Original Article

Cardiac Sympatho-vagal Activity and Cognitive Status of Individuals with Hemophilia – A Pilot Study

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Abstract

Background and Aim: Cardiac autonomic imbalance and cognitive deficits are known to exist in chronic hematological disorders. This pilot study was done to assess and compare the cardiac autonomic activity and cognitive status of patients suffering from hemophilia with age matched healthy male volunteers.

Methods: Fourteen males (18-45 years) suffering from Hemophilia and fourteen age-matched healthy male volunteers were included in test and control groups respectively. Mann-Whitney U test was done to detect the difference between the two groups with respect to blood pressure, time domain and frequency domain Heart rate variability (HRV) indices, descriptors of Poincare plot, Auditory Reaction Time (ART) and Visual Reaction Time (VRT). The level of significance was set at p<0.05.

Results: There was no significant difference between the test and control groups with respect to blood pressure, HRV indices and descriptors of Poincare plot. Visual reaction time for red and green light was significantly prolonged in the Hemophiliacs.

Conclusion: This pilot study did not reveal any significant alterations in the cardiac autonomic activity of people with Hemophilia. However, future studies with a larger sample size are necessary to confirm the results of the study.

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(Received on March 12, 2017)

Introduction

Hemophilia A and B are rare X chromosome linked hereditary bleeding disorders caused due to mutations in factor VIII (FVIII) and factor IX (F IX) genes (1).

Hemophilia may be classified as severe, moderate or mild when the plasma levels of the factor are 1% or less, 2 to 5% and 6 to 40% respectively. The prevalence of hemophilia A is 1 in 5000 male live births, and that of hemophilia B is 1 in 30,000 (1, 2).

Recent studies have documented an increased incidence of cardiovascular events among individuals suffering from Hemophilia (3). Similarly, high prevalence of atherosclerotic plaques (4, 5), hypertension (6, 7) and cardiovascular disorders (8, 9) have also been reported in people with Hemophilia (PWH). While cardiac autonomic derangement is known to exist in various hematological (10-15) and non-hematological disorders (16-21), knowledge regarding the cardiac autonomic activity of PWH is very limited.

Cognitive and neuropsychological impairments and increased risk of neurotoxicity affecting the auditory, visual and somatosensory pathways have been reported in patients with blood disorders such as thalassemia and sickle cell disease (22-27). However, there is paucity of research on the cognitive status of people with Hemophilia.

It is well known that cardiac autonomic imbalance is associated with increased morbidity and mortality (28). Hence, there is a felt need to address the lacunae existing with respect to the knowledge on the cardiac autonomic status of PWH. Therefore, the primary objective of this study was to assess and compare the cardiac autonomic activity of PWH and normal healthy volunteers using Heart Rate Variability Analysis, and, our secondary objective was to assess and compare the cognition of PWH and healthy volunteers using simple Auditory and Visual Reaction Times.

Methodology

The study was carried out in the Department of Physiology, Indira Gandhi Medical College and Research Institute, Puducherry, after obtaining the approval from the Institutional Ethical Committee and Hemophilia Society Centre, Puducherry. A written informed consent was obtained from all the study participants.

Fourteen males aged 18-45 years suffering from Hemophilia were recruited from the Hemophilia Society Center, Puducherry and were included in the Test group. Similarly, fourteen age-matched healthy male volunteers working within the college campus were included in the Control group. Smokers, alcoholics and individuals suffering from known acute or chronic illness or receiving any form of medications that are known to affect the heart rate were excluded from the study.

Assessment of Study Parameters:

Subjects were asked to report to the Research lab in the Department of Physiology, 2-3 hours after a light breakfast on the day of recording. They were requested to refrain from consuming alcohol and caffeinated beverages 12 hours prior to the recording.

Assessment of Body Mass Index (BMI):

Following standardised procedures, participant's height (nearest 0.1 cm) was measured using a stadiometer and weight (nearest 0.5 kg) using a portable weighing scale. BMI was calculated by dividing the weight in Kg by the square of the height in meters (29).

Recording of Blood pressure:

Following the AHA guidelines (30), blood pressure was measured in all the individuals in sitting posture using a manual sphygmomanometer. Two readings were taken at an interval of one minute and the average of the two readings was taken as the blood pressure of the subject.

