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Cardiovascular Reflex Tests in Adolescents with Type 1 Diabetes Mellitus

Tip 1 Diabetes Mellituslu Adolesanlarda Kardiyovasküler Refleks Testleri

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ABSTRACT

Aim: *Diabetic autonomic neuropathy (DAN) is one of the serious, microvascular complications of diabetes mellitus. Clinically the most important form of DAN is cardiovascular autonomic neuropathy (CAN). Determination of the presence of CAN is usually done by a battery of cardiovascular reflex tests. This study was aimed to compare the standard cardiovascular reflex tests between adolescents with diagnosed type 1 diabetes mellitus (T1 DM) and healthy controls.*
Methods: *A total of 64 subjects were recruited for this study. Mean age of the case group was 16.15±2.66 years and the mean age of the control group was 17.13±1.34 years. Mean duration of diabetes was 54.81±33.57 months. Standard cardiovascular tests such as deep breathing test (DBT), Valsalva ratio (VR), Lying to standing test (LST or 30: 15 ratio) and cold pressor test (CPT) were performed.*
Results: *We found a significantly reduced LST (30: 15 ratio) in diabetic group. We also found a significantly reduced change in diastolic blood pressure (ΔDBP) at 1 minute of CPT in diabetic group. Furthermore, greater the duration of disease lesser was the DBT.*
Conclusions: *A significantly reduced LST (30: 15 ratio) in diabetic group signifies reduced parasympathetic activity. Significantly a reduced value of Δ DBP at the end of 1 minute of CPT implies a reduced sympathetic activity in this group of patients. It can be said that in these patients autonomic activity is compromised. Furthermore, the impairment in parasympathetic activity (DBT) is directly related to the duration of diabetes.*

Keywords: *Cardiovascular reflex tests, cardiac autonomic neuropathy, diabetes mellitus*

ÖZ

Amaç: *Diyabetik otonom nöropati (DAN), diyabetes mellitusun bir mikrovasküler komplikasyonu olup, ciddi komplikasyonlarından biridir. DAN'ın klinik olarak en önemli formu kardiyovasküler otonom nöropatidir (KAN). KAN'ın varlığının belirlenmesi genellikle bir grup kardiyovasküler refleks testleri ile yapılmaktadır. Bu çalışma, tip 1 diyabetes mellitus (T1 DM) tanılı adolesanlar ile sağlıklı kontrol bireylerinde standart kardiyovasküler refleks testlerinin karşılaştırmasını amaçlamaktadır.*
Yöntem: *Toplamda 64 birey bu çalışmaya dahil edilmiştir. Vaka grubunda ortalama yaş 16,15±2,66 olup kontrol grubunda ortalama yaş 17,13±1,34'tür. Hastalarda ortalama diyabet süresi 54,81±33,57 aydır. Standart kardiyovasküler testler olarak derin solunum testi (DST), valsalva oranı (VO), ayağa kalkma testi (AKT veya 30:15 oranı) ve soğuk stres testi (SST) uygulanmıştır.*
Bulgular: *Bu çalışma ile diyabetik olgularda anlamlı olarak azalmış AKT (30:15 oranı) elde ettik. Ayrıca diyabetik grupta 1 dk.'lık SST'de diyastolik kan basıncı değişiminde (ΔDKB) anlamlı azalma bulduk. Buna ek olarak, hastalık süresinin uzaması DKB'nin daha düşük olması ile ilişkili idi.*
Sonuç: *Diyabetik grupta AKT (30:15 oranı)'deki anlamlı düşüş, azalmış parasempatik aktiviteyi göstermektedir. Yine bu grup hastalarda 1 dk.'lık SST sonunda ΔDKB değerindeki anlamlı azalma, azalmış sempatik aktiviteyi işaret etmektedir. Bu sonuçlarla bu hastalarda otonom aktivitede bozulma olduğu söylenebilir. Ayrıca parasempatik aktivitedeki bozulmanın (DST) direkt olarak diyabet süresi ile ilişkili olduğu sonucu ortaya çıkmıştır.*

