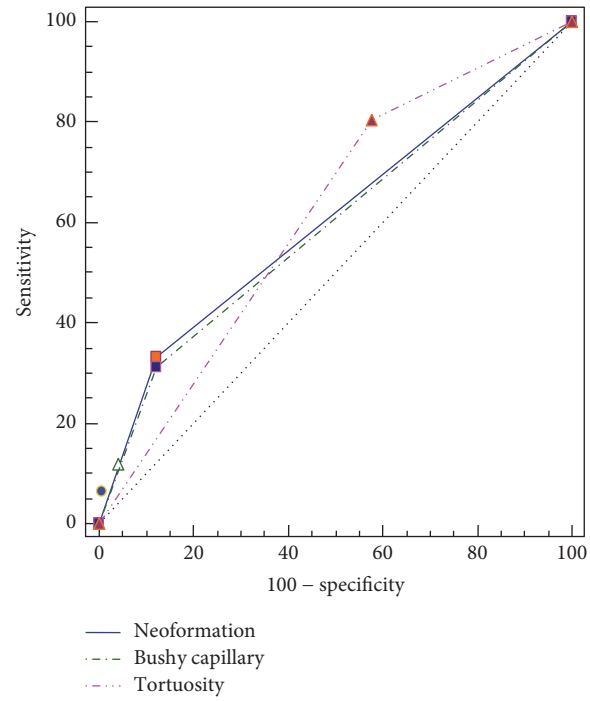

Figure 2: ROC curve of tortuosity, bushy capillary, and neoformation.

give findings on NVC and there have been many studies assessing capillaroscopic findings in different diseases [11–14]. Therefore, capillaroscopic investigations in DM patients were started in the 1960s [15]. However, there are limited data in literature related to this subject, especially in respect of T2DM due to complex nature of retinopathy [16–18].

In the present study, we demonstrated that tortuosity, bushy capillary, neoformation, bizarre capillary, microhemorrhage, capillary ectasia, and aneurysm were significantly higher in patients with T2DM than healthy controls. Capillaroscopic findings including tortuosity, bushy capillary, neoformation, and capillary ectasia were also significantly higher in patients with proliferative DR than patients with

nonproliferative DR and without DR. These findings show that there is a microvascular involvement in T2DM and a strong correlation with DR, and NVC can detect changes in the nailfold capillaries precisely. In 1997, Chang et al. evaluated 35 patients with diabetes (10 without DR, 10 with background DR, and 15 with proliferative DR) and 20 healthy controls. Tortuosity was the highest findings in group of proliferative DR (68%). It was found %20 in control group and %23 in group of without DR [16]. Meyer et al. studied density, diameters, and morphology of nailfold capillaries in 16 patients with T1DM and 19 with T2DM. They found tortuous and dilated capillaries that could indicate microangiopathy by means of NVC [17]. In another study, 49 patients (21 with T1DM and 28 T2DM) and 39 controls were evaluated by Barchetta et al. They performed NVC, ophthalmoscopy, and retinal fluorangiography to all subjects and used quantitative evaluation of NVC and score. They found that increased density, irregular length and distribution of capillary loss, aberrant morphological alterations such as tortuosity, ramifications, and bushes, presence of exudates, oedema, and flux abnormalities were detected by NVC in diabetic patients. Moreover, according to Barchetta et al. study, NVC was capable of identifying alterations in almost %50 of patients with diabetes without retinopathy [18]. Our study is the most comprehensive study in this subject; more patients with T2DM and more healthy controls were evaluated. Furthermore, more and actual capillaroscopic findings were evaluated in this study. These findings can be an indicative of microvascular involvement for further research in T2DM patients.

Diabetes years of patients with tortuosity, bushy capillary, aneurysm, neoformation, and bizarre capillary were longer than diabetic patients lacking these findings regardless of retinopathy in our study. When we compared the median diabetes years of significantly present capillaroscopic findings by the presence of DR, diabetes years of patients having tortuosity, aneurysm, bizarre capillary, and microhemorrhage were significantly longer in patients with DR than patients without DR. Positive correlation of capillaroscopic findings and diabetes duration was also stated in Chang et al. and Meyer et al. studies, whereas Barchetta et al. found that NVC findings were independent from duration of diabetes [16–18]. Although these findings does not predict when the microvascular changes formed, there is a significant correlation between diabetes years and capillaroscopic findings in our study. It can be assumed that early detection of tortuosity, aneurysm, bizarre capillary, and microhemorrhage may be a precursor of DR.

Although tortuosity was the most valuable capillaroscopic finding in logistic regression analysis, it cannot be used as a diagnostic tool since the AUC value was less than 80% in ROC curve (Figure 2).

Compared to previous studies, this cross-sectional study has more T2DM patients and more capillaroscopic findings were evaluated. Our study population was homogeneous in terms of age and hypertension history between groups. HbA1c level was higher in patients with DR as expected and more patients were using antilipid drug in DR negative group. Relationship of other diabetic complications, such as nephropathy and neuropathy, and other comorbidities of patients with NVC findings were not evaluated in this study as a limitation. Also, quantitative evaluation of NVC findings and NVC score were not performed.

5. Conclusions

Our data have showed that there is a significant correlation with capillaroscopic findings and DR, and NVC can detect microvascular changes in T2DM patients without clinically apparent retinopathy. These findings may guide the detection of T2DM associated retinopathy and microvascular complications earlier. Capillaroscopic findings including tortuosity, bushy capillary, aneurysm, neoformation, and bizarre capillary were significantly linked with a longer DM duration and DR positive patients in our study. We suppose that tortuosity may be the leading finding for diagnosis of early DR according to our data. The evaluation of nailfold capillaroscopic findings may be a new modality for vascular assessment of diabetic patients to diagnose and follow up microvascular complications. Further NVC studies are needed to determine the timing and relationship of other comorbidities of DM.

Competing Interests

The authors declare that there are no competing interests.

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