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Cardiac autonomic impairment and low serum vitamin B12 and high homocysteine level in Parkinson's disease -Frequency domain analysis of heart rate variability

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Abstract

Background: Parkinson's disease (PD)adversely affects autonomic control as well as alteration of serum vitamin B12 and homocysteine level. Heart rate variability (HRV) is a reliablemethod for assessing autonomic function.**Objective:**To investigate the role of serum vitamin B12 and homocysteine level for cardiac autonomic impairmennt by analyzing frequency domain measures of HRV in male patients with PD. **Methods:** This observational analytical crosssectional study was involved 30 newly diagnosed male PD patients of 50-60 years age visiting the Out Patient Department of Neurology, Bangabandhu Sheikh MujibMedical University (BSMMU), Dhaka. Thirty age and BMI matched apparently healthy male subjects were included as control. Serum vitamin B12 and homocysteine were determined by chemiluminescent immunoassay method and HRV were measured by using a data acquisition device, Powerlab 8/35, AD instruments with labchart software analysis.. Independent sample t-test and Pearson's correlation coefficient test were used for statistical analysis. **Results:** In this study, resting pulse rate,low frequency power in normalized unit (LF norm), ratio of low frequency and high frequency power (LF/HF) were found significantly higher ($p<0.001$) and total power (TP), low frequency power (LF power),high frequency power (HF power), HF power in normalized unit (HF norm), were found significantly lower ($p<0.001$) in PD patients compared to control. In addition, serum vitamin B12 level was found significantly lower ($p<0.01$) and homocysteine level was found significantly

higher ($p<0.001$) in PD patients than that of control. On correlation analysis,serum vitamin B12 was significantly negatively correlated with LF/HF ($p<0.05$). Besides, serum homocysteine was significantly negatively correlated with HF power($p<0.01$), HF norm ($p<0.001$) and significantly positively correlated with LF norm ($p<0.001$), LF/HF ($p<0.001$). **Conclusion:**Low serum vitamin B12 and high serum homocysteine level are associated with reduced HRV in male patients with PD.

Keywords: Parkinson's disease, Heart rate variability, VitaminB12, Homocysteine.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative type movement disorderwith male predominance.[1] Prevalence of this diseaseincreases with advancing age.[2] Besides motor dysfunction non motor features has been increased exponentially in PD from last 3 decades.[3]In PD degeneration of dopamineregic neuron with deposition of abnormal proteinaceous spherical bodies as Lewy bodies occur in basal ganglia and alsoother areas of brain.[1-2] This pathological involvement of autonomic nervous system(ANS)leading to autonomic dysfunctionwhich is the commonest non-motor features of PD.[4,5]Both sympathetic and parasympathetic nervous systemare affected in PD[5]butvagal impairmentis more prominent in PD.[4,6]To assess functional status of ANS heart rate variability (HRV)is considered one of the most reliable tools.[7]Reduced HRV is a marker of cardiac autonomic dysfunction whereas increased HRV is an indicator of improved health profile.[7-9] Among different methods used for analysis of HRV[7] frequency domain method is the most precise method in which power of an original HRV signal is distributed as power of different frequency bands and reflects amplitude of power against different frequency ranges.[7] Two branches of autonomic nervous system that is sympathetic and parasympathetic operating at individual frequency ranges could be analysed through this method.[10]Commonfrequency domain measures includetotal power (TP), low frequency power (LF power),low frequency power in normalized unit (LF norm),high frequency power (HF power), HF power in normalized unit (HF norm), ratio of low frequencyand high frequency power (LF/HF).[7-8]TPreflects total variability,LF power, LF norm reflect sympathetic activity whereas HF power, HF norm reflectparasympathetic activity and LF/HF represents sympatho-vagal balance.[7]Besides autonomic dysfunctionPD is also associatedwith higher level of homocysteine with or without lower level of vitamin B12which play a conducive role in further progression of this disease itself also.[11-16] In several studiessome researchers found association of autonomic dysfunction with altered levels of vitamin B12and homocysteine[17-18] whereas some found no such association in this parameters.[19]As no published data is found regarding to observe the association of these biochemical variables with HRV in patients with PD. Therefore, the present study has been designed to assess the association of serum vitamin B12 and homocysteine level with frequency domain measures of HRV in PD.