Anahtar kelimeler: *Kardiyovasküler refleks testleri, kardiyak otonom nöropati, diyabetes mellitus*

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INTRODUCTION

It is a well-known fact that diabetic autonomic neuropathy (DAN) is one the most lethal and common complication of diabetes mellitus[1] and has a capability to involve the entire autonomic nervous system[2]. Impaired functioning of various organ systems such as cardiovascular system, genitourinary system, gastrointestinal system etc occurs as a consequence of DAN[2]. Since cardiovascular system is most commonly affected by DAN, the term cardiac autonomic neuropathy (CAN) is used in place of DAN[3,4]. The prevalence of CAN varies from 1 to 90% in patients with type 1 diabetes mellitus depending on the diagnostic method, type of diabetes and stage of diabetes[5]. Symptoms associated with CAN are tachycardia at rest, intolerance to exercise, orthostatic hypotension, loss of variation in blood pressure during day and night, hypoglycemic unawareness, ventricular arrhythmias, silent myocardial ischemia and sudden death[6]. There is evidence that mortality in patients with CAN is many times higher than the mortality in patients without CAN[7]. Cardiac autonomic neuropathy can progress rapidly during adolescence. It may lead to poor metabolic control and irreversible neuropathic changes during puberty[8]. Thus, early detection of CAN and motivation of the patients to improve their glycaemic control is extremely important[8]. Early detection of CAN and strict follow up during adolescence could decrease complications that may otherwise arise as a result of decreased autonomic flexibility[8]. One of the earliest sign of CAN is reduced heart rate variation i.e. shortening of R-R interval[9]. The presence of CAN is usually based on a battery of cardiovascular reflex tests[1]. Clinically CAN is detected early by decreased heart rate variation as a response to deep breathing, decreased heart rate variation to Valsalva maneuver and diminished heart rate response to standing[8]. Therefore cardiovascular reflex tests may lead to early detection of CAN and improve quality of life[1]. Thus the main aim of this study was to determine the functioning of autonomic nervous system in adolescent diabetics by performing cardiovascular autonomic reflex tests since these tests reflect the autonomic reserve in our body and also to detect early CAN in them.

MATERIAL and METHODS

After obtaining clearance from the institutional ethics committee (Institutional Ethics Committee of Government Medical College & SMHS Hospital, Srinagar, J&K, India, 5t October, 2017, Approval number; 2017/389) we retained 64 adolescents for this study. Thirty-two patients (mean age 16.15±2.66 years) including 16 males and 16 females diagnosed with T1 DM were selected among the patients presented to the Endocrinology outpatient department. Mean duration of diabetes in them was 54.81±33.57 months. Age-, and sex- matched 32 controls which included 17 males and 15 females (mean age 17.13±1.34 years) were investigated in this study. Subjects having diabetic ketoacidosis, neuropsychiatric disorders, comorbid medical conditions such as thyroid disorders, anemia, glaucoma and any history of intracranial hemorrhage were excluded from this study. Informed written consent was obtained from all the subjects or their accompanying guardians. The principles of Helsinki declaration were followed throughout the study. All the subjects were seen in the outpatient clinics of endocrinology department. Biochemical parameters such as fasting glucose, postprandial glucose and glycemic control (HbA1c) were recorded. Subjects were adequately explained the procedure and requirements of autonomic function tests. They were told to refrain from intake of tea/coffee, anti-hypertensive drugs, β blockers/agonists on the day of testing. All the subjects were then invited to the autonomic function testing laboratory in the department of physiology. Subjects were advised to be accompanied by their guardians. Standardized conditions were maintained in the laboratory throughout the procedure. Before beginning the autonomic function testing all the subjects were made to lie supine on a table and a rest of 15 minutes was given. Parasympathetic activity was accessed by cardiovascular reflex tests such as DBT, Valsalva ratio (VR) obtained from Valsalva maneuver and LST (30:15 ratio) which were performed on POWER LAB 26 T (AD

Instruments; Sydney, Australia) whereas CPT, which assesses sympathetic activity, was performed using digital sphygmomanometer OMRON (HEM-8712; Taiwan).

