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# Relationship between cardiac autonomic dysfunction and iron status in metabolic syndrome

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## Abstract

**Background:** Metabolic syndrome (MetS) is one of the major public health challenge with potential risk for cardiac autonomic dysfunction. Iron overload in MetS further increase the risk of cardiovascular morbidity. **Objectives:** To observe the relationship between cardiac autonomic nerve function and iron status in MetS patients. **Methods:** This cross-sectional study was conducted on 35 MetS female patients aged 25 to 45 years and equal number of age and sex matched apparently healthy subjects constituted comparison group. Cardiac autonomic nerve function was assessed by analyzing frequency domain parameters of Heart Rate Variability (HRV). HRV was recorded and analyzed by a data acquisition device, Powerlab 8/35, AD instruments, Australia. For evaluation of iron status, serum iron, serum ferritin, total iron binding capacity (TIBC) and transferrin saturation (Tsat) were measured. Data were expressed as mean±SD. Statistical analysis was done by Independent sample 't' test and Pearson's correlation coefficient test as applicable. **Results:** In this study, resting pulse rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), low frequency (LF) norm and LF/HF(high frequency) were significantly higher ($p \leq 0.001$) and total power, LF power, HF power, HF norm were significantly lower ($p \leq 0.001$) in MetS patients compared to healthy subjects. Among the parameters of iron status, serum ferritin was significantly higher ($p \leq 0.05$) and serum TIBC was significantly lower ($p \leq 0.05$) in MetS patients than healthy subjects. On correlation analysis, only the serum TIBC showed significant ($p \leq 0.05$) positive correlation with total power, LF power, HF power, HF norm in MetS patients. **Conclusion:** This study concludes that cardiac autonomic dysfunction may be related to higher iron status in MetS.

**Keywords:** Metabolic syndrome, iron status, heart rate variability.

## Introduction

Over last few decades, MetS has become a major public health concern because of its association with higher risk of cardiovascular morbidity and mortality. According to the new International Diabetes Federation (IDF) definition, MetS must have central obesity and any two of following- raised triglyceride, reduced HDL cholesterol, hypertension, raised fasting plasma glucose These patients have additional twofold risk of developing cardiovascular disease.[1]

Cardiac autonomic dysfunction is an early and important feature of cardiovascular disorders and is significantly related with higher risk of cardiovascular mortality[2,3]. HRVis a measure of variation of instantaneous heart rate or normal to normal interval (N-N interval or RR interval)[4]. As variation in heart rate is influenced by ANS (both sympathetic and parasympathetic nervous system), analysis of HRV provides a measure of outlook into ANS imbalance[4,5].

High HRV resembles optimum cardiac autonomic activity. Whereas low HRV, a marker of cardiac autonomic neuropathy (CAN),indicated increased sympathetic and reduced para-sympathetic activity[4,6] reported in MetS.[5,7-,9]

Frequency domain is one of the most widely used analytical method for analyzing HRV. It reflects the amount of heart rate variation at any specific frequency[4]. In a short term HRV recording of 5 minutes, the main frequency domain parameters are –total power (≤0·4 Hz), Low frequency power (LF power 0.04-0.15 Hz), Low frequency power in normalized unit (LF norm),High frequency power (HF power- 0.15-0.4 Hz), High frequency power in normalized unit (HF norm) andLF/HFratio.[4]

Total power(TP) reflects the periodic variation ofcardiac autonomic function. Relative contribution of each of the LF power and HF power to total power is reflected by LF normand HF norm respectively. Here, LF norm acts as a sympathetic marker solely, whereas, LF power has both sympathetic and parasympathetic contributions[4]. In recent context, LF power more likely reflects vagally mediated baroreflex activity than to be a sole sympathetic indicator[10]. Parasympathetic division is corresponded by HF power and HF norm. LF/HF ratio is used as a measure of sympatho-vagal balance.[4]

Among the microminerals Iron is crucial for$O_2$transport process, ATP biosynthesis, immunity and many other essential metabolic processes of body. Body iron status can be measured by some parameters including serum iron, serum ferritin, total iron binding capacity (TIBC) and transferrin saturation.[12]

Previous studies reported both relatively higher and lower iron status in MetS.[13-15] Moreover, Iron overload was found to be involved in pathogenesis of MetS via insulin resistance.[16]

Research evidence confirmed that iron overload can be responsible for cardiac autonomic dysfunction via producing reactive oxygen species-ROS (Fenton and Haber-Weiss reaction) in several diseases like thalassemia, hemochromatosis.[17-18] Yet no relationship linking these two has been confirmed in MetS. The present study was undertaken to see the relationship between cardiac autonomic dysfunction and iron overload. The result of this study may play rolein reducing cardiovascular morbidity in this mass population.