Mean arterial pressure (MAP) was calculated using the formula,

MAP = DBP+1/3PP (DBP- Diastolic blood pressure, PP- Pulse pressure, the difference of Systolic and Diastolic Blood Pressure)

Rate Pressure Product, (RPP), a measure of myocardial workload and oxygen consumption was calculated using the formula, RPP = systolic pressure × heart rate × 10^{-2} (31).

Recording of Short-term Heart Rate Variability (HRV):

Subjects were made to rest in supine posture for 10-15 minutes, and following this, a five minute ECG was recorded in lead II configuration. ECG signal was acquired at a sampling rate of 1024 samples per sec using the "INCO Digital Physiography" HRV Apparatus. The ECG recordings were done between 9-11 am in a light and noise minimized room and a room temperature of 25°-28°C was maintained for all the recordings.

Heart rate variability (HRV) analysis:

Short-term HRV analysis was done as per the Guidelines of the Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (32). R-R intervals obtained from the 5 minute ECG recording were screened for ectopic beats and noise and were then fed to Kubios HRV version 2.0, software for HRV analysis. Both time domain and frequency domain HRV indices were included in the analysis.

The time domain indices included in the study were:

1. Standard deviation of all normal to normal (NN) R-R intervals (SDNN) - measure of total heart rate variability

2. Square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD) - measure of cardiac parasympathetic activity.

3. Percentage of the number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals (pNN50) - measure of cardiac parasympathetic activity.

Frequency domain indices were obtained by performing Fast Fourier transformation of the RR interval data. The following Frequency domain indices were included in the study:

1. Low frequency (LF) power – indicative of cardiac sympathetic and parasympathetic activity

2. LF normalized units (LF nu) – indicative of cardiac sympathetic activity

3. High frequency (HF) power – indicative of cardiac parasympathetic activity

4. HF normalized units (HF nu) – indicative of cardiac parasympathetic activity

5. LF power+ HF power – indicative of total heart rate variability

6. LF/HF ratio – indicative of cardiac sympatho-vagal balance

Poincare Plot Analysis:

Poincare plot analysis (PPA) is a method which helps to analyse the non-linear dynamics of Heart Rate Variability. It serves as a quantitative technique by providing information on the short-term and long-term HRV and as an effective visual tool for summarizing the entire RR intervals derived from the ECG data (33, 34).

In this study, Poincare plot analysis was performed as per the guidelines of the Task Force for HRV analysis (32). The Poincare plot and its descriptors namely the Standard Deviation 1 (SD1) and Standard Deviation 2 (SD2) were obtained by feeding the RR intervals into the Kubios HRV version 2.0, software.

Standard deviation 1 (SD1): represents the standard deviation (SD) of the instantaneous (short-term) beat-to-beat R-R interval variability (minor axis of the ellipse).

Standard deviation 2 (SD2): represents the standard deviation (SD) of the long-term R-R interval variability (major axis of the ellipse) (35).

Additionally, the following parameters were computed and were also included in the analysis:

SD2/SD1: ratio of the standard deviations – measure of long-to-short-term balance of heart rate variability (36)

Area of the ellipse (S): represents the amount of area covered by the ellipse and is calculated as a product of π, SD1, and SD2. S represents the total heart rate variability (36).

Assessment of Auditory and Visual Reaction Time:

Auditory and Visual Reaction time was assessed using the instrument "Response Analyzer" by Anand Agencies System (RTM-608, Medicaid Systems, Chandigarh). Auditory reaction time (ART) was recorded as the time taken to respond to high and low frequency sound and Visual reaction time (VRT) as the time taken to respond to colour stimuli namely, red and green light. Sufficient trials were given to the subject to familiarize them with the procedure and then the actual recording of the reaction time was done. As soon as the individual perceived the stimulus (auditory or visual), they were asked to press the response switch with the index finger of their dominant hand. The quickness with which they performed was obtained from the display of the instrument in milliseconds which was a measure of their reaction time. Three such readings were taken at an interval of 2-5 seconds between the stimuli, and the average of the three readings was taken as the reaction time of the subject (37).