Procedure

After an initial rest of 15 minutes, parasympathetic activity was assessed from the R-R intervals of ECG obtained from POWER LAB 26 T using by following methods.

1. Deep breathing test (DBT): This test was done with the patient in the lying position. A respiratory belt was tied to the thoracic cavity. Long lead II ECG was acquired. Subjects were asked to inspire and expire for 5 seconds each thus completing 6 cycles of breathing in a minute. Both audio and visual cues were provided. Results were expressed in terms of heart rate variation i.e maximum R-R interval during expiration to the minimum R-R interval during inspiration. Values ≥ 1.21 were considered normal.

2. Valsalva maneuver: This test was performed on a tilt table at an angle of 20°. Subjects were instructed to blow into the sphygmomanometer and maintain a pressure of 40 mmHg for 15 seconds. Heart rate variation was recorded in terms of R-R intervals throughout the procedure. VR was calculated from the maximum R-R interval during phase IV to the minimum R-R interval during phase II. Value of ≥1.21 was considered normal.

3. Lying to standing test (30:15 ratio): Subjects were asked to lie supine on a table, and asked to stand up within 3 seconds. Both audio and visual cues were provided. R-R interval at around 30^{th} beat to the R-R interval at around 15^{th} beat was calculated. Value of ≥ 1.04 was taken as normal.

4. Cold pressor test: This test was carried in sitting position. Temperature of water was maintained at around 4°C. Subjects were asked to immerse their hand in water for at least 60 seconds while blood pressure changes were recorded from the contralateral arm. Resting blood pressure was measured before the test and diastolic blood pressure (DBP) was measured at the end of 60 seconds of CPT. DBP ≥15 mmHg was considered normal.

A rest of 5 minutes was allowed between all tests.

Statistical Analysis

SPSS 22 software (Statistical Package for the Social Sciences, IBM systems inc; California, U.S.A) was used to analyze data. Shapiro-Wilk and Kolmogorov-Smirnov normality tests were employed to check the column statistics. Unpaired t-test was used to compare the data between two groups. Non-parametric, Mann Whitney test, was used wherever needed. Correlations of cardiovascular reflex tests with various biochemical parameters were assessed by Pearson's correlation coefficient or Spearman's correlation coefficient. P value <0.05 was considered significant.

RESULTS

We analyzed the data from 64 subjects. Comparison of various characteristics between controls and adolescent diabetics are given in Table 1. In our study we did not observe any significant difference in age and gender distribution between the two groups as shown in Table 1. Mean duration of diabetes was 54.81±33.57 months. BMI was significantly reduced ($p<0.001$) in diabetic group as compared to the controls. We also observed that the resting heart rate ($p=0.001$) and fasting blood glucose ($p<0.001$) were significantly higher in the diabetic group as shown in Table 1. It is also evident from our findings that resting systolic blood pressure was significantly reduced in diabetic group ($p<0.01$) but there was no difference between the resting diastolic blood pressure between the two groups as shown in Table 1. The results of cardiovascular tests between the controls and patients are shown in Table 2. A significantly reduced LST/30:15 ratio was seen in diabetic group as compared to the controls ($p<0.05$). Change in diastolic blood pressure (Δ DBP) at the end of 1^{st} minute of CPT was significantly lower in diabetic group when compared to the controls ($p<0.01$). Table 3 shows corre-

Table 1. Comparison of various characteristics between controls and cases.

