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# Vitamin $B_{12}$, Folic acid and Homocysteine Levels in female metabolic syndrome patients and their relationship with Heart rate variability.

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## Abstract

**Background:** Metabolic syndrome (MeS) is a well-recognized risk factor for cardiovascular diseases. It is associated with hyperhomocysteinemia resulting from deficiency of vitamin $B_{12}$ and folic acid. Both the MeS and hyperhomocysteinemia adversely affect heart rate variability. **Objectives:** To assess vitamin $B_{12}$, folic acid and homocysteine levels in female metabolic syndrome patients and their relationship with heart rate variability. **Methods:** After taking ethical clearance from Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU) this cross sectional study was conducted on 80 female subjects of 25-45 years of age. Among them 40 metabolic syndrome patients were included in the study group and 40 age matched apparently healthy female constituted control group. All the patients were enrolled from Outpatient Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh according to selection criteria. Serum vitamin $B_{12}$, folic acid, homocysteine level was estimated by chemiluminescent immunoassay and frequency domain parameters of Heart Rate Variability (HRV) was recorded by a data acquisition device, Powerlab 8/35, AD instruments, Australia. Statistical aalysis was done by independent sample 't' test and pearson correlation test.

**Results:** In this study, among frequency domain HRV variables Total power(TP), low frequency power(LF), high frequency power (HF), HF normalized unit(nu) were significantly lower ($p<0.05$) and LF nu, LF/HF were significantly higher ($p<0.05$) in MS patients compared to those of control. In addition, no significant changes ($p>0.05$) were found in serum vitamin $B_{12}$, folic acid and homocysteine levels between these two groups. But on correlation analysis only the folic acid level was significantly negatively correlated ($p<0.05$) with LF and HF power. **Conclusion:** The present study revealed that the folic acid level but not the vitamin $B_{12}$ and homocysteine levels has significant negative correlation with heart rate variability in female MS.

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

## Introduction

Metabolic syndrome (MS) is a cluster of multiple cardiovascular risk factors[1] affecting about 20-25% of global adult population around and in Bangladesh, its overall prevalence is 30% which is higher among female.[2] According to International Diabetes Federation (IDF) definition a person must have central obesity (defined as waist circumference, ≥90 cm for men or ≥80 cm for women of South Asia) plus 2 or more of the following criteria to be diagnosed as having metabolic syndrome – (1) Hyperglycaemia (Fasting plasma glucose ≥100 mg/dl) or previously diagnosed type 2 diabetes, (2) Hypertriglyceridaemia (≥150 mg/dl) or specific 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 treatment for previously diagnosed hypertension.[3] MeS adversely affects all the organ and system of the body but the involvement of cardiovascular system are more pronounced.[4] Multiple metabolic abnormalities related to MeS may increase sympathetic activity may favor the pathogenesis of cardiovascular disease(CVD).[5] Analysis of HRV has been emerged as most reliable and non-invasive method for assessment of cardiac autonomic activity. Reduced HRV as a marker of autonomic imbalance is strongly related to the pathogenesis of cardiac arrhythmia and sudden cardiac death. Among time domain, frequency domain and nonlinear (Poincare plot) methods of HRV analysis[6] Power spectral density in frequency domain method, is most widely used which distinguish the original HRV signal into specific frequency band and reflects the amplitude of the signals of heart rate fluctuation at different frequency ranges. The sympathetic and parasympathetic component of autonomic nervous system operate at individual frequency ranges. So this difference in frequency ranges in frequency domain analysis can differentiate the two component of autonomic nervous system and therefore represents more precise assessment of cardiac autonomic activity.[7] The main frequency domain parameters are Total power(TP) reflects total variability of cardiac autonomic activities, Low frequency power(LF) considered as a marker of sympathetic modulation, High frequency power(HF) a specific index of cardiac parasympathetic activity, Low frequency power in normalized unit (LF norm) and High frequency power in normalized unit(HF norm) represents the relative

contribution of LF power and HF power to total power accordingly, Low frequency and high frequency ratio(LF/HF) is an important marker of sympathovagal balance.[7] Previous studies observed that Metabolic syndrome was associated with significantly reduced HRV.[8,9,10] Over past few decades Hyperhomocysteinemia emerged as a new, independent risk factor for CVD which increase the risk of CVD by two fold especially when present with other cardiovascular risk factor. [11,12] Vitamin $B_{12}$ and Folic acid are essential for the metabolism of homocysteine and also they play a protective role against the development of CVD. Deficiency of these vitamin may results in hyperhomocysteinemia.[13,14] In MS hyperhomocysteinemia can have synergistic effect to accelerate the development of cardiovascular complication.[15] Some researcher reported higher level of homocysteine and/or lower level of vitamin $B_{12}$ and folic acid, whereas some found no significant changes of this parameter in metabolic syndrome.[16,17,18,19] Vitamin $B_{12}$ deficiency was found to be associated with autonomic dysfunction.[20,21] Whereas no association was also observed between homocysteine level and cardiovascular autonomic function.[22] On the other hand, study on MS patients and type 2 diabetic patients showed that hyperhomocysteinemia was negatively correlated with HRV.[23,24] But no published data was found regarding the relationship of vitamin $B_{12}$, folic acid and homocysteine level with heart rate variability in patients with metabolic syndrome. Therefore, the present study has been designed to assess the relationship of vitamin $B_{12}$, folic acid and homocysteine level with heart rate variability in metabolic syndrome.

