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Relationship of vitamin B_{12}, folic acid and homocysteine level with time domain measures of heart rate variability in female Metabolic Syndrome

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Abstract

Background: Metabolic syndrome (MetS) is a major risk factor for cardiac mortality and morbidity. Hyperhomocysteinemia and folic acid are related with MetS. Both MetS and hyperhomocysteinemia adversely affect heart rate variability (HRV). **Objectives:** To assess the relationship of vitamin B_{12}, folic acid and homocysteine levels with HRV in female MetS patients. **Methods:** This cross sectional study was carried out on 40 female MetS patients of 25-45 years of age. Forty apparently healthy female with similar age were control. Chemiluminescent immunoassay measured serum vitamin B_{12}, folic acid, homocysteine level and time domain parameters of HRV were recorded by Powerlab 8/35, AD instruments, Australia. Independent sample 't' test and pearson correlation coefficient test was used for statistical analysis . **Results:** In this study, all the time domain HRV variables (Standard deviation of all RR intervals-SDRR, Coefficient variation of RR interval-CVRR, The square root of mean squared difference of RR intervals-RMSSD and Number of R-R interval differing >50ms from adjacent intervals divided by the total number of all R-R intervals-pRR50%) were significantly lower ($p<0.05$) in MetS patients compared to control. But correlation analysis showed only the folic acid level was significantly negatively correlated ($p<0.05$) with SDRR, CVRR, RMSSD and pRR50%. **Conclusion:** The result of this study showed inverse relation of low HRV with low serum folic acid levels in female MetS patients.

Keywords: Metabolic syndrome, Heart rate variability, Vitamin B_{12}, Folic acid, Homocysteine.

Introduction

Metabolic syndrome (MetS) is condition of metabolic disorder with a potential threat for cardiovascular events.[1] Its worldwide prevalence is 20-25% and 30% in Bangladesh with female predominance.[2] According to International Diabetes Federation (IDF) MetS included central obesity (defined as waist circumference, >90 cm for men or >80 cm for women of South Asia) plus at least 2 or more of the following conditions to be diagnosed as MetS – (1) Hyperglycaemia (Fasting plasma glucose >100 mg/dl) or previously diagnosed type 2 diabetes, (2) Hypertriglyceridaemia (>150 mg/dl) or any particular treatment for this lipid abnormality, (3) Low HDL cholesterol (< 40 mg/dl in men, < 50 mg/dl in women) or specific treatment for this lipid abnormality, (4) Hypertension (Systolic BP >130 or diastolic BP >85 mm Hg) or any treatment for previously diagnosed hypertension.[3] All the organs and systems of the body are badly affected by MetS. However, its effects on cardiovascular system are much more prominent.[4] Autonomic dysregulation of heart characterized by sympathetic hyperactivity closely linked to cardiovascular disease in MetS.[5] Analysis of HRV has been found to be the most effective and non-invasive method for determining cardiac autonomic activity. Reduced HRV caused by autonomic imbalance is associated with the pathogenesis of cardiac arrhythmia and sudden cardiac death. In time domain method some simple and statistical variables are calculated from statistical analysis of a series of instantaneous heart rate or interbeat interval which are recorded from continuous ECG tracing. But this method cannot discriminate specific changes in sympathetic and parasympathetic activity. Time domain variables reflects overall variability as well as cardiac vagal tone.[6-7] Several studies revealed relationship between MetS and reduced HRV.[8-10] Within past few decades, hyperhomocysteinemia has become another risk factor, increasing the risk of CVD by two fold especially in the presence of other cardiovascular risk factor.[11-12] Insufficiency of Vitamin B_{12} and folic acid also lead to hyperhomocysteinemia as these vitamins are necessary for metabolism of homocystein that help to prevent CVD.[13-14] On the other hand, in MetS hyperhomocysteinemia can have cooperative effect to worsen the cardiovascular complications.[15] According to some research, in MetS, there was higher level of homocysteine and/or lower level of vitamin B_{12} and folic acid, whereas other found no such association between these two variables in MetS.[16-19] Also lack of vitamin B_{12} was found to be connected with autonomic dysfunction.[20-21] No relation was observed between homocysteine level and cardiovascular autonomic function.[22] On the other hand, study on MetS patients and type 2 diabetic patients showed that hyperhomocysteinemia was negatively correlated with HRV.[23-24] Little is known about the relationship of vitamin B_{12}, folic acid and homocysteine level with heart rate variability among patients with MetS. Therefore, the present study has been designed to observe the relationship of vitamin B_{12}, folic acid and homocysteine level with HRV in MetS.

Methods

Study design & setting

This cross sectional study was carried out in 2019 in Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Ethical aspects of protocol was approved by Institutional review board of BSMMU.

