Original Article

Assessment of the relationship of systemic vascular dysfunction and cardiac autonomic neuropathy (CAN) with diabetic retinopathy

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Abstract

Context: Diabetic retinopathy, a form of microvasculopathy, is the leading cause of the visual abnormality. However, there is no conclusive evidence of the relationship of systemic vascular dysfunction with retinal microvasculopathy. In addition, diabetes-associated cardiac autonomic neuropathy may also compromise vascular function. **Aims:** The present study intends to correlate arterial stiffness, endothelial function, and heart rate variability (HRV) as a standardized measure of cardiac autonomic neuropathy with diabetic retinopathy. **Settings and Design:** The present cross-sectional, observational study was conducted in the Department of Physiology. **Materials and Methods:** Twenty subjects were recruited in group 1 (T2DM, type 2 diabetes mellitus patients, without retinopathy) and group 2 (T2DM with retinopathy). The vascular parameters such as heart rate, peripheral and central blood pressure, augmentation index [AIx (%)], brachial -ankle pulse wave velocity (baPWV), and reactive hyperaemia index (RHI) were recorded. **Statistical Analysis Used:** Independent sample *t*-test (for parametric data) and Mann–Whitney *U* test (for non-parametric data) were employed to compare the variables of two groups. Spearman correlation was used to examine the relationship among the parameters. Linear regression analysis was performed to examine the important vascular predictor for diabetic retinopathy. **Results:** baPWV was significantly higher in group 2 than in group 1 and positively associated with group 2. RHI was significantly less in group 2 than group 1 and negatively associated with group 2. Among HRV metrics, standard deviation of successive differences (SDSD), root mean square of successive differences between normal heartbeats (RMSSD), and high frequency (HF) power were significantly decreased in group 2 than in group 1. SDSD, RMSSD, and HF *power* were negatively associated with group 2. RHI emerged as a significant predictor of diabetic retinopathy following linear regression. **Conclusions:** Overall, the result of the present study indicates that metabolic dysregulation of glucose may affect the normal functioning of the autonomic nervous system and vascular function. Therefore, screening of vascular function and cardiac autonomic tone may be advocated in diabetic patients in routine clinics to examine the existence of any comorbid condition, such as diabetic retinopathy, as systemic vascular changes may also affect ophthalmic vasculature.

Keywords: Arterial stiffness, cardiac autonomic neuropathy, diabetic retinopathy, reactive hyperaemia

Introduction

Micro and macro-vasculopathy are common complications of type 2 diabetes mellitus (T2DM). Among the microvasculopathies, T2DM and diabetic retinopathy (DR) are the most common complications that may lead to vision impairment.[1] The clinical diagnosis of DR is based on the fundoscopic findings of

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Received: 03-06-2023 **Revised:** 03-07-2023
Accepted: 17-07-2023 **Published:** 21-12-2023

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_909_23

How to cite this article: Hari Prakash KJ, Parija S, Kar M. Assessment of the relationship of systemic vascular dysfunction and cardiac autonomic neuropathy (CAN) with diabetic retinopathy. J Family Med Prim Care 2023;12:3236-41.

vascular abnormalities in the retina. It is divided into two stages: nonproliferative T2DM retinopathy (NPDR) and proliferative T2DM retinopathy (PDR). NPDR represents the early stage of DR, characterized by loss of pericytes, increased vascular permeability, formation of microaneurysms, and breakdown of the blood-retinal barrier (BRB). PDR is a more advanced stage of the disease, characterized by neovascularization, leading to vitreous hemorrhage and tractional retinal detachment. It causes impairment of the patient's vision and decreases their quality of life.[2,3]

Arterial stiffness may decrease intra-arterial wave reflection and increase pulsatile pressure, which may cause damage to retinal microcirculation.[4] Several studies tried to understand the association of arterial stiffness and endothelial dysfunction with DR or the severity of DR.[5–7] But there is no conclusive answer to it. Moreover, the prevalence of cardiac autonomic neuropathy (CAN) at the time of diagnosis of T2DM is around 7%, and it increases with the duration of diabetes by 4.6– 6% per year.[8] It is known that insulin resistance may give rise to insulin-driven sympathetic overactivity conditions affecting cardiovascular functions.[9] Therefore, the present study intends to determine arterial stiffness, endothelial function, and heart rate variability (HRV) in T2DM with and without T2DM retinopathy and examine their association with DR.