## Methods

This cross sectional study was conducted in Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from September, 2018 to August, 2019. Protocol was approved by Institutional review board of BSMMU. For this study, after taking informed written consent 40 female metabolic syndrome patients aged 25-45 year who were diagnosed according to International Diabetes Federation (IDF) criteria[3] were enrolled by purposive sampling from Outpatient Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka according to selection criteria. The patients who had history or suffering 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 under 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 other tobacco products were excluded from this study. 40 age and sex matched apparently healthy female subjects were collected as controls through personal contact. Then detailed history was taken and thorough physical examination was done and documented in a preformed standard data sheet. In all person waist circumference (WC) was measured and body mass index (BMI) was calculated from their measured weight and height for detection of obesity, pulse and blood pressure were measured to assess their baseline cardiovascular status. Then 6ml of venous blood was collected for estimation of 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] in Department of Biochemistry and Molecular Biology, BSMMU, Dhaka. 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 requested to take complete bed rest in supine position for 10-15 minutes in a 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 prohibited. 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 comparable between two groups but BMI and WC, Resting pulse rate, systolic and diastolic blood pressure were significantly higher ($p<0.01$) in metabolic syndrome patients compared to healthy subjects. In this study, significantly lower values of total power, LF power, HF power norm and higher value of LF nu, LF/HF ratio were observed in metabolic syndrome patients compared to healthy control ($p<0.01$) (Table II). In addition, no significant ($p>0.05$) changes 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 LF power and HF power in metabolic syndrome patients (Table IV, V, VI).

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

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; ns- non significant ($p>0.05$); $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; n= number of subject.

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

| Variables | MS (n=40) | Control (n=40) | p value |
|---|---|---|---|
| Total Power ($ms^2$) | 801.22±231.10 (275-3084.00) | 1762.17±509.67 (575-6901.00) | 0.000*** |
| LF power ($ms^2$) | 239.82±93.26 (125-972.00) | 579.60±173.57 (215-3072.00) | 0.000*** |
| HF power ($ms^2$) | 137.69±71.46 (85-864.00) | 587.59±128.68 (336-1902.00) | 0.000*** |
| LF norm (n.u.) | 66.56±11.63 (49.90-89.93) | 52.92±10.30 (29.95-71.32) | 0.000*** |
| HF norm (n.u.) | 31.34±10.79 (10.60-48.28) | 46.20±9.17 (32.68-69.79) | 0.000*** |
| LF/HF ratio | 2.68±1.84 (1.06-8.49) | 1.23±0.46 (0.43-2.18) | 0.000*** |

Data were expressed as Mean ± SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MS-Metabolic syndrome; TP- Total power; LF power- Low frequency power; HF power- High frequency power; LF norm- Low frequency power in normalized unit; HF norm- High frequency power in normalized unit; LH/HF ratio- Low frequency & High frequency power ratio; ($p > 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n= number of subject.

**Table III: S**erum 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($p > 0.05$); n= number of subject.

**Table IV:** Correlations of frequency domain HRV measures with serum $B_{12}$ level in two groups (N=80)

| | MS (n = 40) | | Control (n = 40) | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Total Power (ms²) | -0.188 | 0.244$^{ns}$ | -0.291 | 0.068 |
| LF power (ms²) | -0.158 | 0.330$^{ns}$ | -0.177 | 0.275 |
| HF power (ms²) | -0.160 | 0.324$^{ns}$ | -0.152 | 0.348 |
| LF norm(n.u.) | 0.106 | 0.517$^{ns}$ | -0.050 | 0.760 |
| HF norm(n.u.) | -0.179 | 0.269$^{ns}$ | 0.046 | 0.778 |
| LF/HF ratio | 0.135 | 0.405$^{ns}$ | -0.070 | 0.670 |

Statistical analysis was done by Pearson's correlation (r) test. MS- Metabolic syndrome; TP- Total power; LF power- Low frequency power; HF power- High frequency power; LF norm- Low frequency power in normalized unit; HF norm- High frequency power in normalized unit; LH/HF ratio- Low frequency & High frequency power ratio; ($p > 0.05$); $^{*}p < 0.05$; n = number of subjects.