Variables included in the study:

i.   Anthropometric variables-height, weight,

ii. BMI

iii. Cardiovascular (CV) parameters – systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), rate pressure product (RPP)

iv. Short-term HRV parameters

    a.   Time domain parameters (Mean RR, SDNN, RMSSD, pNN50)

    b.   Frequency domain parameters (LF power, LF nu, HF power, HF nu, LF+HF, LF/HF)

v.   Descriptors of Poincare plot- SD1, SD2, SD2/SD1, S

vi. Auditory and Visual Reaction Time

Statistical Analysis:

Statistical analysis was done using SPSS 16 (SPSS Software Inc., Chicago, IL, USA). The study variables are presented as median with inter-quartile ranges. Mann-Whitney U test was done to compare the variables of the test and control groups. P value less than 0.05 was considered to be statistically significant.

Results

Fourteen males suffering from Hemophilia and fourteen healthy male volunteers were included in test and control groups respectively. There was no significant difference between the subjects of the test group and control groups with respect to age (P=0.227) and BMI (P=0.603) (Table I).

Blood pressure, mean arterial pressure and rate pressure product were not significantly different between the test and control groups. Similarly, comparison of time domain and frequency domain indices of HRV (Table I) and descriptors of Poincare plot (Table II) also did not reveal any significant difference between the two groups. However, Visual reaction time for green (P=0.035) and red light (P=0.024) was significantly prolonged in individuals with Hemophilia (Table III).

Discussion

Fourteen males with hemophilia (Test group) and fourteen age and sex matched healthy volunteers (control group) were included in the study. Cardiovascular parameters, time and frequency domain indices of Heart rate variability, descriptors of Poincare plot, and Auditory and Visual Reaction time of the test and control group subjects were assessed and compared.

Prevalence of cardiovascular risk factors and its associated cardiovascular disorders (CVD) have been reported among people with Hemophilia (6, 8, 9, 38). Hypertension is one of the known potential risk factor

TABLE I : Comparison of body mass index (BMI), cardiovascular (CV) parameters, time domain indices (TDI) and frequency domain indices (FDI) of heart rate variability (HRV) analysis between test group and control group.

Parameters	Test group (n=14) Median (IQR)	Control group (n=14) Median (IQR)	P value
AGE	24.50 (21.00 – 29.00)	23.00 (19.75 – 30.00)	0.227
BMI (kg/m^2)	21.47(18.60 – 22.54)	19.94 (18.94 – 21.73)	0.603
CV Parameters			
SBP (mmHg)	121.00 (109.50 – 128.50)	114.00 (106.00 – 118.00)	0.164
DBP (mmHg)	73.00 (60.00 – 90.00)	70.00 (63.00 – 78.50)	0.401
MAP (mmHg)	90.67 (77.17 – 101.00)	84.33 (75.83 – 91.83)	0.306
RPP	71.13 (64.96 – 89.97)	70.21 (59.12 – 76.95)	0.401
TDI of HRV			
Mean RR (ms)	925.90 (827.23 – 1050.35)	989.05 (857.68, 1045.85)	0.454
RMSSD (ms)	41.85 (24.50 – 53.95)	57.15 (36.65, 76.20)	0.085
pNN50 (%)	21.35 (4.73 – 39.05)	33.55 (16.90, 49.20)	0.210
SDNN (ms)	37.70 (32.35 – 62.05)	60.15 (35.63, 87.50)	0.094
FDI of HRV			
LF Power (ms^2)	304.50 (257.50 – 987.00)	894.00 (367.50 – 1485.00)	0.069
HF Power (ms^2)	539.00 (177.25 – 1030.50)	924.00 (629.00 – 1731.50)	0.085
LF nu	57.15 (31.75 – 68.25)	49.25 (37.20 – 57.20)	0.454
HF nu	42.80 (31.30 – 68.23)	50.75 (42.70 – 62.65)	0.454
LF + HF (ms^2)	1328.50 (926.25 – 2870.50)	2776.00 (1492.75 – 6566.25)	0.085
LF/HF	1.35 (0.47 – 2.18)	0.970 (0.594 – 1.339)	0.454

Values are expressed as Median (IQR-Inter-quartile range). Mann -Whitney U test- p value

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; RPP = rate pressure product; Mean R-R= mean duration of R-R interval; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; pNN50 = percentage of NN50 count divided by the total number of all NN intervals; SDNN = standard deviation of normal-to-normal intervals; LF power = low frequency power; HF power = high frequency power; LF nu = low frequency power, normalized units; HF nu = high frequency power, normalized units; LF + HF = sum of LF and HF power; LF/HF = ratio of low to high frequency power.