Methods

Study design & setting

This was an observational analytical cross sectional study which was done during 2023 in the Department of Physiology, BSMMU, Dhaka.

Study participants & sampling

According to selection criteria 30 newly diagnosed male patients with PD (50-60 years &BMI:18-24.9kg/m^2)upto stage III (Hoehn & Yahrscale)visiting the Out Patient Department of Neurology, BSMMUwere enrolled purposively. Also 30 age and BMI matched apparently healthy male subjects were enrolled as control.

Exclusion criteria

Patients with history of cardiovascular disorders, respiratory disorders, renal insufficiency, liver disease, arthritis, neurological disorders (migraine, epilepsy, stroke), thyroid disorders, psychiatric disorders, malignancy, gastric surgery, vegan diet, medications for cardiac, respiratory or other reasons which may interfere with autonomic nervous system balance or current use of vitamin supplementation, current smokers and consumption of alcohol or any substance abuse were excluded from this study.

Data collection procedure

After selection of eligible participants by history taking, physical examination and screening tests baseline characteristics were recorded.Then finally selected subjects were requestedproperly to prepare forrecording of HRV.They were instructed to finish their meal by 9:00 pm and to have a sound sleep in the previous night as well as to avoid any physical or mental stress, sedatives, hypnotics medication. Theywere also advised to take light breakfast in the morning without tea or coffee and then attend to the Noorzahan Begum Neurophysiology Laboratory, Department of Physiology, BSMMUbetween 8-9 a.m. After taking rest for 15-20 minutes frequency domain measures of HRV were recorded in a noise free and comfortable laboratory environment in supine position by a data acquisition device, PowerLab 8/35, AD instruments, Australia. During recording of HRVany talking, eating or drinking as well as performing physical or mental activity even sleep were strictly prohibited.Finally 02 ml of venous blood was collected and immediately sent to the laboratory of the Department of Biochemistry and Molecular Biology, BSMMU for the estimation of serum vitamin B12 and homocysteine levels.

Statistical analysis

All quantitative data were expressed as mean ± SD. For statistical analysis independent sample t-test, Chi-square test and Pearson's correlation coefficient test were done by using SPSS version 25 and p value<0.05 wasconsidered as statistical significance.

Results

Both PD patients and control were found age and BMI matched (p>0.05) in Table Ibut the mean value of resting pulse ratewas found significantly higher (p<0.01) in PD patients than that of control. In this study,LF norm, LF/HFwere found significantly higher (p<0.001) and TP, LF power, HF power, HF normwere found significantly lower (p<0.001) in PD patients compared to control (Table II). In addition, serum vitamin B12 level was found significantly lower (p<0.01) and homocysteine level was found significantly higher (p<0.001) in PD patients than that of control (Table III). Comparison of frequency domain measures of HRV betweenPD patients with normal and low levels of serum vitamin B12 significantlyhigher values of LF/HF, LF norm (p<0.01, p<0.05 respectively)and lower value of HF norm were observed (p<0.05)(Table IV)in PD patients with low level than that of normal level whereasSDSDand RMSSDwere found significantly lower(p<0.05) in PD patients with high homocysteine level than that of PD patients with normal homocysteine level(TableV). On correlation analysis, only LF/HF was significantly negatively correlated (p<0.05)with serum vitamin B12 (Figure1) and HF power, HF norm weresignificantly negatively correlated (p<0.01, p<0.001 respectively)(Figure2,3 respectively)and LF norm, LF/HF were significantly positively correlated (p<0.001)with serum homocysteine (Figure 4,5 respectively).

