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A correlation study of arterial stiffness, cardiac autonomic neuropathy and lipid profile in type 2 diabetes mellitus patients

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ABSTRACT

Objectives: Micro and macrovasculopathy are common complications of undertreated or undiagnosed type 2 diabetes mellitus (T2DM) patients. One of the underlying factors of macrovasculopathy is arterial stiffness, which may lead to cardiovascular and cerebrovascular diseases. Understandably, diabetic micro and macrovasculopathy affect vital functions, which may affect the well-being of the individual. However, few studies have attempted to determine arterial stiffness, cardiac autonomic neuropathy (CAN) and lipid profile separately in South Asian population and examined its associations with T2DM. Moreover, there is a need to understand the mechanistic links among cardiovascular risk factors. This forms the basis of the present study.

Materials and Methods: T2DM patients of 53–62 years and age- and gender-matched healthy control subjects were recruited in the cross-sectional and observational study (n = 30 each, eight women). Anthropometric measurements, physiological parameters such as resting heart rate, peripheral blood pressure (PBP), central blood pressure (CBP), augmentation index% (AIx%), brachial-ankle pulse wave velocity and lead II ECG for analysis of heart rate variability parameters were recorded after obtaining the consent of the study participants. The lipid profile and fasting blood glucose were also analysed.

Results: Peripheral systolic blood pressure was significantly higher (P = 0.05) in T2DM patients. Dyslipidaemia was evident in T2DM patients. Atherogenic index of plasma (AIP) was also significantly higher in T2DM patients. Correlation analysis revealed a positive association between AIx% with PBP and CBP as well as between AIP index and central systolic blood pressure, serum triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels. AIP index was found to be negatively associated with HF (nu). Serum TG, high-density lipoprotein cholesterol (HDL-C) levels and AIP index have emerged as significant independent predictors of T2DM vasculopathy by multiple regression analysis.

Conclusion: In the present study, atherogenic dyslipidaemia was observed in T2DM patients in combination with increased serum levels of TG, VLDL-C and decreased serum levels of HDL-C. Moreover, AIP index, a predictor cardiovascular risk, was found to be significantly higher in T2DM patients. Dyslipidaemia was found to be associated with dysregulation of autonomic nervous system in those patients. A positive association between non-invasive, surrogate markers of arterial stiffness with PBP and CBP indicates that enhanced arterial stiffness may elevate systemic arterial pressure. Therefore, early screening of T2DM patients for the estimation of serum lipid profile, arterial stiffness and cardiac autonomic neuropathy may be performed to unravel diabetic vasculopathy.

Keywords: Type 2 diabetes mellitus, Arterial stiffness, Cardiac autonomic neuropathy (CAN), Heart rate variability, Lipid profile

INTRODUCTION

Diabetes mellitus has emerged as one of the greatest disease burdens of mankind. It is a metabolic disorder that incapacitates each organ system of the body and, thereby, causes increased morbidity and mortality. The complex pathophysiology of type 2 diabetes mellitus (T2DM) gives rise to heterogenous disease syndromes, but at the core of its varied presentations lie hyperglycaemia.[1] Globally, the prevalence of diabetes is estimated at 463 million, which is predicted to rise to 642 million in the next 25 years.[2] In India, the number of diabetic patients is expected to cross 123.5 million by 2040.[3] Some undiagnosed patients present late with long-term complications of untreated chronic hyperglycaemia.

The vasculatures are affected by the ongoing slaughtering of various pathological processes involved in this disease process. These are advanced glycation end products, oxidative stress and mediators of low-grade chronic inflammation. These may affect proliferation, migration and function of endothelial cells and vascular smooth muscle cells. Moreover, it may cause structural changes in extracellular matrix. In this way, micro- and macrovessels are affected. The pathological changes in microvessels give rise to nephropathy, retinopathy and neuropathy. The macrovascular complications give rise to accelerated atherosclerosis which finally leads to ischaemic heart disease, increased risk of cerebrovascular disease and severe peripheral vascular disease. Understandably, vasculopathy itself can impose a great burden on the health delivery system.