Materials and Methods

The present study was a cross-sectional, observational study conducted from July 2022 to October 2022. Twenty T2DM patients diagnosed without retinopathy and 20 T2DM patients with retinopathy were recruited on the basis of inclusion and exclusion criteria from the outpatient department of Ophthalmology. The study was approved by Institute Ethics Committee (IEC/AIIMS BBSR/SS/2022-23/01).

Assessment of T2DM retinopathy at the Department of Ophthalmology

A comprehensive ocular examination was performed on diabetic patients to rule out diabetic retinopathy. The visual acuity for distant and near vision was noted. The slit-lamp biomicroscopy in the anterior segment was conducted to examine for the presence of corneal and lenticular changes. The patient's pupil was dilated using plain tropicamide (0.8%) eye drops for the examination of the fundus.

The indirect or direct ophthalmoscope was used to examine the fundus. As per the Early Treatment T2DM Retinopathy Study (ETDRS) classification and the findings such as microaneurysms, tortuosity of veins, hard or soft exudates, and macular edema, the patient was graded as nonproliferative T2DM retinopathy (NPDR) or proliferative T2DM retinopathy.

After recruitment, the study participants were requested to report to the Clinical Physiology Laboratory. They were asked to refrain from tea, coffee, smoking, and physical exertion for 2–3 h before the test. After obtaining written consent from the recruited study participants, their demographic profile, anthropometric assessment, and relevant family and medication history were recorded in the pre-approved proforma. The adopted study protocol for the present study is shown in Figure 1.

Recording of physiological parameters

The resting heart rate, peripheral and central blood pressure, and augmentation index (AIx) of the study participants were recorded by central blood pressure recording instrument [USCOM make BP + (CardioScope II), Australia]. For the recording of brachial-ankle pulse wave velocity (baPWV), the subjects were asked to lie in supine position for 5 min. Then, pulse waveforms of the brachial artery and posterior tibial artery were recorded with the help of a pulse transducer in reference to ECG (lead II) recording for 5 min using Powerlab™ 4/35 hardware. LabChart™ 8 reader software was used to analyze the data (AD Instruments, Sydney, Australia).[10] Lead II ECG was recorded from the study participants using disposable Ag-AgCl electrodes. The signal acquisition was performed using Powerlab™ 4/35 hardware (AD Instruments, Sydney, Australia). The data were analyzed with the help of software to obtain power spectral density and low frequency (LF)/HF ratio. The HRV was analyzed in the time domain also. Both ECG and pulse signals were acquired using the digital data acquisition system Powerlab™ 4/35 hardware (AD Instruments, Sydney, Australia). Following this, the recording of the estimation of reactive hyperaemia index (RHI) was conducted by fixing the pulse probe to the middle finger of the left hand. Baseline lead II ECG and pulse signals were recorded for 5 min. Arterial occlusion was produced by raising cuff pressure to 50 mm Hg above baseline systolic blood pressure and maintained for 5 min.[11] The cuff was deflated, whereas finger pulse wave amplitude (PWA) was recorded digitally for another 5 min. The data were analyzed offline to determine RHI.