Paticipants & sampling

For this study, 40 female MetS patients aged 25-45 year who were diagnosed according to International Diabetes Federation (IDF) criteria[3] were enrolled by purposive sampling from the

Outpatient Department of Endocrinology according to selection criteria. Forty age matched apparently healthy female volunteers were control.

Exclusion criteria

The patients were free from- cardiovascular disorders, respiratory disorders, renal insufficiency, liver disease, arthritis, any neurological disorders (migraine, epilepsy), thyroid disorders, psychiatric disorders, malignancy, pregnancy and also the patients who had history of medications for cardiac disease or respiratory disease or for other reasons which may interfere with autonomic nervous system balance or, current use of vitamin supplementation, current smokers and consumption of any other tobacco products.

Data collection procedure

Informed written consent was taken from them. Then detailed history was taken and anthropometric measurement of all subjects was done. Pulse and blood pressure were measured to assess their baseline cardiovascular status. Under aseptic precaution 6ml of venous blood was collected for estimation of biochemical parameters - fasting plasma glucose (GLUC method)[25], lipid profile (Enzymatic method)[26], plasma creatinine (CRE2 method)[27] and serum vitamin B_{12}, folic acid, homocysteine level (Chemiluminescent immunoassay)[28]. The finally selected subjects were then given instruction to prepare for HRV. For HRV recording, the subjects were instructed to take their meal by 9:00 pm and to have a sound sleep in the previous night, avoid any physical or mental stress, and not to take any sedatives, hypnotics medication. The patients were requested to take light breakfast in the morning without tea or coffee and then attend to the autonomic nerve function laboratory in the Department of Physiology, BSMMU between 8-9 a.m. After that, the subjects were allowed to take rest in a bed in supine position for 10-15 minutes in a noise free and comfortable temperature controlled laboratory environment. Then HRV was recorded by a data acquisition device, powerlab 8/35, AD instruments, Australia[6] in Department of Physiology to assess cardiac autonomic nerve function status. During the procedure, any talking, eating or drinking and as well as performing physical or mental activity even sleep were strictly prohibited.

Statistical analysis

Data were expressed as mean ± SD. For statistical analysis independent sample 't' test and pearson's correlation coefficient test were done by using SPSS version 16. p value <0.05 was considered as level of significance.

Results

General characteristics of the subjects were presented in Table I. In this study, age was similar between two groups but BMI and WC, resting pulse rate, systolic and diastolic blood pressure were significantly higher ($p<0.01$) in MetS patients as per criteria of MetS. In this study, among the time domain HRV variables significantly higher value of mean HR and lower value of mean RR interval, SDRR, CVRR, RMSSD and pRR50% ($p<0.01$) were found in MetS patients compared to healthy control (Table II). In addition, no significant ($p>0.05$) differences were found in serum vitamin $B_{12,}$ folic acid and homocysteine level between the two groups (Table III). Among serum vitamin $B_{12,}$ folic acid and homocysteine level, only the folic acid level showed statistically significant negative correlation ($p<0.05$) with SDRR, SDSD, CVRR, RMSSD in MetS patients (Table IV). Data for vitamin B_{12} and homocysteine are not presented.

Table I: Age, BMI, WC, Resting pulse rate and blood pressure in two groups (N=80)

Variables	MS (n=40)	Control (n=40)	p value
Age	37.98±6.71	35.60±5.99	0.099
(years)	(25.00-45.00)	(25.00-45.00)	
BMI	29.33±3.94	22.22±1.92	0.000
(Kg/m^2)	(25.00-37.60)	(18.50±24.90)	
WC	90.68±7.46	75.45±4.56	0.000
(cm)	(81.00-119.00)	(63.00-80.00)	
Pulse	78.65±11.20	71.00±7.51	0.001
(beats/min)	(60.00-100.00)	(62.00-88.00)	
SBP	131.00±17.66	112.50±10.06	0.000
(mm of Hg)	(100.00-200.00)	(100.00±140.00)	
DBP	86.00±9.62	73.55±7.68	0.000
(mm of Hg)	(70.00-120.00)	(60.00-90.00)	

Data were expressed as Mean ± SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MS-Metabolic syndrome; BMI- body mass index; WC- waist circumference; SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure; n= number of subject.