Very few studies have been conducted to assess the association between serum lipids and degrees of glucose intolerance in the South Asian population.[4] The study conducted in the rural, Bangladeshi population, has documented that among 2293 study participants, high triglyceride (TG) level was observed in 26–64% of the participants. The same study reported low high-density lipoprotein cholesterol (HDL-C) levels in more than 90% of cases.[4] Moreover, T2DM was significantly associated with high total cholesterol (OR: 2.43, $P < 0.001$), high TG (OR: 3.91, $P < 0.001$) and low HDL-C (OR: 2.17, $P = 0.044$).[4] A meta-analysis of 15 eligible studies, with a total sample size of 4010, revealed a positive association between T2DM and atherogenic index of plasma (AIP, [$\log_{10}$TG/$\log_{10}$ HDLc]) is stronger than other lipid parameters. Moreover, the study concluded that AIP may be used as a simple, easily calculated parameter in assessing the risk of T2DM.[5] Asian Indians are known to have a unique pattern of dyslipidaemia with lower HDL-C, increased TG levels and a higher proportion of small dense low-density lipoprotein cholesterol (LDL-C). Phase I of a large-scale representative study conducted in India documented that there exists regional variation in the profile of dyslipidaemia with the highest rates of hypercholesterolemia observed in Tamil Nadu (13.9%), hypertriglyceridemia in Chandigarh (38.6%), highest rates of low HDL-C in Jharkhand (76.8%) and highest rates of high LDL-C in Tamil Nadu (15.8%).[6]

Arterial stiffness is regarded as an independent cardiovascular risk factor besides dyslipidaemia. It was reported that arterial stiffness is closely related to the progression of complications of T2DM, such as nephropathy, retinopathy and neuropathy. It was also detected in pre-diabetic patients.[7] It can be assessed by employing several surrogate non-invasive markers such as augmentation index (AIx [%]) and pulse wave velocity (PWV). The heart is also inflicted due to diabetic vasculopathy and cardiac autonomic neuropathy. There are studies in the literature that determined cardiac autonomic neuropathy, and arterial stiffness as measured by PWV and lipid profile in T2DM patients separately especially in South Asian population and examined its association with T2DM.[4,8-10] Moreover, all these studies emphasised on conduction of further studies for further understanding of mechanistic linkage among multiple cardiovascular risk factors. However, there is a lack of study in the literature which has determined all three components in T2DM patients and examined their association. This is the basis of the present study.

MATERIALS AND METHODS

It was a cross-sectional and observational study. A total of 30 T2DM patients aged between 53 and 62 years ($n = 30$, 22 men and eight women) were recruited from the outpatient department of endocrinology. T2DM patients were diagnosed based on American Diabetes Association criteria. Diabetic patients with a history of systemic disorder affecting liver, autoimmune disease, or any endocrinological disorder affecting blood vessels and peripheral vascular disease were excluded from the study. Out of them, 28 study participants were suffering from the disease for 1–10 years and two were suffering from it for the past 15 years. Age- and gender-matched controls ($n = 30$) were enrolled in the present study through advertisement. The ethical clearance for this study was obtained from the Institutional Ethics Committee (IEC). The recruited subjects were asked to visit Clinical Physiology Laboratory at around 10 a.m.

Procedures

The study participants were requested to refrain from tea and coffee for at least 2 h before laboratory tests. On arrival at the laboratory, the participants were informed of the study protocol in detail and written consent was obtained from them in the prescribed format as approved by IEC. Afterward, the anthropometric measurements of the subjects were taken.

Recording of non-invasive physiological surrogate markers of arterial stiffness

The study participants were examined in the supine position after a rest of 5 min. Resting peripheral blood pressure (PBP), central blood pressure (CBP), AIx% and pulse rate were recorded with the help of the Central Blood Pressure Recording Instrument (USCOM make BP + [Cardioscope II], Australia). For the recording of brachial-ankle pulse wave velocity (baPWV), pulse waveform of the brachial artery and posterior tibial artery was recorded simultaneously with the help of pulse transducers for 5 min using Powerlab™ 4/35 hardware. Labchart™ 8 reader software was used to analyse the data (AD Instruments, Sydney, Australia).[11]

Recording of heart rate variability (HRV) to ascertain cardiac autonomic neuropathy (CAN)

HRV was also recorded with PowerLab 4/35 system (AD system, Sydney, Australia). The disposable Ag-AgCl surface electrodes were placed on four limbs for the recording of lead II ECG. The ECG data were digitised at a sampling frequency of 1 kHz. The data were passed through a bandwidth filter, in which low pass filter was set at 50 Hz and high pass filter was set at 0.3 Hz with 50 Hz notch 'ON'. The standard time domain, frequency domain and non-parametric analysis were performed from the recorded HRV.[12]

Biochemical analysis of serum for fasting blood sugar and lipid profile

Five millilitres of whole blood were collected from the study participants and serum was separated following standard protocol. Serum was analysed to estimate fasting blood sugar and lipid profile by the colorimetric method (Erba Diagnostics, Germany).