Statistical analysis

The Kolmogorov–Smirnov normality test was performed to examine the normality of the data. The data, which were found to be normally distributed, are expressed as mean ± sd, whereas non-normally distributed data were presented as median (interquartile range). The independent sample *t*-test was employed to compare variables that were normally distributed. The Mann–Whitney *U* test was employed to compare the variables of the two study groups, which were not normally

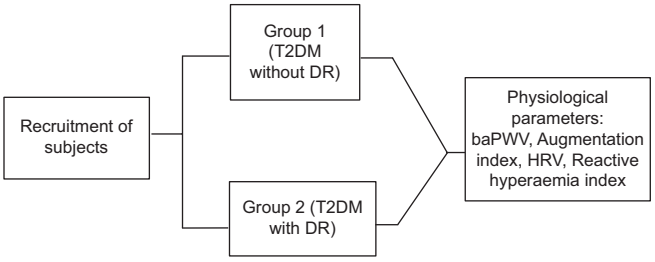

Figure 1: The flow diagram of the study protocol adopted for the present study

distributed. Spearman correlation was used to examine the relationship among the parameters. Linear regression analysis was performed to examine the important vascular predictors for diabetic retinopathy.

Results

In the present study, 20 T2DM patients diagnosed with and without retinopathy were recruited on the basis of inclusion and exclusion criteria, as stated in the methodology section. Out of them, 15 were male participants and 5 were female participants, irrespective of the study group. The basic characteristics of the participants are presented in Table 1.

It is evident from Table 1 that the mean age of T2DM patients with retinopathy was 58.6 (5.6) years and that of T2DM patients without retinopathy was 54.5 (8.6) years. The mean body mass index (BMI) of the study participants reflects that they were mostly overweight, although the BMI of some of them was in the normal or obese range as per World Health Organization (WHO) Asian criteria. The mean duration of diabetes was found to be around 10 years in both groups. There was no significant difference in anthropometric measurements of the two study groups except weight. The weight of group 1, that is, T2DM without retinopathy, is significantly higher than group 2 ($P = 0.043$).

Following the normality test, it was observed that most of the data was distributed normally except for HRV metrics. Therefore, appropriate statistical tests were applied to compare the means of variables between the two study groups, as mentioned in the methodology section. The following graphs display the comparison of various physiological variables between the two groups.

Figures 2–4 display the comparison of anthropometric measures such as weight, height, and BMI between T2DM without and with retinopathy. The mean ± standard deviation data of the variables are presented in graphical form ($*P \leq 0.05$, $*P \leq 0.001$).

It is evident from the above graphs [Figures 1–3] that the weight of T2DM patients without retinopathy is significantly higher ($P = 0.043$) than T2DM patients with retinopathy.

Among the vascular parameters, the baPWV value is significantly higher ($P = 0.021$) in group 2 (T2DM with retinopathy) than in group 1 (T2DM without retinopathy). Moreover, RHI is significantly blunted ($P = 0.001$) in group 2 than in group 1. The results are being displayed in Figures 5 and 6, respectively.

Analysis of HRV data revealed that time domain metrics of HRV, such as standard deviation of successive differences (SDSD) ($P = 0.033$), root mean square of successive RR interval differences (RMSSD) ($P = 0.035$) and the frequency domain metrics such as high frequency (HF) power ($P = 0.016$) are significantly lower in group 2 (T2DM with retinopathy) than group 1 (T2DM without retinopathy). The results are shown in the following graphs [Figures 7–9].

The correlation study reveals that baPWV ($r = 0.364$, $P = 0.021$) is positively associated with T2DM with the retinopathy group, that

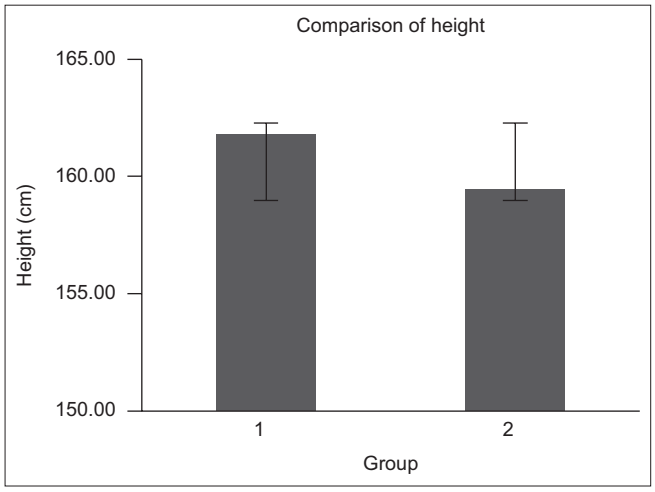

Figure 2: Comparison of anthropometric measures – height between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.409)

