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Research Article

THE COMBINED UTILITY OF ANKLE-BRACHIAL INDEX, HEART RATE VARIABILITY AND LIPID PROFILE IN PREDICTING THE RISK OF AUTONOMIC NEUROPATHY AND FOOT ULCERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Context: Diabetic foot ulcer is one of the most dreaded complications with the major risk factor being distal sensorimotor polyneuropathy combined with autonomic neuropathy.**Aims**: This study was done to assess the combined utility of ankle-brachial index (ABI), heart rate variability (HRV) and lipid profile in predicting the risk of autonomic neuropathy in diabetic population.**Settings and Design**: This is a cross-sectional study done in our hospital.**Methods and Material**:Forty five type 2 diabetic patients of age 35-60 years, without signs and symptoms of diabetic foot ulcers dividedin three groups based on the diabetic age,were investigated for their glycaemicindex and fasting lipid profile, Ankle-brachial index and short-term resting HRV. **Statistical analysis used**: The various parameters of investigations were compared between the three groups and statistically analysed by one-way ANOVA and post-hoc analysis using SPSS statistical software version 21.0.**Results**: Serum LDL was significantly increased in groups II and III (P 0.0001), pNN50 and total power of HRV were found to be significantly decreased as the duration of diabetes increases ($p<0.05$) and there was an increasing trend of ABI from group I to group III ($p=0.003$). **Conclusions**: In spite of the absence of neuropathic symptoms, periodic assessment of serum LDL, short term HRV analysis and ABI is recommended to predict the risk of diabetic neuropathy in patients with type 2 diabetes mellitus.
Key-words: Distal sensorimotor polyneuropathy, Autonomic neuropathy, Ankle-Brachial index and Heart rate variability

INTRODUCTION

Majority of the microvascular complications in both type 1 and type 2 DM can be attributed to neuropathy which involves central, peripheral and/or autonomic nervous system [1, 2]. It is estimated that the life time risk of developing foot ulcers in diabetic patients is around 15%, [3] which may be attributed to distal sensorimotor polyneuropathy.Autonomic neuropathy leads to a diminution in sweat and oil gland functionality. As a result, the foot loses its

natural ability to moisturize the overlying skin and becomes dry and increasingly susceptible to tears and the subsequent development of infection[4].

The risk factors recognized for the development of diabetic neuropathy are increasing age, longer duration of diabetes and poor glycaemic control, while retinopathy, hypertension, hyperlipidaemia, micro-albuminuria and cigarette smoking have also been reported as the potential risk factors[5, 6, 7].Even though the lipid profile of most diabetic patients shows is 'Borderline-high' (130-159 mg/dL)[8],the normal LDL has the tendency to get converted to smaller and denser particles which is highly atherogenic and this can eventually lead to all major cardiovascular events[9, 10].

Blood flow in the peripheral limb arteries is influenced to a greater extent by the sympathetic division of autonomic nervous system to the arterioles and arterio-venous shunts which directly influences the peripheral circulation [11, 12]. The failure of the sympathetic fibres causes a reduction in the peripheral resistance through arteriolar vasodilatation and increased shunt flow. Thus there is an imbalance in the ankle blood pressure and hence ankle brachial index (ABI). In a large study, it was stated that higher ABI is strongly related to the risk of foot ulceration [13]. Thus ABI is an indirect measure of peripheral autonomic neuropathy.

Heart rate variability (HRV) is a simple non-invasive test used to assess the autonomic function. Decreased HRV is a classical feature of diabetic autonomic neuropathy[14].In this study, a comparison of ABI, HRV, lipid profile and glycemic status of three different group of diabetic patients was studied with the aim of risk prediction of autonomic neuropathy and hence occurrence of foot ulcers.

SUBJECTS AND METHODS

This cross-sectional study was designed and done following the ethical considerations of Helsinki's declaration and was approved by the Institutional Ethics Committee. Informed and written consent was obtained from all the patients included for the study. The inclusion criteria to participate in this study were patients with biochemically proven Type 2 diabetes mellitus of age group 35 – 60 years, who were not on statin therapy due to some contraindications. Patients having present and past history of diabetic foot ulcers, myocardial infarction and ischemia, other causes of autonomic neuropathy like chronic alcoholism, cancer chemotherapy etc., advanced renal failure and diabetic retinopathy were excluded from the study. The final sample size following the exclusion criteria was 45. This sample was divided into 3 groups based on the duration of diabetes since diagnosis, viz. group I (duration ≤ 5 yrs; n=26), group II (duration 6 – 10 yrs; n=9) and group III (duration > 10 yrs; n=10).

Biochemical Assessment: All the patients were subjected to biochemical analysis. Blood samples were taken from the patients to determine fasting and post-prandial blood glucose, HbA1c and fasting lipid profile including estimation of Low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC) and serum triglycerides (TGL).