Statistical analysis

Shapiro–Wilk normality test was applied to assess the distribution pattern of the data. The data were found to be non-normally distributed. The data were presented as median (interquartile range). Differences between variables were compared using Mann–Whitney U-test. Association between parameters was examined by Spearman's correlation test. Multiple linear regression analyses were performed to examine the independent risk factors for diabetic vasculopathy. A two-tailed $P \leq 0.05$ was considered statistically significant. The data were analysed using the appropriate statistical tools (SPSS software, version 20 SPSS, IBM Inc., Chicago, IL).

RESULTS

The analysis of anthropometric data of T2DM patients and age- and gender-matched controls did not reveal any significant difference in BMI [Table 1]. The vascular, HRV parameters and biochemical parameters of the study participants are displayed in [Tables 1-3], respectively. Among vascular parameters, peripheral systolic blood pressure (PSBP) was significantly higher ($P = 0.05$) in T2DM patients as compared to age- and gender-matched control. High serum TG ($P = 0.023$), very low-density lipoprotein cholesterol (VLDL-C) ($P = 0.005$) and AIP index ($P = 0.01$), and low serum HDL-C ($P = 0.004$) were found in T2DM patients in comparison to control [Table 1]. Fasting blood glucose is significantly more ($P = 0.0005$) in T2DM patients than in control [Table 1].

A significant correlation between BMI and serum TG level ($r = 0.369$, $P = 0.024$) was observed. A positive association between AIx% with peripheral diastolic blood pressure ($r = 0.331$, $P = 0.010$), PSBP ($r = 0.484$, $P = 0.0005$), central diastolic blood pressure ($r = 0.318$, $P = 0.013$) and central systolic blood pressure (CSBP) ($r = 0.511$, $P = 0.0005$) was observed. A strong positive association between AIP index and CSBP ($r = 0.364$, $P = 0.031$), baPWV ($r = 0.353$, $P = 0.032$), serum TG level ($r = 0.894$, $P = 0.0005$), serum LDL-C level ($r = 0.371$, $P = 0.024$) and serum VLDL-C level ($r = 0.736$, $P = 0.0005$) was found. Interestingly, AIP index was found to be negatively associated with HF (nu) ($r = -0.388$, $P = 0.018$). Serum TG, HDL-C levels and AIP index have emerged as significant independent predictors of T2DM vasculopathy by multiple regression analyses [Table 4]. No association was found between the duration of the disease and physiological and biochemical parameters.

DISCUSSION

The prevalence of T2DM has increased largely in the Asian population during recent decades. Moreover, a larger proportion of young- and middle-aged individuals are the sufferers of this condition, which lead to a great socioeconomic burden in the country. Undoubtedly, increased incidence of T2DM enhances the occurrence of micro and macrovasculopathy in the patients. However, few numbers of studies have attempted to estimate systematically the vascular status in T2DM patients. The present study has aimed to determine arterial stiffness, cardiac autonomic neuropathy and lipid profile in T2DM patients.

30 T2DM patients and the same number of age- and gender-matched control subjects have participated in the present study. The data analysis revealed that T2DM patients have significantly higher peripheral systolic blood pressure than age- and gender-matched control subjects. However, there was no significant difference in central systolic pressure and other arterial stiffness markers, between these study groups. This may be attributed to the age-associated changes of vasculature in the control group and intake of medicines in T2DM patients. It is known that various factors such as

Table 1: Comparison of BMI and biochemical parameters of the study participants.