Table I: Age, BMI, resting pulse rate and blood pressure in two groups (N=60)

Variables	PD (n=30)	Control (n=30)	p value
Age(Years)	55.37±3.24 (50-60)	54.30±3.12 (50-60)	0.199
BMI(Kg/m^2)	22.52±1.21 (20.48-24.62)	22.92±0.79 (21.45-24.56)	0.131
Pulse rate (beats/min)	81.27±3.95 (70-90)	78.67±5.64 (68-88)	0.001
SBP(mm Hg)	128.17±5.17 (120-135)	128.33±5.14 (120-135)	0.901
DBP(mmHg)	78.83±4.29 (70-85)	79.00±3.57 (70-85)	0.871

Data were expressed as mean ± SD. Values in parentheses indicate ranges. Statistical analysis was done by Independent sample t-test. BMI- Body Mass Index; SBP-systolic blood pressure; DBP- diastolic blood pressure; PD-Parkinson's disease;Control-Apparently healthysubjects; N- Total number of subjects; n- Number of subjects in each group.

Table II: Frequency domain measures of HRV in two groups (N=60)

Variables	Group-A (PD) (n=30)	Group-B (Control) (n=30)
Total Power (ms^2)	589.44±285.57*** (176.7-1149)	1521.23±320.58 (923-2274)
LF power (ms^2)	187.28±98.76*** (60.56-427.4)	499.74±150.95 (218.8-755.9)
HF power (ms^2)	87.59±50.10*** (15.44-211.4)	539.67±182.24 (235.3-865.3)
LF norm (n.u.)	67.73±10.83*** (44.85-84.17)	47.68±10.64 (26.11-66.11)
HF norm	31.93±10.60*** (16.15-54.41)	50.61±10.10 (33.23-72.95)
LF/HF	2.48±1.18*** (0.82-5.21)	1.02±0.42 (0.36-1.99)

Data were expressed as mean ± SD. Values in parentheses indicate ranges. Statistical analysis was done by independent sample t-test. 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 of low frequency power and high frequency power; Group-A/ Study group- Male patients with Parkinson's disease; Group B/Control -Apparently healthy male subjects;N- Total number of subjects;n- Number of subjects in each group.*This depicts comparison with control group;***p<0.001.

Table III: Serum Vitamin B12 and Homocysteine level in two groups (N=60)

Variables	PD (n=30)	Control (n=30)	p value
Vitamin B12 (Pg/ml)	423.10±201.97	548.13±181.70	0.014
	(109-830)	(217-931)	
Homocysteine (µmol/L)	16.76±3.98	7.43±1.69	0.000
	(9.85-24.94)	(3.77-9.85)	

Data were expressed as mean ± SD. Values in parentheses indicate ranges. Statistical analysis was done by Independent sample t-test. PD-Parkinson's disease;Control-Apparently healthy subjects; N=number of subjects; n=Number of subjects in each group.

Table IV: Frequency domain measures of HRV in PD on basis of serum vitamin B12 (N=30)

Variables	Group-A1a (low B12) (n=05)	Group-A1b (normal B12) (n=25)
Total Power (ms^2)	542.08±377.88	598.91±272.36
	(219.10-1149)	(176.7-1124)
LF power (ms^2)	180.67±82.95	188.60±103.09
	(67.46-265.3)	(60.56-427.4)
HF power (ms^2)	52.19±26.25	94.67±51.04
	(15.44-80.90)	(24.05-211.40)
LF norm (n.u.)	77.89±6.65*	65.70±10.42
	(67.27-84.17)	(44.85-82.67)
HF norm (n.u.)	22.25±6.28*	33.87±10.29
	(16.15-32.17)	(18.26-54.41)
LF/HF	3.77±1.20**	2.22±1.02
	(2.10-5.21)	(0.82-4.53)

Data were expressed as mean ± SD. Values in parentheses indicate ranges. Statistical analysis was done by independent samplet-test. 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 of low frequency and high frequency power;N- Total number of subjects; n- Number of subjects in each group.*This depicts comparison with normal B12 group of PD; *$p<0.05$;**$p<0.01$.