## Method

*Design and setting*

This cross-sectional study was conducted in Department of Physiology, BSMMU, Shahbag, Dhaka from March, 2019 to February, 2020.

*Study participants*

Thirty five (35) female diagnosed patients with MetS were selected from the Department of

Endocrinology, BSMMU and for comparison group, equal number of age and sex matched apparently healthy subjects were selected.

*Sampling*

Al the subjects of MetS group and comparison group were selected by purposive sampling.

*Inclusion criteria*

After diagnosis as MetS following IDF criteria,[1] 35 female patients of 25-45 year were selected from Department of Endocrinology, BSMMU. To minimize hormonal impact, all subjects of both groups were studied during their proliferative phase of menstrual cycle.

*Exclusion criteria*

Subjects having cardiac, respiratory, renal, thyroid disorders, any acute or chronic inflammation, menstrual abnormality, known case of hemochromatosis, thalassemia, women having history of blood transfusion within last 3 months, having history of recent major surgery or illness, taking hormonal contraceptives or iron fortified vitamin, pregnant, lactating mother, women after menopause were kept out from this study.

*Data collection*

After getting informed written consent,detail relevant history (family, menstrual, medical and dietary history) was taken and each subject underwent a full scale physical examination including measurement of resting pulse rate, blood pressure and waist circumference (WC). Weight and heightwere measured for calculation of BMI. Then, 9 ml of venous blood was collected for estimation of fasting plasma glucose(GLUC method), lipid profile (enzymatic method), serum SGPT (Activated ALT Assay method) and serumcreatinine(Automated Picric Acid Assay method), Serum iron (spectrophotometric method), serum ferritin (chemiluminescent microparticle immunoassay method), serum TIBC (spectrophotometric method) in the laboratory of Department of Biochemistry and Molecular Biology andcomplete blood count (CBC) (fluorescence method) in Department of Laboratory Medicine, BSMMU, Dhaka. Serum transferrin saturation (Tsat) was calculated from serum iron and TIBC.

Subjects were advised to finish their meal by 9:00 pm and to have a sound sleep without any sedative hypnotic medication on previous night of HRV recording. On HRV recording day, after having light breakfast without any caffeine drink,they were requested to attend the autonomic nerve function laboratory in the Department of Physiology, BSMMU, between 8:00am to 9:00am. Thencomplete bed rest for 15-20 minutes was ensured. ECG was recorded in lead II for 5 minutes by data acquisition device Power Lab 8/35 (AD Instrument, Australia). Lab chart software was used for analysis of RR interval of HRV. Talking, eating, drinking, any type of physical or mental activity or sleep were disallowed during the recording period.

*Statistical analysis*

Data were expressed as Mean ± SD. Statistical analysis was done using SPSS version 16.Independent sample 't' test and Pearson's correlation coefficient test were done, p value of≤0.05 was considered as statistically significant.

## Result

Table I presented general characteristics and cardiovascular variables of all subjects. Age was matched in subjects of both groups, but subjects of MetS group had significantly ($p<0.001$) higher WC, FPG and serum TG but significantly ($p<0.05$) lower serum HDL-C than those of comparison group (Table I). Also, MetS patients had significantly higher resting pulse rate, SBP and DBP ($p<0.001$) compared to healthy subjects (Table I).

**Table I :** General characteristics in two groups (N=70)

| Variables | MetS (n=35) | Comparison (n=35) | p value |
|---|---|---|---|
| Age(years) | 37.89±6.07(25-45) | 35.34±5.94(25-45) | 0.081 |
| Anthropometric marker | | | |
| WC(cm) | 95.51±5.97(83 -113) | 75.60±3.09(64-79) | 0.000 |
| Metabolic markers | | | |
| FPG(mmol/L) | 8.70±3.45(5.0-16.9) | 5.01±0.35(4.40-5.60) | 0.000 |
| HDL-C(mg/dL) | 39.26±7.04(23-56) | 44.34±10.14(31-68) | 0.017 |
| TG(mg/dL) | 232.89±108.47(98-466) | 95.74±25.95(30-145) | 0.000 |
| Cardiovascular variables | | | |
| Resting pulse rate(beats/min) | 87.40±8.69(65.00-104.00) | 73.97±7.14(60.00-88.00) | 0.000 |
| SBP(mm of Hg) | 132.00±13.02(110.00-160.00) | 116.43±6.92(100.00-125.00) | 0.000 |
| DBP(mm of Hg) | 85.00±9.39(70.00-100.00) | 73.14±6.07(60.00-80.00) | 0.000 |

Data were expressed as mean±SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MetS- Metabolic syndrome; WC- Waist circumference; FPG- Fasting plasma glucose; HDL-C- High density lipoprotein cholesterol; TG- Triglyceride; SBP- Systolic blood pressure; DBP- Diastolic blood pressure; N-Total number of subjects; n- Number of subjects in each group.