Variables	Control (*n*=30)	T2DM case (*n*=30)	*P*-value
BMI (kg/m^2)	24.7 8 (23.30–26.62)	25.25 (23.12–27.95)	0.371
Serum cholesterol (mg/dL)	166.0 (119.0–214.0)	203 (187.8–212.5)	0.162
Serum TG (mg/dL)	109.0 (79.0–147.0)	148.0 (118.0–160.0)*	0.023
Serum LDL (mg/dL)	124.0 (62.0–147.0)	136.5 (123.0–145.0)	0.280
Serum HDL (mg/dL)	48.0 (38.0–54.0)	40.0 (35.8–45.3)**	0.004
Serum VLDL (mg/dL)	21.6 (15.0–26.0)	29.6 (23.6–32.0)*	0.005
AIP	0.07 (0.06–0.14)	0.13 (0.11–0.14)*	0.01
Serum fasting blood glucose (mg/dL)	90.0 (80.0–96.3)	130.3 (112.0–156.5)***	0.0005

Data are presented as median (IQR), * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.0005$. T2DM: Type 2 diabetes mellitus, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, AIP: Atherogenic index of plasma, BMI: Body mass index

Table 2: Comparison of vascular parameters of the study participants.

Variables	Control (*n*=30)	T2DM case (*n*=30)	*P*-value
PDBP (mm Hg)	80.0 (71.0–88.5)	80.5 (76.7–88.0)	0.935
PSBP (mm Hg)	131.0 (120.5–139.3)	137.5 (125.8–151.0)*	0.05
CDBP (mm Hg)	78.5 (72.0–85.7)	81.0 (75.5–86.25)	0.432
CSBP (mm Hg)	120.0 (107.7–129.3)	123.5 (116.5–136.3)	0.082
AIx (%)	70.0 (56.0–92.00)	77.50 (61.25-88.00)	0.701
baPWV (cm/s)	1439.4 (1239.3–1620.2)	1457.18 (1106.7–1867.7)	0.882

Data are presented as median (IQR), * $P \leq 0.05$. T2DM: Type 2 diabetes mellitus, PSBP: Peripheral systolic blood pressure, PDBP: Peripheral diastolic blood pressure, CDBP: Central diastolic blood pressure, CSBP: Central systolic blood pressure, AIx%: Augmentation index, baPWV: Brachial ankle pulse wave velocity

Table 3: Comparison of heart rate variability parameters of the study participants.

Variables	Control (*n*=30)	T2DM case (*n*=30)	*P*-value
Heart rate (bpm)	77.5 (73.50–85.2)	81.5 (71.7–89.3)	0.594
SDNN (ms)	23.3 (18.9–34.9)	26.0 (19.1–40.3)	0.668
SDSD (ms)	14.7 (11.2–31.3)	15.3 (11.6–27.1)	0.824
pNN50%	0.26 (0.00-11.76)	0.28 (0.0–4.0)	0.747
RMSSD (ms)	14.9 (10.9–33.6)	15.3 (11.6–27.1)	0.915
LF (ms^2)	174.4 (94.1-281.00)	134.6 (41.1–540.6)	0.802
LF (nu)	53.2 (45.5–70.8)	42.7 (32.2–66.4)	0.204
HF (ms^2)	125.0 (62.8–447.1)	166.2 (64.7-534.4)	0.626
HF (nu)	45.4 (29.0–53.7)	46.5 (32.2–65.4)	0.506
LF/HF	1.3 (0.9–2.8)	1.1 (0.5–2.1)	0.209

Data are presented as median (IQR). T2DM: Type 2 diabetes mellitus, HR: Heart rate, HF (nu): High frequency (normalised), LF (nu): Low frequency (normalised), LF/HF: Low frequency and high-frequency ratio, SDSD (ms): Standard deviation of differences between adjacent NN intervals, SDRR (ms): Standard deviation of adjacent RR intervals, PNN50%: Percentage of adjacent normal sinus RR intervals with more than 50 ms difference, RMSSD: Root mean square of adjacent RR intervals

age, gender, height, heart rate and disease condition affecting the vasculature may affect systolic pressure amplification.[13] Moreover, dyslipidaemia was documented in T2DM patients as evidenced by significantly high serum TG, VLDL-C levels and low serum HDL-C levels in T2DM patients in comparison to control. This finding corroborates the earlier studies.[4] There is a clear indication in the literature that elevated serum level of LDL-C contributes immensely to the development of cardiovascular disease. However, in the present study, a combination of increased serum levels of TG and VLDL-C and decreased serum levels of HDL-C was found. A combination of elevated TG level and decreased HDL-C level is often a component of atherogenic dyslipidaemia. Moreover, several studies also indicated that elevated serum TG level is associated with an increased risk of CVD. The mechanistic underpinning for this association may be the release of excessive free fatty acid, synthesis of pro-inflammatory cytokines, coagulation factors and impairment of fibrinolysis.[14,15]