Table II: Time domain measures of HRV in two groups (N=80)

Variables	MS (n=40)	Control (n=40)	p value
Mean heart rate	85.39±11.21	77.14±8.89	0.000
(beats/min)	(62.42-113.80)	(61.07-98.17)	
Mean RR	715.53±93.13	790.29±92.36	0.001
interval(ms)	(528.70-962.20)	(611.60-986.30)	
SDRR	26.52±13.16	40.84±17.54	0.000
(ms)	(6.77-58.56)	(15.17±84.18)	
CVRR	0.04±0.02	0.56±0.04	0.005
	(0.01-0.08)	(0.02-0.26)	
SDSD	17.72±11.01	31.94±17.82	0.000
(ms)	(2.89-44.85)	(6.96-76.51)	
RMSSD	17.71±11.00	31.90±17.80	0.000
(ms)	(2.89-44.85)	(6.95-76.41)	
pRR50%	2.58±5.92	12.12±15.94	0.001
	(0.00-25.41)	(0.00-53.56)	

Data were expressed as Mean ± SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MS-Metabolic syndrome; SDRR- Standard deviation of all RR interval; CVRR- Coefficient of variance of RR interval; SDSD- Standard deviation of successive RR interval differences between adjacent RR intervals; RMSSD- Square root of mean of squared differences of successive RR interval; pRR50%- Proportion of RR interval with duration >50ms; ns- non significant (p >0.05); n= number of subject

Table III: Serum vitamin B_{12}, Folic acid and Homocysteine in two groups (N=80)

Variables	MS (n=40)	Control (n=40)	p value
Vitamin B_{12} (pgm/mL)	409.98±152.88 (168-795)	412.78±138.28 (173-798)	0.932
Folic acid (ng/mL)	8.30±3.12 (3.50-14.30)	8.42±3.09 (3.60±15.80)	0.863
Homocysteine (µmol/L)	8.03±3.27 (4.43-22.82)	7.53±2.17 (3.44-12.72)	0.423

Data were expressed as Mean ± SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MS-Metabolic syndrome; p value was >0.05; n= number of subjects.

Table IV: Correlations of time domain HRV measures with serum Folic acid level in two groups (N=80)

	MS		Control	
	r value	p value	r value	p value
Mean heart rate (beats/min)	0.064	0.696	0.254	0.114
Mean RR interval(ms)	-0.048	0.770	-0.264	0.099
SDRR (ms)	-0.390	0.013*	-0.154	0.341
CVRR	-0.410	0.009**	0.136	0.401
SDSD (ms)	-0.395	0.012*	-0.214	0.184
RMSSD (ms)	-0.395	0.012*	-0.214	0.184
pRR50%	-0.290	0.069	-0.309	0.052

Statistical analysis was done by Pearson's correlation (r) test. MS- Metabolic syndrome; SDRR- Standard deviation of all RR interval; CVRR- Coefficient of variance of RR interval; SDSD- Standard deviation of successive RR interval differences between adjacent RR intervals; RMSSD- Square root of mean of squared differences of successive RR interval; pRR50%- Proportion of RR interval with duration >50ms; n = number of subjects.

Discussion

The present study investigated the relationship of serum vitamin B_{12}, folic acid and homocysteine level with time domain measures of HRV in 25-45 years of females metabolic syndrome patients. Almost similar age range[29] and higher BMI[8,10,29] in MetS were reported by different researcher. In this study higher resting pulse rate, SBP and DBP in MetS patients agree to others suggesting dysregulation of cardiovascular control in MetS.[4,8,10,29] Among the results of time domain HRV variables, significantly higher Mean HR and lower Mean RR interval, SDRR, RMSSD, pRR50%, CVRR and SDSD suggesting autonomic impairment in MetS which is consistent with the findings of other researcher[4,8,]. Accleration of heart rate is affected both by inhibition of parasympathetic vagus nerve and the stimulation of sympathetic nerve. Among time domain measures SDRR has most significant prognostic value and it represent general measurement of sympathovagal balance,

but it cannot quantify specific changes in sympathetic and parasympathetic activity. In contrast SDSD, RMSSD, pRR50% considered as index of parasympathetic activity[6,7]. So the findings of HRV suggest autonomic imbalance with decreased parasympathetic modulation in current metabolic syndrome patients. The current study also revealed that serum vitamin B_{12} and folic acid were lower and serum homocysteine level was higher in MetS patients though these were statistically nonsignificant. The results of different study supported this findings.[18-19,30-31] On correlation analysis among HRV measures and serum vitamin B_{12}, folic acid and homocysteine, only folic acid showed significant negative correlation with SDRR, SDSD, CVRR and RMSSD which suggests that decreasing level of folic acid is related with parasympathetic hypo-activity in MetS though no previous data was available to compare this findings. But animal experimental evidence demonstrated that lower level of folic acid cause increased superoxide and decreased nitric oxide (NO) production.[32] This low NO tonically inhibit the firing activity of paraventricular nucleus of hypothalamus that ultimately inhibit the autonomic outflow from the dorsal vagal complex (DVC) and rostral ventrolateral medulla (RVLM). Therefore decreasing bioavailability of NO may results in inactivation of both the sympathetic and parasympathetic nervous system.[33-34]

Conclusion

From the result of this study, it may be concluded autonomic dysfunction with low parasympathetic activity is related to lower serum folic acid level in MetS patients.

Conflict of interest: None

Ethical clearance

Ethical clearance obtained from Institutional Review Board(IRB) of Bangabandhu Sheikh Mujib Medical University(BSMMU).

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