Analyzing the frequency domain parameters of HRV shows, the mean values of TP, LF power, HF power and HF norm were significantly lower ($p \leq 0.001$ for all) whereas LF norm and LF/HF ratio were significantly higher in MetS subjects ($p \leq 0.001$) than that of healthy subjects (Table II).

**Table II:** Frequency domain measures of HRV in two groups (N=70)

| Variables | MetS (n=35) | Comparison (n=35) | p value |
|---|---|---|---|
| Total Power($ms^2$) | 491.29±516.05 (12.72-2522.00) | 2459.9±1907.97 (121.80-7586.00) | 0.000 |
| LF power($ms^2$) | 136.18±175.84 (1.66-854.60) | 671.47±673.43 (33.31-3072.00) | 0.000 |
| HF power($ms^2$) | 112.52±213.85 (1.82-1138.00) | 896.16±809.14 (18.67-3360.00) | 0.000 |
| LF norm(n.u.) | 62.82±15.69 (39.64-84.34) | 42.57±10.75 (21.34-62.78) | 0.000 |
| HF norm(n.u.) | 36.28±14.41 (16.44-58.63) | 55.04±10.89 (33.42-76.73) | 0.000 |
| LF/HF | 2.23±1.37 (0.68-5.08) | 0.83±0.41 (0.05-1.78) | 0.000 |

Data were expressed as mean±SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MetS-Metabolic syndrome; 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- Low frequency and high frequency power ratio; ; n- Number of subjects in each group; N- Total number of subjects.

Analysis of iron status revealed, MetS group has significantly ($p<0.05$) higher serum ferritin and significantly ($p<0.05$) lower TIBC than that of comparison group (Table III). But on correlation analysis of MetS group, only TIBC had significant correlation with total power ($p<0.05$), LF power ($p<0.01$), HF power ($p<0.05$), HF norm ($p<0.05$) (Figure 1-4). There was no significant relationship between all frequency domain measures and serum iron, serum ferritin and also Tsat..

**Table III:** Serum iron, ferritin, TIBC, transferrin saturation in two groups (N=70)

| Variables | MetS (n=35) | Comparison (n=35) | p value |
|---|---|---|---|
| Iron(µg/dL) | 61.83±28.40 (25-144) | .49±20.59 68(29-110) | 0.266 |
| Ferritin (ng/mL) | 122.57±143.47 (7.60-526.90) | 60.55±40.82 (5.53-172.33) | 0.017 |
| TIBC(µg/dL) | 286.63±99.75 (102-494) | 325.77±49.65 (215-407) | 0.041 |
| Tsat(%) | 24.43±15.41 (5.47-69.23) | 21.65±7.22 (7.80-40.74) | 0.337 |

Data were expressed as mean±SD. Values in parentheses indicate ranges; Statistical analyses were done by Independent sample 't' test; MetS- Metabolic syndrome; TIBC- Total iron binding capacity; Tsat- Transferrin saturation; ; n- Number of subjects in each group; N- Total number of subjects.

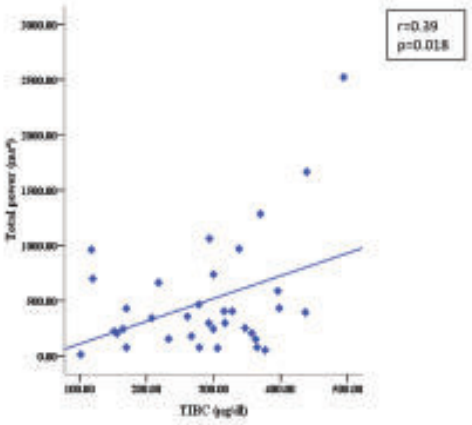


**Figure 1:** Correlation of total power (ms²) with serum TIBC level (µg/dl) in group A (n=35). MetS- Metabolic syndrome patients; TIBC- Totaliron binding capacity; *p≤0.05.