Biochemical parameters	Controls (n=32)	Cases (n=32)	P value
Age (in years)	17.13±1.34	16.15±2.66	>0.05
Duration of diabetes (in months)	N/A	54.81±33.57	N/A
BMI (kg/m^2)	21.16±1.91	18.49±3.25	<0.001***
Gender distribution (male: female)	17:15	16:16	>0.05
Fasting blood glucose (mg/dl)	90.10±7.26	241.7±120.2	<0.001***
Glycaemic control (HbA1c)	N/A	10.44±2.93	N/A
Resting heart rate (bpm)	76.80±12.23	86.76±13.27	0.001**

BMI: body mass index. mg/dl: milligram per deciliter. bpm: beats per minute. HbA1c: glycaemic control. Kg/m^2: kilogram per meter square.
 Chi square test
*significant, **highly significant, ***Very highly significant
Data expressed as mean ± SD

Table 2. Comparison of cardiovascular reflex tests between healthy controls and adolescent subjects diagnosed with T1 DM.

Cardiovascular reflex tests	Controls (n=32)	Cases (n=32)	P value
DBT (E/I ratio)	1.57±0.17	1.51±0.25	>0.05
Valsalva ratio	1.54±0.23	1.60±0.35	>0.05
LST (30:15 ratio)	1.34±0.22	1.22±0.19	<0.05*
DBP (mmHg) at 1 min of CPT	14.16±6.51	10.42±11.69	<0.01**

DBT: deep breathing test. E/I ratio: expiration to inspiration ratio. LST: lying to standing test. 30:15 ratio: ratio of maximum R-R interval at around 30th beat to minimum R-R interval at around 15th beat. DBP: change in diastolic blood pressure. CPT: cold pressor test. mmHg: millimeters of mercury. n: number of subjects.
*Significant, **Highly significant.
Data expressed in terms of mean ± SD.

Table 3. Correlation of cardiovascular tests with duration of diabetes and other biochemical parameters.

Cardiovascular tests	Duration of disease	Fasting glucose	Postprandial glucose	HbA1c
DBT (E/I ratio)	r=-0.532 p<0.01**	r=0.081 p>0.05	r=-0.021 p>0.05	r=0.215 p>0.05
Valsalva ratio	r=-0.015 p>0.05	r=0.263 p>0.05	r=0.296 p>0.05	r=0.234 p>0.05
LST (30:15 ratio)	r=-0.204 p>0.05	r=0.121 p>0.05	r=0.098 p>0.05	r=0.039 p>0.05
DBP at 1 min of CPT	r=-0.055 p>0.05	r=-0.314 p>0.05	r=-0.178 p>0.05	r=-0.133 p>0.05

DBT: deep breathing test. E/I ratio: expiration to inspiration ratio. LST: lying to standing test. 30:15 ratio: ratio of maximum R-R interval at around 30th beat to minimum R-R interval at around 15th beat. DBP: change in diastolic blood pressure. CPT: cold pressor test. HbA1c: glycaemic control.
** Highly significant.
"r" Coefficient of correlation

lation of cardiovascular reflex tests with duration of diabetes, fasting glucose, postprandial glucose and HbA1c values. We observed a significantly negative correlation of DBT with the duration of disease as shown in Figure 1. We did not find any correlation of cardiovascular tests with fasting glucose, postprandial glucose and HbA1c in our study.

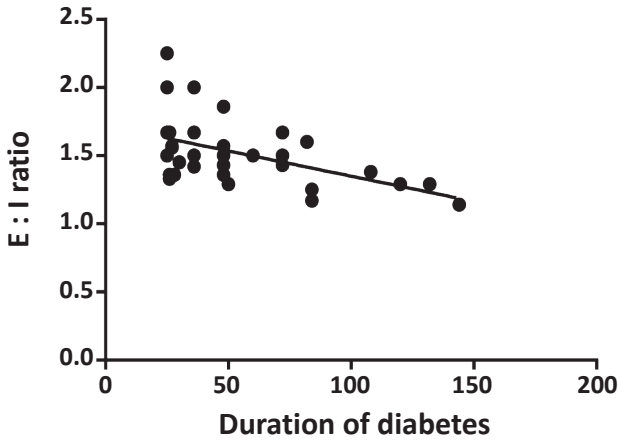

Figure 1. Shows negative correlation of DBT (E:I ratio) with duration of diabetes in adolescents with type 1 diabetes mellitus. E:I ratio; expiratory to inspiratory ratio.