TABLE II : Comparison of descriptors of Poincare plot analysis between test and control group.

Parameters	Test group (n=14) Median (IQR)	Control group (n=14) Median (IQR)	P value
SD 1 (ms)	29.65 (17.33 – 38.18)	40.50 (25.95 – 53.96)	0.081
SD 2 (ms)	47.65 (39.85 – 78.33)	74.85 (44.30 – 97.93)	0.089
SD2/SD1	2.01 (1.61 – 2.36)	1.75 (1.61 – 2.02)	0.312
S (ms^2)	3875.03 (2479.51 – 9504.50)	9513.34 (3510.35 – 19497.55)	0.060

Values are expressed as Median (IQR-Inter-quartile range). Mann - Whitney U test - p value.

SD: Standard deviation; SD1: Standard deviation 1 (Minor axis of the ellipse); SD2: Standard deviation 2 (Major axis of the ellipse); SD2/SD1: ratio of Standard deviation 2 to Standard deviation 1; S: Product of π, SD1 and SD2.

TABLE III : Comparison of Visual Reaction Time and Auditory Reaction Time between test and control group.

Parameters	Test group (n=14) Median (IQR)	Control group (n=14) Median (IQR)	P value
VRT- green (msec)	212 (187 – 228)	186 (177 – 200)	0.035
VRT- red (msec)	212 (203 – 221)	183 (176 – 197)	0.024
ART- HF (msec)	171 (162 – 198)	164 (153 – 192)	0.246
ART- LF (msec)	175 (166 – 194)	168 (158 – 178)	0.137

Values are expressed as Median (IQR-Inter-quartile range). Mann - Whitney U test - p value.

VRT-green=Visual reaction time for green light; VRT-red= Visual reaction time for red light; ART-HF= Auditory reaction time for high frequency; ART-LF= Auditory reaction time for low frequency.

for cardiovascular disorders, and hence, in this study, the blood pressure of individuals with Hemophilia was assessed and compared with that of the healthy volunteers. It was seen that there was no significant difference in SBP, DBP, MAP and RPP values of the test and control group, although the median values of these parameters were high among the test group individuals.

Blood disorders due to genetic abnormalities such as Thalassemia (10-13) and Sickle cell anemia (14, 15) are known to be associated with autonomic imbalance. However, to the best of our knowledge,

there is not much report on the cardiac autonomic status of individuals with Hemophilia, the reason being the decreased prevalence of this disorder compared to other clinical disorders.
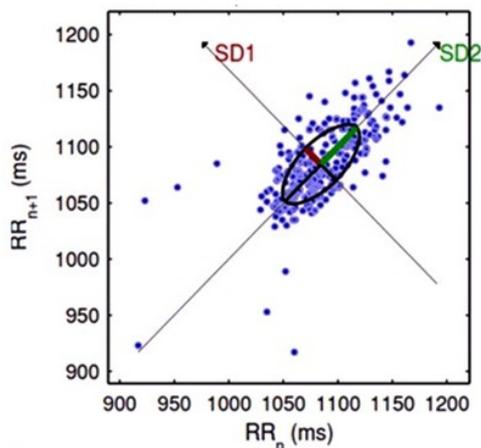
Hence, in this study, Heart rate variability analysis, a simple non-invasive tool which helps to assess the cardiac sympathetic and parasympathetic activity (32) was done to see if there were any alterations in the cardiac autonomic activity of individuals suffering from Hemophilia. We observed that the HRV indices indicative of cardiac parasympathetic activity (pNN50, RMSSD, HF power, HF nu) and total heart variability (SDNN, LF+HF) were not significantly different between the test and control group, although, the absolute median values of these indices were less among the test group individuals. Similarly, there was no significant difference between the test and control group with respect to LF nu, a measure of cardiac sympathetic activity and LF/HF, a measure of cardiac sympatho-vagal balance, although the median values for these indices were found to be high among Hemophliacs. The apparently increased sympathetic activity may be stated as a cause for the reduced total HRV and the increased blood pressure values observed in the Hemophliacs.