AIP, which was calculated as the logarithm of TG and HDL-C ratio, was also significantly higher in T2DM patients in comparison to the control subjects. This finding is also concordant with the previous study.[5] The present study also confirms the previous notion that AIP may be used as an easy-to-calculate parameter to assess vascular risk in T2DM patients.[5] AIP reflects the composition of lipoprotein in plasma and is regarded as a predictor of atherosclerosis and cardiovascular risk. An AIP value below 0.11 has been associated with low, from 0.11 to 0.24 with intermediate, and values exceeding 0.24 with high cardiovascular disease

Table 4: Multiple regression analyses to examine independent risk factors for diabetic vasculopathy.

Variables	Unstandardised coefficients	Standardised coefficients	t-values	*P*	R^2
					0.552
Serum Triglyceride (mg/dl)	0.009	1.138	2.867	0.008**	
Serum HDLc (mg/dl)	−0.042	−0.826	−3.422	0.005**	
AIP	0.490	0.132	2.252	0.032*	

HDLc: High-density lipoprotein cholesterol, AIP: Atherogenic index of plasma, *P<0.05, **P<0.01, R^2 : Coefficient of determination

risk.[15] In the present study, T2DM patients were found to be at intermediate cardiovascular risk based on AIP value [Table 1].

The significant correlation between BMI and serum TG in the present study may be explained by the availability of excess free fatty acid from the fat depot. Moreover, a strong positive association was observed between AIP index and CSBP and baPWV. AIP is regarded as a predictor of plasma atherogenicity. Therefore, the higher the AIP value, the more there is a chance of arterial stiffness. Interestingly, there was no significant difference in parameters of arterial stiffness between control and T2DM patients in the present study. The age-associated changes in arterial stiffness may not be ignored given the age group of both control and T2DM patients. AIP index was also found to be associated with the parameters of lipid profile. This finding is expected as AIP index reflects metabolic interaction within the lipoprotein complex.[16] AIP index was found to be negatively associated with HF (nu) in the present study. However, there was no significant difference in HF (nu) value between control and T2DM patients. HF (nu) signifies the functioning of the parasympathetic limb of the autonomic nervous system (ANS). It may be inferred that derangement of lipid metabolism in T2DM patients may affect the normal functioning of ANS. It may be possible that dyslipidaemia may negatively affect the myelination status of the nerves, and thereby, it may cause dysregulation of ANS.[17,18] AIP index was also positively associated with CSBP and baPWV, which are non-invasive surrogate markers of arterial stiffness. Understandably, enhanced atherogenicity of plasma may escalate arterial stiffness. A positive association between AIx%, which is another non-invasive surrogate marker of arterial stiffness with PBP and CBP, was observed in the present study. It may be concluded from this observation that increased arterial stiffness may enhance the possibility of the development of hypertension. Multiple regression analysis revealed serum TG, HDL-C levels and AIP index as significant independent predictors of diabetic vasculopathy in the present study. This study highlights the emergence of AIP as a screening parameter to assess vascular risk in T2DM patients. The sample size of the present study is a limiting factor. Hence, the findings of the study cannot be generalised. Moreover, the present study was a cross-sectional and observational study. Therefore, no cause-effect relation can be established based on the present study. However, the accumulated data of the present study emphasise early screening of T2DM patients for arterial stiffness and cardiac autonomic neuropathy along with serum lipid profile estimation.

CONCLUSION

In the present study, a combination of increased serum levels of TG and VLDL-C and decreased serum levels of HDL-C was observed in T2DM patients in comparison to the control group. This finding indicates the presence of atherogenic dyslipidaemia in T2DM patients. Moreover, AIP index, a predictor of atherosclerosis and cardiovascular risk was found to be significantly higher in T2DM patients. All these observations point toward the increased cardiovascular risk in diabetic patients. AIP index was found to be negatively associated with one of the parameters of activation of the parasympathetic nerve in T2DM patients. Dyslipidaemia may affect the myelination of the nerves, which may lead to dysregulation of ANS in those patients. A positive association between non-invasive, surrogate markers of arterial stiffness with PBP and CBP indicates that enhanced arterial stiffness may elevate systemic arterial pressure. Therefore, early screening of T2DM patients for arterial stiffness and cardiac autonomic neuropathy may be recommended along with serum lipid profile estimation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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