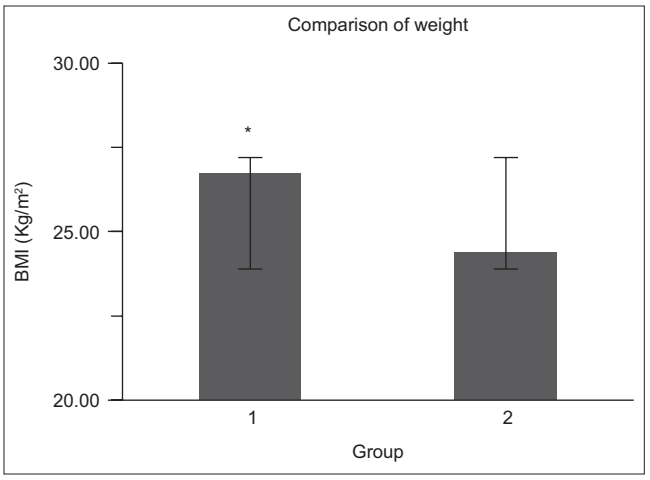

Figure 3: Comparison of anthropometric measure – weight between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus (T2DM) without or with retinopathy, respectively, with *P* value *P* = 0.043)

Table 1: Basis characteristics of the study participants

Group	Age (years)	Duration of diabetes (years)	Height (cm)	Weight (kg)	BMI (kg/m²)
Group 1 (T2DM without retinopathy) [*n*=20]	58.6±5.6	11.2±7.2	161.8±8.4	69.8±11.6*	26.7±3.9
Group 2 (T2DM with retinopathy) [*n*=20]	54.5±8.6	9.2±4.0	159.4±9.2	61.9±12.3	24.3±4.5

n=participants number, T2DM=type 2 diabetes mellitus, BMI=body mass index (Data are presented as mean±standard deviation with *P*<0.05)

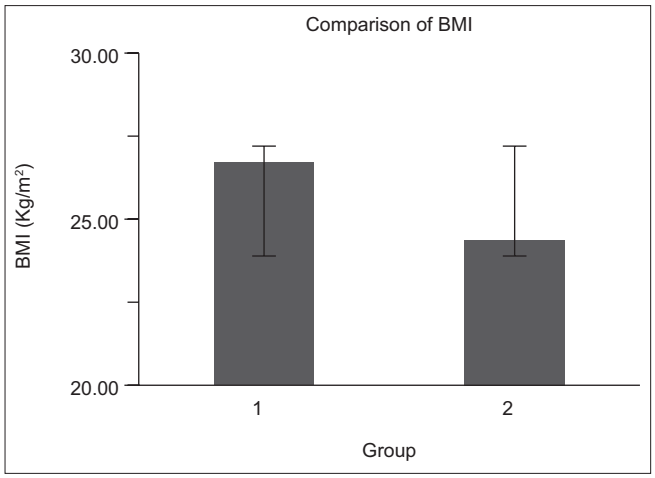

Figure 4: Comparison of anthropometric measure – body mass index (BMI) between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus (T2DM) without or with retinopathy, respectively, with *P* value = 0.089

