ORIGINAL ARTICLE

CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS AND CONTROL SUBJECTS

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Background: Cardiac autonomic neuropathy (CAN) is a common but frequently undiagnosed complication of diabetes mellitus (DM). Up to 15% of the patients have been reported to have CAN at the time of diagnosis of DM. The objective of this study was to determine correlation between glycaemic control, microalbuminuria (MAU) and cardiac autonomic neuropathy in type 2 diabetes mellitus (T2DM). **Method:** This was a cross-sectional comparative study conducted on 100 subjects (50 diabetics, 50 controls) at Services Institute of Medical Sciences, Lahore. Glycated haemoglobin (HbA1c) levels were measured by quantitative caloric method, MAU was quantified by using QuicKey human microalbuminuria ELISA kit and cardiac autonomic functions were assessed using PowerLab® 26T Teaching System. **Results:** All the diabetic subjects in our study had cardiac autonomic neuropathy. No significant correlation between duration of diabetes and glycaemic control (p=0.230), duration of diabetes and microalbuminuria (p=0.891), and glycaemic control and microalbuminuria (p=0.698) was found. **Conclusion:** Cardiac autonomic neuropathy and microalbuminuria are highly prevalent in T2DM. Duration of diabetes, glycaemic control, and microalbuminuria are not significantly correlated to each other.

Keywords: CAN, Microalbuminuria, T2DM, PowerLab, Neuropathy, Autonomic, HbA1c

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INTRODUCTION

Diabetes mellitus (DM) is a global health problem. Approximately 462 million people corresponding to 6.28% population of the world are affected by type 2 diabetes mellitus (T2DM).[1] The pooled prevalence of diabetes in Pakistan is 13.7% with a slight preponderance towards male gender and urban living.[2] Despite this high prevalence, more than half of the patients remain unaware of their disease due to which complications are often present at the time of the diagnosis.[3] Damage to various body organs including blood vessels, eyes, kidney, nervous tissue and heart has been observed with chronic hyperglycaemia of T2DM.[4] The damage to nerve fibres both somatic and autonomic including cardiac autonomic nerve fibres is a feature of DM and is responsible for various neuropathies.[5] Cardiac autonomic neuropathy (CAN) is often found in T2DM patients but it frequently remains undiagnosed.[6]

The *CAN Subcommittee of Toronto Consensus Panel on Diabetic Neuropathy* defines CAN as an "impairment of cardiovascular autonomic control in patients with established diabetes after excluding other causes". It has been proposed that autonomic nerve fibres that innervate heart and blood vessels are damaged with long-standing diabetes which in turn cause abnormalities in heart rate and vascular dynamics.[7] Based on the available data, the reported prevalence of CAN is highly variable and ranges from 25% to 75% in T2DM.[8]

The pathophysiology of CAN in DM is still unclear but it has been proposed that diabetes triggers multiple reactions that promotes neuropathic changes.[7] Microvascular changes of diabetes, including retinopathy and albuminuria, are associated with progression of CAN based on the results from the EURODIAB study.[9] Microalbuminuria (MAU) has been defined as 'albumin excretion rate (AER) of $\geq$30 mg/ 24 hour of urine sample collection'.[10] A review of literature indicates strong association between MAU and increased risk of cardiovascular complications.[11]

Most of the studies reporting this association have been done in type 1 diabetes but similar studies in type 2 diabetes are relatively few in number.[12] Pakistan ranks at sixth position in the world regarding the burden of DM.[13] Although CAN manifests approximately after 20 years of disease initiation, its pathology starts in early years of DM.[5] Increased urinary albumin excretion in diabetic patients with CAN has been observed. However, little is known about correlation between glycaemic control, microalbuminuria and CAN in our local population. The present study aimed at determining correlation between glycaemic control, microalbuminuria and cardiac autonomic neuropathy.