Measurement of Ankle-brachial Index: ABI was measured using a pocket Doppler Ultrasound with 8 MHz handheld transducer (PC based Vascular Doppler Recorder - Versalab Dx, Diabetik Foot Care India Pvt. Ltd.) ABI was calculated for both feet separately. ABI was also graded into low, normal and high values according to American Diabetic Association[15].

Assessment of Short-term HRV: All the patients were then subjected for short term HRV assessment after 15 minutes of rest in supine position. ECG signals were acquired in all the patients in lead II using polygraph, the analogue signals were digitized using NI-DAQ Signal Acquisition device from National Instruments, India and the data stored in a PC. The HRV

was then analysed using HRV Soft, version 1.1, developed by AIIMS, New Delhi. The time domain parameters analysed were Resting heart rate, Standard deviation of the R-R Intervals [SDNN], Root square of the mean of the sum of the squares of differences between adjacent RR intervals [RMSSD], percentage of number of R-R interval differences ≥ 50 ms [pNN50]. The frequency domain parameters included Low frequency (LF), High Frequency (HF), the ratio of LF and HF and the total power (TP). The various parameters were then compared among the three groups.

Statistical Analysis: The mean and standard deviation of all the parameters of biochemical profile, ankle-brachial index and HRV were compared and correlated between the 3 groups using One-way ANOVA using SPSS Software version 21.0. The post-hoc test was done to confirm the significance using Tukey-Kramer multiple comparison test. A probability value of less than 0.05 was considered to be significant in all the statistical tests.

Results

Among the 45 diabetic subjects included for the studythere were 21 males and 24 females. On comparing the basic parameters (Table 1) between the three groups using one-way ANOVA, the mean age and duration of diabetes were found to be statistically significantly (p=0.001).

Table 1. Clinical characteristics of study participants

Parameters	Group 1	Group 2	Group 3
Age (mean±SD)*	45.92308±7.99	55.33±5.19	54.02±4.96
Males	12 (46.15%)	5(55.5%)	4(40%)
Females	14 (53.8%)	4(44.4%	6(60%)
Duration of diabetes* (mean±SD)	2.45 ± 1.69	8 ± 1.41	13.9 ± 2.47

*p=0.001, using One-way ANOVA

Among the various biochemical parameters (Table 2) using one-way ANOVA, LDL was found to be significantly higher among the groups (p = 0.0001). Post-hoc analysis using Tukey-Kramer's test confirmed significance in LDL between Groups 1 & 3 and groups 2 & 3 with p=0.0001 and 0.001 respectively.

Ankle-brachial index (Fig 1) was found to be highly significant (p=0.0001 byone-way ANOVA). Post-hoc analysis performed by Tukey's test for ABI showed significance between groups 1 and 2 (p = 0.02 and 0.01 for right and left limbs, respectively) and between groups 1 and 3 (p=0.0001 for both limbs). The distribution of ABI among the groups based on the grading given by American Diabetic Association is shown in the table 3.

Analysis of HRV parameters among the groups using one-way ANOVA showed pNN50 of time domain and total power of frequency domain (Fig 2) to be significant with p values 0.001 and 0.0001 respectively. Tukey's test showed significant decrease in pNN50 between groups 1 & 2 (p = 0.02) and between groups 1 & 3 (p=0.002). Total power was also significantly decreased on comparing groups 1 & 2 (p = 0.006) and between groups 1&3 (p=0.0001).