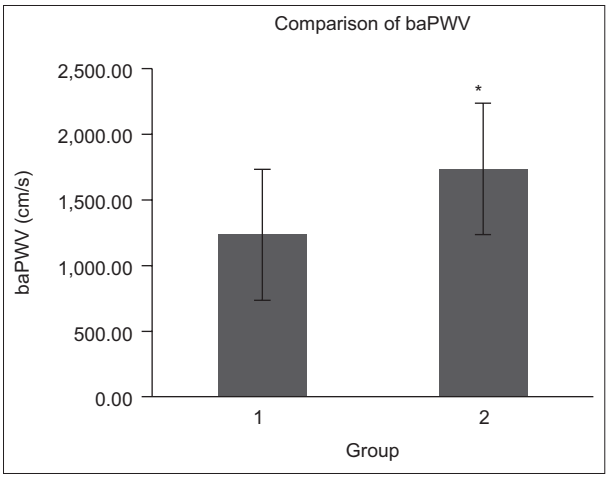

Figure 5: Comparison of vascular parameter – brachial-ankle pulse wave velocity (baPWV) between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.021). vascular parameters

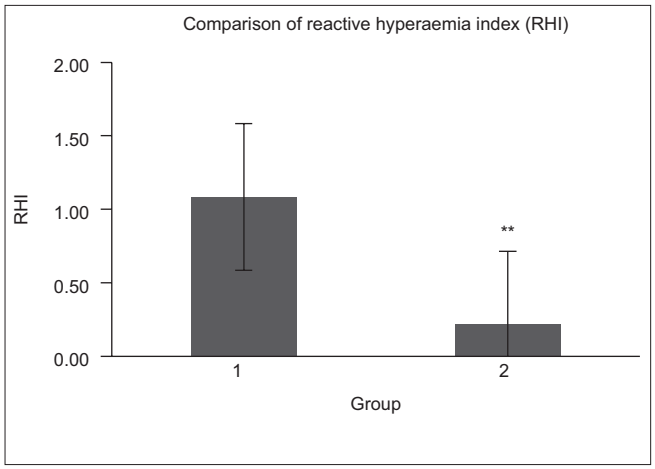

Figure 6: Comparison of vascular parameter – reactive hyperaemia (RHI) between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.001)

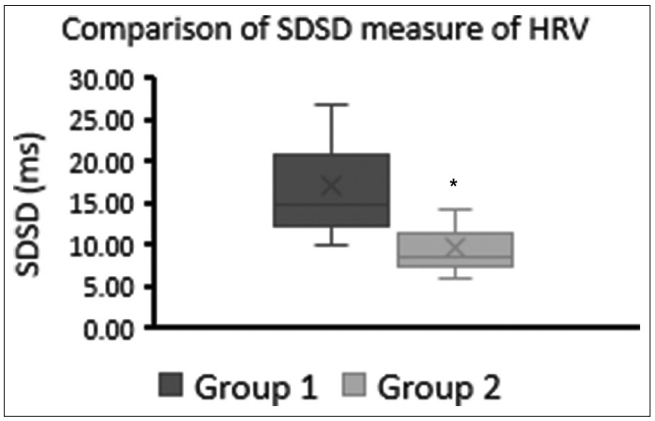

Figure 7: Comparison of time domain metricsof heart rate variability (HRV) - standard deviation of successive differences (SDSD) between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.033)

is, group 2. RHI ($r = -0.724$, $P = 0.001$) is negatively associated with group 2. Among HRV metrics, time domain metrics such as SDSD ($r = -0.342$, $P = 0.031$), RMSSD ($r = -0.338$, $P = 0.033$), and frequency domain metrics such as HF power ($r = -0.386$, $P = 0.014$) are negatively associated with group 2. Linear regression analysis was performed to understand the significant predictor of diabetic retinopathy. The significant results are presented in Table 2.

It is evident from Table 2 that blunted RHI is a significant predictor of diabetic retinopathy if other variables remain constant.

Discussion

The present study intends to determine arterial stiffness, endothelial function, and heart rate variability (HRV) in T2DM patients with and without T2DM retinopathy and examine the association of arterial stiffness, endothelial dysfunction, and HRV with T2DM retinopathy (DR). Twenty T2DM patients with retinopathy (NPDR and PDR) and another 20 T2DM patients without retinopathy were recruited in the present study. Both the study groups, that is, T2DM patients with and without retinopathy, were comparable on the basis of age and gender. The duration of diabetes was more than 10 years in the case of 14 T2DM patients with retinopathy and seven T2DM patients without retinopathy. The weight of T2DM patients is significantly higher in comparison to the T2DM patients with retinopathy. It reflects inadequate lifestyle treatment of the patients and/or glycemic dysregulation.