Table V: Frequency domain measures of HRV in PD on basis of serum homocysteine (N=30)

Variables	Group-A2c (high homocysteine) (n=18)	Group-A2b (normal homocysteine) (n=12)
Total Power	534.95±255.99	671.16±318.69
(ms²)	(219.10-975.60)	(176.7-1149.0)
LF power	196.31±114.14	173.74±72.42
(ms²)	(60.56-427.4)	(64.43-265.3)
HF power	65.64±34.18**	120.53±53.30
(ms²)	(15.44-127.6)	(51.59-211.4)
LF norm	73.82±6.49***	58.60±9.63
(n.u.)	(62.22-84.17)	(44.85-80.73)
HF norm	25.77±5.84***	41.18±9.40
(n.u.)	(16.15-35.21)	(20-54.41)
LF/HF	3.07±0.99***	1.59±0.86
	(1.82-5.21)	(0.82-4.04)

Data were expressed as mean ± SD. Values in parentheses indicate ranges. Statistical analysis was done by independent sample t-test. 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 of low frequency and high-frequency power;N- Total number of subjects; n- Number of subjects in each group. *This depicts comparison with normal homocysteine group of PD;**p<0.01;***p<0.001.

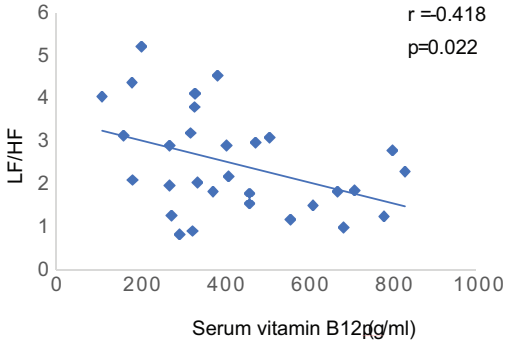

Figure 1: Correlation of LF/HF with serum vitamin B12 level (pg/ml) in study group (N=30)

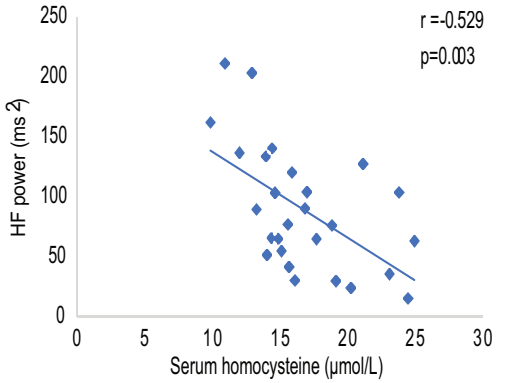

Figure 2: Correlation of HF power (ms^2) with serum homocysteine level (µmol/L) in study group (N=30)

Statistical analysis was done by Pearson's correlation coefficient (r) test. H Study group-Male patients with Parkinson's disease; N- Total number of subjects.

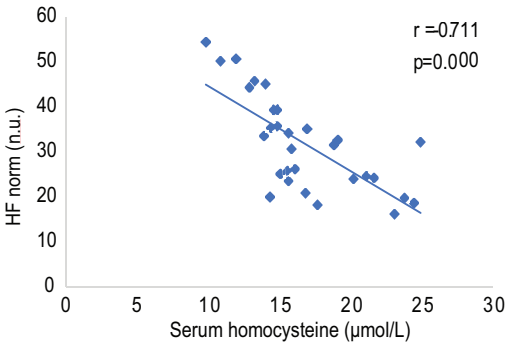

Figure 3: Correlation of HF norm (n.u.) with serum homocysteine level (µmol/L) in study group (N=30)

Statistical analysis was done by Pearson's correlation coefficient (r) test. HF norm- High frequency power in normalized unit; Study group-Male patients with Parkinson's disease; N- Total number of subjects.

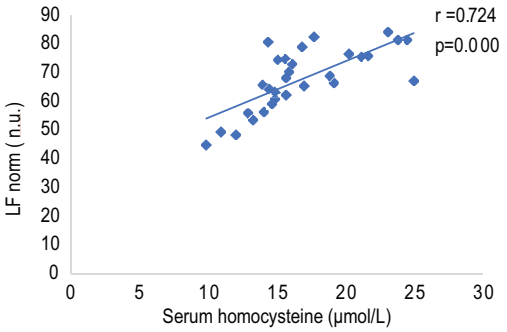

Figure 4: Correlation of LF norm (n.u.) with serum homocysteine level (µmol/L) in study group (N=30)

Statistical analysis was done by Pearson's correlation coefficient (r) test. LF norm- Low frequency power in normalized unit; Study group-Male patients with Parkinson's disease; N- Total number of subjects.