Analysis of Poincare plot did not reveal any significant difference between the test and control group, although, the median values of the descriptors indicative of total HRV (S), short-term (SD1) and long-term variability of heart rate (SD2) were found to be reduced among the Hemophliacs. Visual analysis of Poincare plots revealed a lesser dispersion of RR intervals, both beat-to-beat and in the long term, indicating a lower HRV in individuals with Hemophilia (Fig. 1.A) in comparison with the Poincare plots of normal healthy volunteers (Fig. 1.B) which revealed a greater dispersion of RR intervals, both in the short-term and in the long term.

It may be hypothesized that the frequent transfusion of clotting factor in these individuals may lead to increased stimulation of cardiac sympathetic afferents resulting in enhanced cardiac sympathetic activity. Moreover, it has been reported that individuals with Hemophilia of all ages, irrespective of the severity of the disorder often have a lower than average values for hemoglobin (39). Hence, this state of chronic subclinical anemia may lead to persistent sinus tachycardia (due to increased sympathetic activity) and decreased autonomic modulation in these individuals as observed in this study.
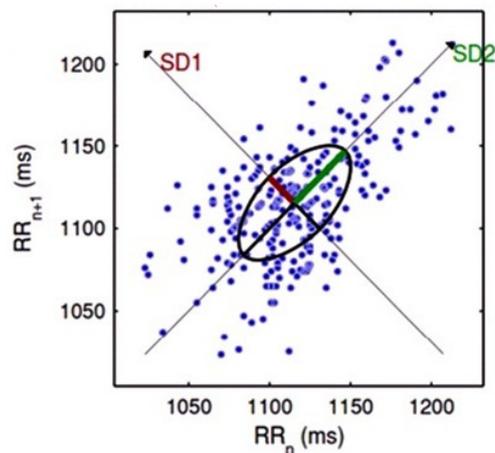

Fig. 1: A sample of Poincare plot with its descriptors SD1 and SD2 in (A). an individual with Hemophilia (Test group) and in (B). a normal healthy volunteer (Control group). SD 1: minor axis of ellipse, SD 2: major axis of ellipse.

Chronic health related disorders are known to affect the cognition of the individual (40, 41). The disease per se or the social and mental impact of the disease is considered as major cause for alterations in the cognition of individuals suffering from various chronic disorders. Blood disorders such as Thalassemia (22-25) and Sickle cell disease (26, 27) are known to be associated with impaired cognition and alterations in the auditory, visual and somatosensory evoked potentials. A study by Usner et al., in 1998 (42) reported that Hemophiliac patients with co-ordination and gait (CG) abnormalities achieved less scores in the Revised- Wide Range Achievement Test than those without CG abnormalities. Similarly, a study by Zanon et al. (43) in 2014 on patients with Hemophilia reported that these individuals had mild cognitive impairment irrespective of the severity of their disease and that these individuals also had asymptomatic cerebral microbleeds which were shown to be associated with their impaired cognition. In line with these reports, we observed that the visual reaction time for red and green light was significantly prolonged among the individuals with Hemophilia. The auditory reaction time was also prolonged in these individuals, but was not statistically significant. Thus, prolongation of the visual reaction time in PWH points to the fact that the disease does seem to have an effect on the cognition of these individuals.

The small sample size of this pilot study could be a major reason for the non-significant changes observed in the HRV indices and descriptors of Poincare plot analysis. Considering the fact that low total heart rate variability is a major risk factor for increased cardiovascular morbidity and mortality (44), similar studies with a larger sample size are required to substantiate the results of this pilot study on Hemophliacs. This would help the clinicians to identify the cognitive abnormalities and cardiac autonomic derangements if any at an early stage, so that appropriate measures can be taken to avoid complications in future.

Conclusion:

In this pilot study, the cardiac sympatho-vagal activity seems to be unaltered in individuals with Hemophilia. However, cognition as assessed by visual reaction time is impaired in these individuals.

Limitations:

The small sample size of this pilot study could be a major limitation of the study. Estimation of hemoglobin and plasma levels of Factor VIII & IX would have helped in assessing the severity of the disorder and its correlation with the changes observed in cardiac autonomic and cognitive status of individuals with Hemophilia.

Acknowledgements

Conflicts of interest, in any:

Nil

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