DISCUSSION

In this study we compared the cardiovascular reflex tests i.e. DBT, VR obtained from Valsalva maneuver, LST (30: 15 ratio) and ΔDBP at the end of 1 minute of CPT between adolescent subjects diagnosed with T1 DM and age-, and sex- matched controls. We found a significantly reduced LST (30:15 ratio) in diabetic group as compared to the controls ($p<0.05$). Also ΔDBP at the end of 1 minute of CPT was significantly reduced in the diabetic group ($p<0.01$). Cardiovascular tests such as DBT, Valsalva maneuver and LST (30: 15 ratio) are standard parasympathetic tests[10] whereas CPT is a standard test to measure generalized sympathetic activity characterized by increase in heart rate, systolic and diastolic blood pressure[11]. In the diabetic group reduced LST (30:15 ratio) suggests a decrease in heart rate variation, R-R interval, at around 30th beat. Vagal or parasympathetic activity predominates at around 20th beat and becomes maximum at around 30th beat after standing from a supine position i.e. LST[12]. In our study, diabetic group had decreased variation of R-R interval at around 30th beat during LST which may be attributed to decreased vagal or parasympathetic activity in them. Similar findings were reported by Elamin et al.[13] who found a significantly reduced LST / 30:15 ratio ($p<0.05$) in T1 DM patients as compared to the control group. The DBT (E:I ratio) and the Valsalva ratio between the diabetic and healthy children and adolescents were not significantly different in their study. Barkai et al.[14] observed that diabetic children had lower LST /30:15 ratio ($p<0.05$) compared with the controls. They also observed that longer duration of diabetes and HbA1c were independently predictive of CAN. Khatoon M et al.[15] studied the cardiovascular reflexes such as LST (30:15 ratio), DBT and CPT of diabetic subjects and age-, and sex-matched healthy controls. Their results showed that diabetics had significantly impaired cardiovascular reflexes compared to non-diabetics, which increases with the duration of diabetes. We also had similar findings in our study, unlike other studies, we did not find any relation between cardiovascular reflexes with fasting glucose, postprandial glucose and HbA1c which may be attributed to a small sample size of our preliminary study. In our study we found that DBT had a significant negative correlation with the duration of diabetes which means that greater the duration of diabetes the lesser is the E:I ratio hence leading to an impaired DBT in these patients. The decrease in E:I ratio may be caused by a decreased parasympathetic activity in them. This finding is similar to what was found by Khatoon M et al.

In the diabetic group a significantly reduced ΔDBP at the end of 1 minute of CPT means that the diabetic group had reduced sympathetic activity which led to the impaired response of DBP in them. In a healthy subject CPT response stimulates sympathetic response that leads to increased blood pressure and heart rate[11]. Our findings were similar to that of Sayinalp S et al.[16] who applied CPT to a group of diabetic patients and controls. They found that ΔDBP during CPT was smaller in patients with autonomic neuropathy than the controls ($P<0.05$). Krishna BH et al.[17] performed CPT in 30 diabetics and 30 controls and found that the changes in DBP during CPT were significantly reduced in diabetics. In conclusion adolescents with T1 DM in our study had significantly reduced autonomic reactivity characterized by a significant decrease in both parasympathetic and sympathetic activity thus leading to reduced heart rate variability in these patients. However, there are some limitations in this study such as a small sample size and poor glycemic

control of the patients. It may be concluded that cardiovascular reflex tests help in timely diagnosis of CAN. Thus proper management and quality of life in patients with diabetes mellitus can be increased if CAN is detected in earlier stages.

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