The vascular parameters such as heart rate, peripheral and central blood pressure, Aix (%), baPWV, and RHI were recorded in both study groups. Out of these parameters, baPWV and RHI were found to be significantly different between the two study groups.

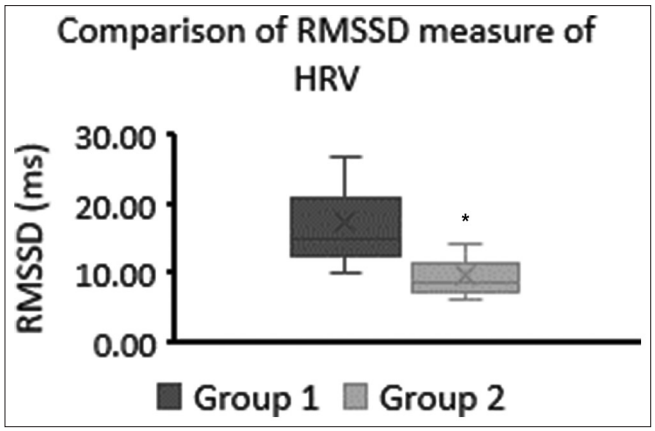

Figure 8: Comparison of time domain metrics of heart rate variability (HRV) - root mean square of successive differences between normal heartbeats (RMSSD) between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.035)

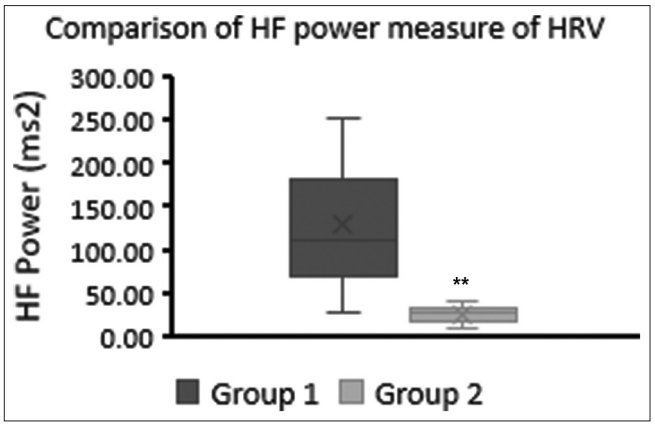

Figure 9: Comparison of frequency domain metrics of heart rate variability (HRV) – high frequency (HF) power between two participant groups (1 and 2 denotes the groups with type 2 diabetes mellitus [T2DM] without or with retinopathy, respectively, with *P* value = 0.016)

Table 2: Linear regression analysis

Variable	Unstandardized coefficient	Standardized coefficient	*t*	*P*	R^2
RHI	-0.246	-0.295	-2.286	0.035*	0.925

*$P<0.05$

RHI, the noninvasive, surrogate markers of endothelial function reflects endothelium-dependent vasodilatation. It is mostly mediated by NO after a transient increase in systolic pressure. RHI was found to be significantly less in T2DM patients with retinopathy than in T2DM patients without retinopathy in the present study. Furthermore, decreased RHI was significantly associated with the T2DM retinopathy group. This finding indicates that endothelial function is blunted more in T2DM patients with retinopathy. A previous study reported that peripheral endothelial function, as assessed by peripheral arterial tonometry, was associated with retinal vascular reactivity. Moreover, this study suggested that peripheral endothelial function is reflective of endothelial dysfunction in the retina.[7] Though in the present study, retinal vascular reactivity has not been ascertained, it is interesting to note that T2DM patients with retinopathy exhibited blunted endothelial function. Linear regression analysis revealed that blunted RHI is a significant predictor of diabetic retinopathy based on the present study. Therefore, assessing endothelial function in diabetic patients at the primary care level is of paramount importance as its presence may suggest the existence of retinopathy in diabetic patients.