METHODOLOGY

This was a cross sectional comparative study carried out in the department of Physiology, Services Institute of Medical Sciences, Lahore. A sample size of 72 was calculated at 95% confidence level, and 5% margin of error while taking probability of CAN in 29% of the diabetic patients and zero in control subjects.[5] However, we enrolled a total of 100 subjects (50 cases

and 50 controls) by non-probability consecutive sampling based on inclusion and exclusion criteria. The subjects having type 2 DM ≥5 years of duration were included as cases while controls were non-diabetic subjects. Subjects with known ischemic heart disease, malignancies, renal failure, limb amputation and cerebrovascular stroke were excluded from the study. Height of all the subjects was measured in centimetres from the highest point of the vertex while the subject was standing in the anatomical position. Weight was measured in kilograms by using weighing machine floor type model RGZ-160 (SERICO, China) while all the subjects were bare-footed and wearing minimal clothing. Body mass index (BMI) was calculated from height and weight using formula:

$$\text{BMI} = \frac{\text{Weight (Kg)}}{[\text{Height (m)}]^2}$$

HbA1c levels were measured by quantitative caloric method using diabetes chemistry reagents developed by Stanbio laboratory, USA. The diagnosis of the diabetes was based on American Diabetes Association recommended glycated haemoglobin (HbA1c) level of ≥6.5%.[14] MAU was quantified by using QuicKey human microalbuminuria ELISA kit by Elabscience®, USA. MAU was categorized into mild (30–50 mg/24 hr), moderate (50–100 mg/24 hr), and severe (100–300 mg/24 hr) categories.[15] The cardiac autonomic functions were assessed using PowerLab® 26T Teaching System ADInstruments, UK. The diagnosis of CAN was established if two or more of the following cardiac autonomic functions were abnormal.[9]

Beat to beat heart rate variation (HRV) was measured by monitoring heart rate on ECG while patient was at rest and lying supine. The patient was then asked to breathe in and out at 6 breaths/minute (deep breathing). R-R interval during three successive breaths was measured and mean value was obtained. The difference between beats per minute during rest and deep breathing was calculated. A difference of >15 bpm between rest and deep breathing was taken as normal.

Heart rate response to standing was recorded using continuous ECG monitoring and the subject was asked to stand up. R-R interval on ECG was calculated at beats 15 and 30 after standing. The 30:15 of >1.03 was taken as normal.

In heart rate response to the Valsalva manoeuvre, subjects were asked to exhale forcibly into the mouthpiece of a manometer at a pressure of 40 mmHg for 15 sec while ECG was monitored. Healthy subjects develop tachycardia during the manoeuvre while bradycardia with release. The ratio of R-R interval after release and during Valsalva manoeuvre of >1.2 was considered normal.

Systolic blood pressure (SBP) response to standing was checked by measuring SBP of the subject in supine position. The patient was then asked to stand up and blood pressure was again monitored after two minutes. A fall of <10 mmHg is normal, 10–29 mmHg is border line while a fall of >30 mmHg on standing was considered abnormal.

Diastolic blood pressure (DBP) response to isometric exercise was evaluated by first taking the blood pressure of the subject in one arm. The subject was then asked to squeeze a handgrip dynamometer to his/her maximum force and value was calibrated on PowerLab®. The patient was then asked to squeeze the same dynamometer with 30% of the maximum for 5 minutes. A rise of >16 mmHg in diastolic blood pressure on opposite arm was taken as normal.

RESULTS

A total of 100 study subjects divided in two equal groups (diabetic and controls) were enrolled for the study. More than half of the study subjects (56%) were male while remaining (44%) were females. Mean age of the diabetic patients was 39.5±1.07 Years and that of the controls was 38.5±3.03 Years. The BMI of diabetics was 26.55±1.16 Kg/m^2 while it was 25.12±2.67 Kg/m^2 in healthy controls (Table-1).

Comparison of mean values for parasympathetic (HRV and hear rate response to standing) and sympathetic (systolic blood pressure response to standing and diastolic blood pressure response to isometric exercise) functions showed significant differences between cases and controls, while heart rate response to Valsalva manoeuvre was similar in both groups (Table-2).

Chi-square analysis of sympathetic and parasympathetic parameters indicated highly significant differences between diabetics and controls (Table-3).

Microalbuminuria was found to be of mild, moderate, and severe nature in 6, 14 and 30 cases respectively. None of the sympathetic or parasympathetic parameters were significantly correlated with microalbuminuria. Significant correlations were observed between HRV and heart rate response to standing in relation to duration of diabetes. Only heart rate variation during deep breathing had significant correlation with glycaemic control (Table-4).

We did not find any significant correlation between duration of diabetes and glycaemic control (p=0.230), duration of diabetes and microalbuminuria (p=0.891) and glycaemic control and microalbuminuria (p=0.698).