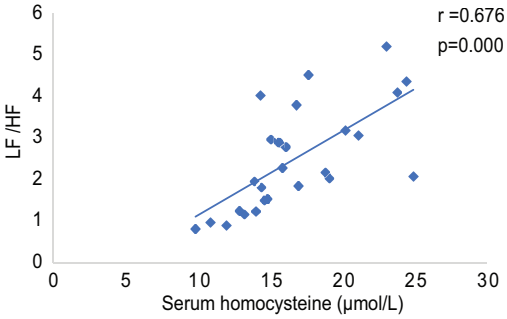

Figure 5: Correlation of LF/HF with serum homocysteine level (µmol/L) in study group (N=30)

Statistical analysis was done by Pearson's correlation coefficient (r) test. LH/HF-Ratio of low frequency and high frequency power; Study group-Male patients with Parkinson's disease; N- Total number of subjects.

Discussion

This study investigated the relationship of altered levels of serum vitamin B12 and homocysteine with frequency domain measures of HRV in 50-60 years of male PD patients. In this study, significantly higher values of resting pulse rate, LF norm, LF/HF and lower values of TP, LF power, HF power, HF norm in PD patientssuggested reduced overall variability, sympathetic hyperactivity as well as parasympathetic hypoactivity which is consistent with the findings of other researchers.[4-6,20-22]In addition, significantly lower value of serum vitamin B12 level as well as higher value of serum homocysteine level in PD patients suggested association of these parameters with PD and also reported bythe results of different studies.[11-16] Besides,frequency domain measures of HRV werealso compared between PD patients with normal and altered levels of serum vitamin B12 and homocysteine in which higher value of LF/HF were observed in PD patients with low serum vitamin B12 level than that of normal suggesting association of low serum vitamin B12 level with sympathetic hyperactivity. Lower values of HF power, HF norm and higher values of LF norm, LF/HF were observed in PD patients with high homocysteine level than that of PD patients with normal homocysteine level suggesting association ofhigh homocysteine with reduced heart rate variability as well as cardiac autonomic dysfunction. On correlation analysis of frequency domain HRV measures with serum vitamin B12 and homocysteine, LF/HF was found negatively correlated with serum vitamin B12 which was statistically significant suggesting that lower level of serum vitamin B12 is associated with sympathetic hyperactivity in PDpatients. Also, HF power, HF normwerefound significantly negatively correlated and LF norm, LF/HF were found significantly positively correlated with serum homocysteine suggesting that higher level of serum homocysteine is associated with parasympathetic hypoactivity as well as sympathetic hyperactivity in PD.Previous evidences also reported the association of low serum vitamin B12 and high serum homocysteinewith PD. In vitamin B12 deficiency homocysteine cannot remethylated back to methionine resulting higher level of serum

homocysteine. Homocysteine undergoes oxidation and produces reactive oxygen species (ROS) as well as decreases nitric oxide (NO) production.[18]NO causes sympathoinhibition via increase release of ã-amino butyric acid (GABA) from presympathetic neuronsin the RVLM which controls the basal central sympathetic outflow. In addition,homocysteine increases production of ROS in RVLM which causes activation of glutamatergic input and inhibition of GABAergic input to the sympathetic promoter neuron in RVLM. GABAergic input tonically inhibits the firing activity of the neurons of RVLM by increasing the release of GABA and ultimately inhibit the sympathetic outflow from the RVLM.Thereby decreasing bioavailability of NO and increase production of ROSin RVLM may results in activation of sympathetic nervous system as well as sympathoexcitation.[23]So, alteration of serum vitamin B12 and serum homocysteine level could be a potent risk factor for cardiac autonomic dysfunction in PD patients.

Conclusion

According to the results of this study, it may be concluded that low serum vitamin B12 level and high serum homocysteine level had significant relationship with reduced heart rate variability in male patients with Parkinson's disease.

Ethical issue

The study protocol was first approved by the departmental ethical and academic committee and then further reviewed and approved by Institutional Review Board(IRB) of BSMMU.

Conflict of interest: None

Acknowledgments

Department of Neurology, BSMMU and Department of Biochemistry and Molecular Biology, BSMMU.

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