Arterial stiffness occurs because of structural changes in the elastic layer of vascular conduits. The most important determining factor of arterial stiffness is age. Additionally, smoking, obesity, hypertension, and diabetes can alter the structural composition of blood vessels. Pulse wave velocity is regarded as one of the most important noninvasive surrogate markers of arterial stiffness. Though carotid-femoral pulse wave velocity (cfPWV) is regarded as the gold standard of arterial stiffness marker,[12] baPWV is used increasingly because of its ease of operation. Previous studies documented higher cfPWV in T2DM patients with retinopathy than only diabetic patients and/or healthy control. Moreover, higher cfPWV was found to be strongly associated with the severity of T2DM retinopathy.[4,6] However, few studies did not find any association between central aortic stiffness and T2DM retinopathy.[13,14] In the present study, baPWV was found to be significantly higher in T2DM patients with retinopathy than in T2DM patients without retinopathy. Moreover, a positive association of baPWV with the T2DM retinopathy group was found. A previous study reported that higher baPWV values are associated with the presence and severity of DR, and baseline baPWV may be an independent predictor in new-onset DR.[5] Liu Sung-Chen *et al.* (2020) reported that 'high baPWV is strongly associated with risk of severe DR, especially PDR'.[15] It can be inferred from the present study that increased systemic arterial stiffness is present in diabetic retinopathy patients and as systemic vascular stiffness increases, the chance of the existence of diabetic retinopathy also increases. It emphasizes the fact that the screening of vascular stiffness in diabetic patients may help to understand any coexisting morbid condition.

Measurement of HRV is helpful in assessing cardiac autonomic neuropathy, and it is one of the easiest ways to assess it. As we know, HRV is a beat-to-beat variation, and higher variation in HRV indicates higher parasympathetic activity.[16] HRV metrics such as standard deviation of the differences between successive NN intervals (SDSD), RMSSD, and HF power were found to be significantly less in T2DM patients with retinopathy than in T2DM patients without retinopathy, which is indicative of decreased parasympathetic activity. The previous study documented that SDNN and RMSSD values are lower in T2DM patients with retinopathy than in T2DM patients without retinopathy.[17] The findings of the present study are in sync with it. Furthermore, a significant negative association was found between SDSD, RMSSD, HF power, and the T2DM retinopathy group. Overall, the result indicates that metabolic dysregulation of glucose may affect the normal functioning of the autonomic nervous system, which is more pronounced

in the parasympathetic nervous system. However, a systematic and meta-analysis review reported that both sympathetic and parasympathetic activity is decreased in T2DM patients.[16] It has also been reported that parasympathetic activity is affected earlier than sympathetic activity in T2DM patients.[18] Therefore, the present study supports the previous notion that glycemic dysregulation may affect autonomic nerves.[18]

There is no significant difference in other vascular parameters such as heart rate, peripheral and central blood pressure, and AIx (%) between T2DM patients with and without retinopathy.

Linear regression analysis reveals that blunted RHI is an important predictor of T2DM retinopathy condition. However, the result of the present study cannot be generalized because of the limited sample size and cross-sectional study design.

Conclusion

It may be concluded from the findings of the present study that RHI was blunted more in T2DM patients with DR than in T2DM patients without DR. Moreover, blunted RHI is a significant predictor of diabetic retinopathy. baPWV was higher in T2DM patients with DR than in T2DM patients without DR. Furthermore, it is found to be positively associated with diabetic retinopathy condition. However, other vascular markers showed no significant difference between the two study groups. Among HRV measures, SDNN, RMSSD, and HF power are found to be decreased in T2DM patients with DR than in T2DM patients without DR. It signifies metabolic dysregulation of glucose, which may affect the normal functioning of ANS. Overall, it may be concluded that screening diabetic patients for cardiovascular risk assessment is an essential component of a Diabetic clinic to understand any existing comorbid condition such as diabetic retinopathy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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