Table-1: Demographic characteristics of the study population (n=100)

Variables	Cases
HbA1c (%)	10.67±1.67
Duration of diabetes (Months)	83.96±15.21
Microalbuminuria (mg/24 hour)	140.13±76.26

Table-2: Comparison of cardiac autonomic functions between cases and controls by Student's *t*-test (Mean±SD)

Cardiac autonomic neuropathy test	Cases	Controls	*p*
Heart rate response to deep breathing	9.84±6.62	23.74±5.56	<0.001*
Heart rate response to standing	0.95±0.16	1.20±0.14	<0.001*
Heart rate response to the Valsalva manoeuvre	1.45±0.38	1.44±0.14	0.823
Systolic blood pressure response to standing	32.40±11.65	4.72±2.25	<0.001*
Diastolic blood pressure response to isometric exercise	8.60±7.24	21.20±3.9	<0.001*

Table-3: Chi-square analysis of CAN among diabetic and control subjects [n (%)]

Cardiac autonomic neuropathy test	Cases	Controls	*p*
Heart rate response to deep breathing	32 (64)	0 (0)	<0.001*
Heart rate response to standing	37 (74)	0 (0)	<0.001*
Heart rate response to the Valsalva manoeuvre	14 (28)	1 (2)	<0.001*
Systolic blood pressure response to standing	35 (70)	0 (0)	<0.001*
Diastolic blood pressure response to isometric exercise	37 (74)	0 (0)	<0.001*

*Highly Significant, 0 cells have expected count <5

Table-4: Pearson correlation analysis for various sympathetic and parasympathetic parameters with microalbuminuria, duration of diabetes and glycaemic control

Cardiac autonomic neuropathy test	Microalbuminuria		Duration of diabetes		Glycaemic control	
	r	*p*	*r*	*p*	*r*	*p*
Heart rate variation during deep breathing	-0.108	0.454	-0.303*	0.03	-0.416*	0.003
Heart rate response to standing (30:15)	-0.043	0.765	-0.297*	0.04	-0.220	0.125
Heart rate response to Valsalva manoeuvre	0.017	0.907	-0.007	0.96	0.143	0.323
Systolic blood pressure response to standing	-0.232	0.105	0.102	0.48	-0.015	0.915
Diastolic blood pressure response to isometric exercise	-0.216	0.131	-0.265	0.06	0.063	0.663

*Correlation is significant at 0.05 level (2-tailed)

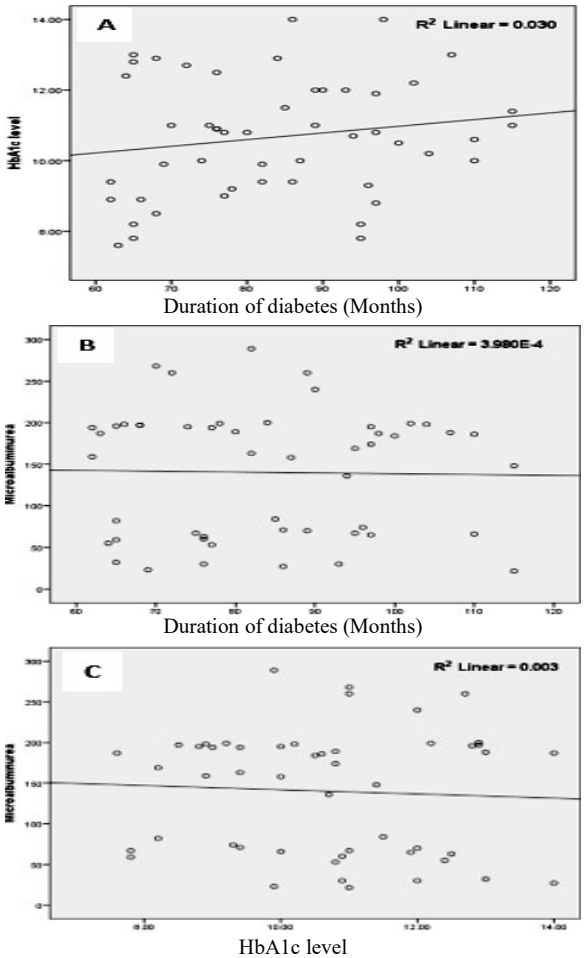

Figure-1: Correlation between A) duration of diabetes and glycaemic control, B) duration of diabetes and microalbuminuria, C) glycaemic control and microalbuminuria