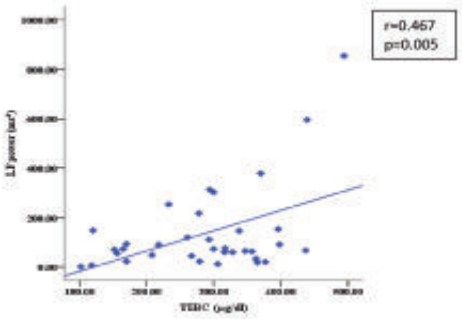


**Figure 2**: Correlation of LF power (ms²) with serum TIBC level (µg/dl) in group A (n=35). MetS- Metabolic syndrome patients; LF power- Low frequency power; TIBC- Total iron binding capacity; ** p≤0.01

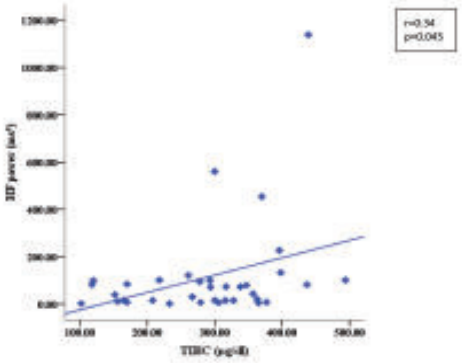


**Figure 3:** Correlation of HF power (ms²) with serum TIBC level (µg/dl) in group A (n=35). MetS- Metabolic syndrome patients; HF power- High frequency power; TIBC- Totaliron binding capacity;

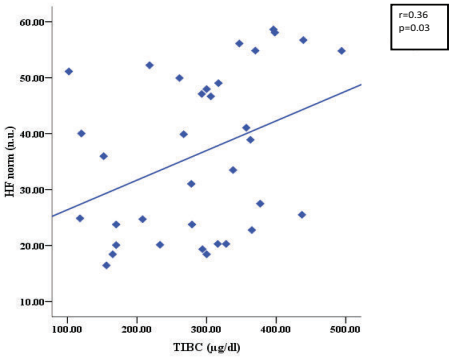


**Figure 4:** Correlation of HF norm (n.u.) with serum TIBC level (µg/dl) in group A (n=35). MetS- Metabolic syndrome patients; HF norm- High frequency power in normalized unit; TIBC- Totaliron binding capacity;

## Discussion

In this study, MetS subjects had significantly higher resting pulse rate, SBP and DBP compared to healthy subjects. This finding is supportive of autonomic impairment in MetS and in accordance with that of other studies.[2,5,8]

Besides, in MetS, significantly lower values of total power and LF power compared to healthy individuals act as evidence of autonomic impairment or low HRV. Also, significantlyhigher LF norm, LF/HF and lower HF power, HF norm in Mets subjects than comparison group indicatehigher sympathetic and lower parasympathetic activityrespectively in MetS subjects. These findings were similar to some other studies.[5,7] Though reverse findings of HF norm and LF/HF in same group of patients were reported in a study.[19]

Our study found significantly higher serum ferritin and lower TIBC indicating higher iron store in MetS compared to healthy individuals. Finding of serum ferritin was similar to some other studies[13,14]whereas some authors reported higherTIBC in MetS.[14] Positive correlation was found between some frequency domain parameters and TIBC and it suggests that higher iron status (lower TIBC) may have relationship with low HRV and reduced parasympathetic cardiac activity in MetS subjects.

Obesity contributes to all the metabolic irregularities found in MetS[20] and also abnormal metabolic process of ironvia releasing various inflammatory mediators such as TNF-á, IL-1, IL-6, leptin etc.[20] These mediators in turn cause excessive hepcidin secretion and reduced hephaestin-ferroxidase activity.[21] Both of them lead to poor iron export into blood and cause higher cellular iron storage as ferritin.[21,22] Due to negative feedback effect of higher ferritin, TIBC is reduced.[23]Ininsulin resistant state of MetS, reduced synthesis and excessive breakdown of transferrin occurs, which may also be responsible for decreased TIBC.[24]

Free iron takes part in Fenton and Haber-Weiss reaction and generate ROS[25]. Transferrin acts as an anti-oxidant which keeps redox-active free iron in check. Reduced transferrin leads to more free iron, which causes excessive ROS production. This, in turn, results reduced nitric oxide (NO) bioavailability.[26]

NO aids cardiac parasympathetic activity both pre-synaptically[27,28]and post-synaptically.[27] It also plays a role in cardio-sympathoinhibition via increasing GABA secretion at RVLM[26], decreasing norepinephrine (NE) release and its action.[27-28] Thus, reduced NO may be the key mechanism by which higher iron store/ status may lead to cardiac autonomic impairmentin MetS.

## Conclusion

Based on this study result, it can be concluded that cardiac autonomic dysfunction with reduced parasympathetic tone is related to higher iron status in metabolic syndrome.

## Conflict of interest

Authors declare no conflict of interest

## Ethical aspects

The ethical aspects were accepted by Institutional Review Board of BSMMU .

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