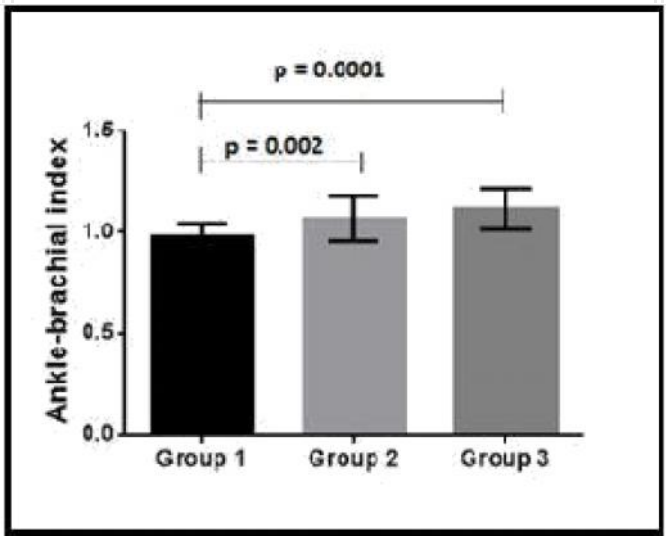

Fig 1 Comparison of Right Ankle-brachial index between groups

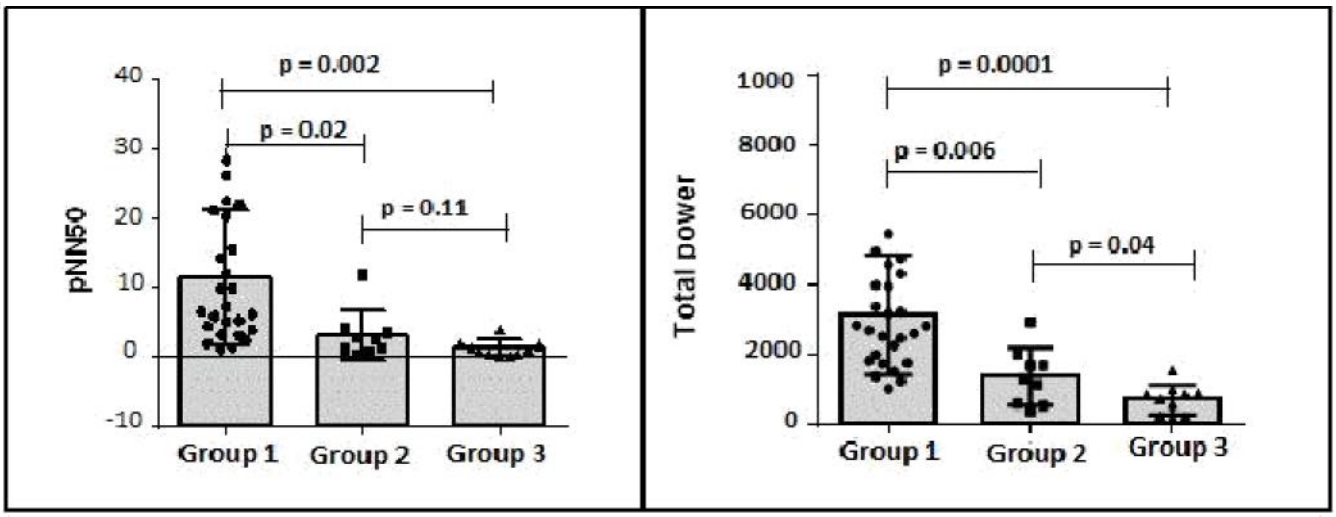

Fig 2: Comparison of pNN50 and total power between groups.

Pearson's correlation between ankle-brachial index and HRV parameters showed strong negative correlation for pNN50 (r= -0.546, p =0.01) and total power (r =-0.48, p = 0.01)

Table 2.Comparison of biochemical profile, ABI and HRV among three groups.

Parameter	**Group 1 (n=26)**	**Group 2 (n=9)**	**Group 3 (n=10)**
FBS (mg/dL)	149.46 ± 48.9	158.22 ± 40.9	187.2 ± 70.64
PPBS(mg/dL)	237.27 ± 73.63	278.89 ± 116.59	271.8 ± 93.60
HbA1c	8.4 ± 1.43	9.4 ± 2.43	9.62 ± 2.16
TC (mg/dL)	170.15 ± 40.75	179.67 ± 33.57	192.9 ± 32.32
TGL(mg/dL)	211.27 ± 127.7	182.56 ± 69.8	265.9 ± 201.14
HDL(mg/dL)	60.27 ± 16.71	50.44 ± 10.68	48.6 ± 12.79
LDL(mg/dL)*	97.19 ± 26.67	101 ± 17.51	143.6 ± 28.59
ABI_Right*	0.97 ± 0.07	1.068 ± 0.11	1.12 ± 0.10
ABI_Left*	0.97 ± 0.09	1.08 ± 0.12	1.13 ± 0.08
Mean heart rate (beats per min)	79.7 ± 13.11	78.17 ± 8.66	83.65 ± 10.33
SDNN (ms)	33.23 ± 13.12	31.43 ± 19.98	21.73 ± 10.7
RMSSD(ms)	26.22 ± 20	21.70 ± 17.63	16.67 ± 9.22
pNN50(ms)*	11.36 ± 9.63	3.16 ± 3.47	1.21 ± 1.14
LFnu	59.77 ± 17.86	60.16 ± 25.21	60.84 ± 23.82
HFnu	40.22 ± 17.86	39.83 ± 25.21	39.15 ± 23.82
LF:HF	2.08 ± 1.72	2.07 ± 1.8	3.63 ± 4.95
Total power*	3135.63 ± 1726.13	1363.70 ± 820.06	710.41 ± 424.97

*Parameters with significant p values <0.05. Significant p values for individual parameters are given in the text under results.

Table 3. Distribution of patients based on grading of ABI[15].