DISCUSSION

Diabetes mellitus is a global health problem having significant impact on quality of life of the patients. Up to 15% of the patients have been reported to have CAN at the time of diagnosis of diabetes.[16] Moreover, before CAN is symptomatic and evident clinically in diabetic patients, sub-clinical CAN may persist for several years.[7] Thus, *Toronto Consensus Panel on Diabetic Neuropathy* has recommended that CAN screening must be done in all asymptomatic T2DM patients at the time of diagnosis.[17]

All of the diabetic subjects in our study were diagnosed with CAN with a mean duration of diabetes and HbA1c level of 83.96±15.21 months and 10.67±1.67% respectively. This indicates a very high prevalence of cardiac autonomic neuropathy in our population. The reported prevalence of CAN from different studies is highly variable. In a recent study, prevalence of CAN in newly diagnosed cases was reported to be 15.3%.[16] while another study reported a prevalence of 20.67% in newly diagnosed cases.[18] Fawwad *et al* reported CAN in 68% and 50% of the subjects with poor and good glycaemic control after 5 years.[19] Another study reported, that not a single case of CAN was found in patients with less than 5 years of diabetes but when the duration was increased to 15 years, 100% of the subjects developed cardiac autonomic neuropathy.[20,21]

Despite good glycaemic control, more than two-third of the newly diagnosed cases of diabetes in Pakistan have some degree of cardiac autonomic neuropathy within one year.[22] This variability can partly be explained by risk factors including age, gender, type and duration of diabetes and glycaemic control.[17] The other possible factors may be non-inclusion of factors,

e.g., obesity, smoking, hypertension, distal polyneuropathy, nephropathy, and retinopathy which were not taken into consideration in our study.[23]

A number of factors including age, gender, ethnicity, glycaemic control, duration of diabetes and microvascular complications play a role in the development of CAN.[24] However, duration of diabetes and glycaemic control have been identified as two of the most prevalent risk factors for development of CAN. In turn, increased risk of cardiovascular diseases, kidney diseases and mortality has been reported with development of CAN.[25] Duration of diabetes is an independent risk factor while chronically elevated levels of HbA1c are associated with increased risk of cardiovascular and renal complications.

In our study, all the patients had poor glycaemic control. Glycaemic control is an independent risk factor for the development of CAN in both types of diabetes.[21] Measurement of glycated haemoglobin (HbA1c) is an important diagnostic and prognostic marker for disease progression.[26]

Most of the diabetic subjects (80%) in our study had moderate to severe microalbuminuria. MAU has been identified as an early sign of cardiovascular and renal diseases in diabetic and non-diabetic individuals.[27] Studies have reported association between glycaemic control and microalbuminuria, but we did not find any significant association between the two variables. Ullah *et al* reported mild association between microalbuminuria and glycaemic control in diabetic patients.[28] In another study, microalbuminuria was reported in type 2 diabetics with poor glycaemic control and elevated blood pressure. On the other hand, no microalbuminuria was reported in cases with poor glycaemic control only.[29] Still another study reported association between microalbuminuria and glycaemic control but the association was significant only at HbA1c levels of >11%.[30] Thus, the differences can be explained on the basis of duration for which diabetes remain undiagnosed in the subjects and degree of uncontrolled hyperglycaemia. The other possible factors for the above differences can be ethnicity, susceptibility of nephropathy and method of assessment of microalbuminuria.

To summarize, none of the sympathetic or parasympathetic parameters were significantly correlated with microalbuminuria. However significant correlations were observed between heart rate variation during deep breathing and standing and diastolic blood pressure response to isometric exercise with duration of diabetes. Beat to beat variation was also associated positively with glycaemic control. Symptoms and signs of autonomic dysfunction, including resting HR, BP responses to standing, and time and frequency measures of HRV in response to deep breathing, standing and Valsalva manoeuvre, should be conducted on all patients with diabetes to allow early detection and intervention.[31]

CONCLUSION

Cardiac autonomic neuropathy and microalbuminuria are highly prevalent in T2DM. Duration of diabetes, glycaemic control and microalbuminuria were not significantly correlated to each other.

RECOMMENDATION

Further large-scale studies with more refined subject selection must be designed to determine the true prevalence of CAN in our population. Moreover, prospective cohorts must also be planned to discover the optimal level of glycaemic control, duration of diabetes and microalbuminuria in order to reduce morbidity and mortality in T2DM.

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