**Table V:** Correlations of frequency domain HRV measures with serum folic acid level in two groups (N=80)

| | MS (n = 40) | | Control (n = 40) | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Total Power (ms²) | -0.308 | 0.053$^{ns}$ | -0.150 | 0.355 |
| LF power (ms²) | -0.337 | 0.033$^{*}$ | -0.245 | 0.128 |
| HF power (ms²) | -0.320 | 0.044$^{*}$ | -0.256 | 0.107 |
| LF norm(n.u.) | 0.238 | 0.140$^{ns}$ | -0.042 | 0.795 |
| HF norm(n.u.) | -0.282 | 0.078$^{ns}$ | -0.013 | 0.936 |
| LF/HF ratio | 0.175 | 0.281$^{ns}$ | -0.058 | 0.724 |

Statistical analysis was done by Pearson's correlation (r) test. MS- Metabolic syndrome; TP- Total power; LF power- Low frequency power; HF power- High frequency power; LF norm- Low frequency power in normalized unit; HF norm- High frequency power in normalized unit; LH/HF ratio- Low frequency & High frequency power ratio; ($p > 0.05$); $^{*}p < 0.05$; n = number of subjects.

**Table VI:** Correlations of frequency domain HRV measures with serum homocysteine level in two groups (N=80)

| | MS (n = 40) | | Control (n = 40) | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Total Power (ms²) | -0.035 | 0.829$^{ns}$ | 0.308 | 0.053 |
| LF power (ms²) | 0.154 | 0.343$^{ns}$ | 0.368 | 0.020$^{*}$ |
| HF power (ms²) | 0.001 | 0.997$^{ns}$ | 0.390 | 0.013$^{*}$ |
| LF norm(n.u.) | -0.159 | 0.327$^{ns}$ | -0.156 | 0.337 |
| HF norm(n.u.) | 0.199 | 0.218$^{ns}$ | 0.215 | 0.183 |
| LF/HF ratio | -0.155 | 0.340$^{ns}$ | -0.217 | 0.179 |

Statistical analysis was done by Pearson's correlation coefficient (r) test. MS- Metabolic syndrome; TP- Total power; LF power- Low frequency power; HF power- High frequency power; LF norm- Low frequency power in normalized unit; HF norm- High frequency power in normalized unit; LH/HF ratio- Low frequency & High frequency power ratio; ($p > 0.05$); $^{*}p < 0.05$; n = number of subjects.

MS – Metabolic syndrome patients. LF power – Low frequency power, $p<0.05$; ns statistically non significant ($p>0.05$); n=number of subjects.

MS Metabolic syndrome patients. HF power- High frequency power; $^{*}p<0.05$; ns - statistically nonsignificant ($p > 0.05$); n=number of subjects.

## Discussion

The present study investigated the relationship of serum vitamin $B_{12}$, folic acid and homocysteine level with HRV in metabolic syndrome patients aged 25-45 years. Almost similar age range[29] and higher BMI[8,10,29] in MS were reported by different researcher. In present study significantly higher resting pulse rate, SBP and DBP were observed which agree to others in respect of SBP and DBP.[4,8,10,29] Among frequency domain HRV variables significantly lower value of TP, LF power, HF power and higher value of LF nu, LF/HF ratio in MS were consistent with the findings of some researcher[4,8,30] which suggests autonomic function impairment with sympathetic dominance and decreased parasympathetic modulation. The current study revealed that serum vitamin $B_{12}$ and folic acid were lower and serum homocysteine level was higher in MS patients but these were statistically nonsignificant. The results of different study supported this findings.[18,19,31] On correlation analysis among HRV measures and serum vitamin $B_{12}$, folic acid and homocysteine, only folic acid showed significant negative correlation with LF and HF power which suggests that decreasing level of folic acid is related with increased cardiac sympathetic and parasympathetic activity though no previous study was available to compare this findings. Lower level of folic acid resulting increased superoxide and decreased nitric oxide (NO) production.[32] This NO tonically inhibit the firing activity of paraventricular nucleus of hypothalamus that ultimately inhibit the autonomic outflow from the dorsal vegal complex (DVC) and rostral ventrolateral medulla (RVLM). Thereby decreasing bioavailability of NO may results in activation of both the sympathetic and parasympathetic nervous system.[33,34]

Obesity and insulin resistance which are the core features of metabolic syndrome also responsible for autonomic function impairment. High plasma level of insulin in insulin resistant state causes sympathoexcitation by decreasing the bioavailability of NO in RVLM whereas increased leptin level in central obesity account for sympathetic activation by acting centrally in nucleus tractus solitarius and hypothalamic arcuate nucleus.[35]

## Conclusion

Results of this study concluded that there is inverse relationship between folic acid level and heart rate variability in female metabolic syndrome.

**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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