Grade	ABI	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)
Low	<0.9	6 (23.08)	3 (33.3)	1 (10)
Normal	0.91-1.3	20 (76.92)	5 (55.6)	6 (60)
High	>1.31	0 (0)	1 (11.1)	3 (30)

DISCUSSION:

The aetiology of foot ulcers in diabetic patients is mostly because of the diabetic sensorimotor polyneuropathy combined with autonomic neuropathy[1, 2, 16]. There is a proven consensus that peripheral circulation is directly influenced by autonomic nervous system[11,17].Forty five patients without clinical signs and symptoms of neither autonomic neuropathynor peripheral vascular disease were selected since the presence of both may lead tobiased interpretation of results. We divided the patients into 3 groups based on the duration of diabetes since diagnosisand the age group was restricted to 35-60 years to exclude the age-related changes in HRV and ABI interpretation.

The serum LDL showed a significantly increasing trend from group 1 to group 3. The HbA1c levels indicatingthe severity of disease also showed an increasing trend though not statistically significant. It was proven by many studies that LDL increases the risk of cardiovascular atherogenic risk in type 2 diabetes patients[18,19].Hence, it can be stated that increasing trend in LDL with increasing duration of diabetes and poor glycaemic control should be considered in the assessment of atherogenic complication spectrum which also includes peripheral vasculardisease. Furthermore, this study was done in patients who were not on statin therapy due to certain contraindications like musculoskeletal pain and GI disturbances. This emphasizes that LDL lowering therapy will be beneficial in diabetic people to prevent the risk of atherogenesis and its complications.

Ankle-brachial index increases significantly from group 1 to group 3, although the mean ABI were found to be within the normal range in each group. Low ABI (< 0.9)were found more in group 2 (33.3%) whereas a high ABI (> 1.3) was found more in group 3 (30%). There is a trend of increasing ABI with increasing duration of the disease and poor glycaemic control. In previous studies in symptomatic diabetic patients ABI < 0.9 or >1.3 indicated peripheral artery disease[20, 21, 22, 23]. William et al.,[21]showed ina group of diabetic patients with an intermediate vascularprofile and without neuropathy that an ABI <0.9 hada sensitivity of 100% and a specificity of 88%in predicting peripheral artery disease. However, high ABI is more often noticed in case of neuropathy. This is attributed to the stiffness of the vessel wall produced by medial artery calcification, [24, 25]which is a strongly feature of diabetic neuropathy. The association of both arteriopathyas evidenced by low ABI and neuropathy as evidenced by high ABIare responsible for the high prevalence of wounds, ulcers andamputations of diabetics' feet. Though, both low ABI and high ABI are the better indicators of impending neuropathy and atherogenic cardiovascular events in diabetic population, the current study emphasizes high ABI as the most important predictor of neuropathy.

HRV analysis done in this study showed decreased pNN50 and total power in groups which ultimately indicate that there is decreased RR variability and sympathovagal imbalance. These are the characteristic features of diabetic autonomic neuropathy [14]. This fact was supported by a wealth of previous studies[26, 27, 28, 29, 30]. Reduced HRV is one of the features associated with early stages of distal sensorimotor neuropathy(DSP)[31]. There areburgeoning literatures stating that HRV decreases with increase in age, diabetic duration and poor glycaemic control, [6, 31, 32]. A study by Orlov et al [29] has stated that a low HRV may be a biomarker for clinical DSP and is associated cross-sectionally with both early and late measures of neuropathy. Considering its potential implications in predicting the early autonomic neuropathy and sensorimotor polyneuropathy, it can be concluded that HRV can itself be a simplest, sensitive, early and betterpredictor than ABI in predicting the future risk of diabetic foot ulcers. On correlating HRV with ABI, both pNN50 and total power showed a significant and strong negative correlation which denotes that as ABI increases, HRV decreases, all of which is seen in established diabetic neuropathy[31, 33]. ABI is done in majority of diabetic clinics as one of the regular follow-up investigation, but HRV, being simpler and less expensive than ABIis done in only very few hospitals that too less frequently in developing countries. Hence, this study emphasizes the importance of short term HRV in the assessment of diabetic neuropathy.

CONCLUSION:

This study concludes HRV decreases with increasing diabetic age depicting the sympatho-vagal imbalance. A high ABI is a more prompt indicator for the occurrence of diabetic neuropathy. Even a borderline high normal value of LDL Cholesterol is to be considered as

significant risk factor for diabetic neuropathy and other atherogenic complications like foot ulcers.

Limitations: One, the small sample size is small to have a firm conclusion. Hence similar studies with larger sample are recommended to draw solid conclusions. Two, the quantification of sensorimotor polyneuropathy was not done and compared with the methods used in the study. Concurrent studies from developing countries are necessary to quantify sensorimotor polyneuropathy the using appropriate tests and correlate them with HRV and ABI.

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