

Original Research Article

## Association of hemoglobin concentration with heart rate variability in young adult males

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**Abstract:** Previous studies have reported decreased heart rate variability in several diseases to be associated with increased cardiac risk including various anemias. The relation of heart rate variability with underlying factors causing anemia needs further elucidation. Thus this study was an attempt to find out and determine the strength of correlation between heart rate variability indices and hemoglobin concentration. The present study was conducted in the Upgraded Department of Physiology, S.M.S. Medical College, Jaipur on sixty healthy, life style matched young adult males aged 20-30 years, recruited from the employees and medical undergraduates. Hemoglobin estimation was done using Sahli's method. HRV assessment was done by recording 5 minute electrocardiogram using RMS ECG 101 instrument and later analyzed by HRV Software Version 1.1. Correlation coefficient was calculated between various heart rate variability indices and haemoglobin concentration using Pearson's correlation coefficient. All the time and frequency domain parameters correlated positively with hemoglobin concentration except the mean NN and LF norm. Only the total power ( $r=0.267$ ;  $p<0.05$ ) and HF band ( $r=0.283$ ;  $p<0.05$ ) of HRV showed statistically significant positive correlation with hemoglobin concentration in the healthy young adult males. Altered levels of hemoglobin concentrations lead to alteration of overall status of heart rate variability along with cardiac parasympathetic activity, subsequently putting anemic subjects with low hemoglobin at greater cardiac risk.

**Keywords:** Anemia, Heart rate variability, Hemoglobin.

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### INTRODUCTION

Anemia is a very common and one of the major public health problems in India with a prevalence of 74.3 percent population being anemic (Hemoglobin<11 g/dL) [1]. WHO has proposed that anemia or deficiency should be considered to exist, when haemoglobin is below 13 g/dL in adult males in venous blood [2].

Anemia has been suggested to be an independent risk factor for adverse cardiovascular outcomes in general population [3]. Moreover, anemia has been found to be associated with low heart rate variability (HRV) in ambulatory patients with stable coronary heart disease indicating that reduced HRV could potentially mediate the association of anemia with greater cardiac risk [4].

Heart rate variability assessment provides a simple and non-invasive tool to assess temporal

variations in interval between consecutive heart beats, reflecting the measurements of the degree of autonomic modulations. HRV can be assessed under two domains: the time domain method and the frequency domain method. The components of time domain included in the present study were mean heart beat interval (Mean NN), standard deviation of all NN intervals (SDNN) and square root of the mean squared differences of successive RR intervals (RMSSD). SDNN reflects all the cyclic components responsible for variability in the period of recording and RMSSD estimates high frequency variations in the heart rate reflecting the action of parasympathetic nervous system [5].

The components of frequency domain include total power (TP), very low frequency (VLF), low frequency (LF), high frequency (HF), normalized low frequency (LF Norm), normalized high frequency (HF Norm) and LF/HF ratio. VLF ( $\leq 0.04$  Hz) is thought to be influenced by the thermoregulation of vasomotor

tone; LF (0.04–0.15 Hz) is affected by the baroreceptor reflex and is thought to reflect sympathetic and parasympathetic tone; HF (0.15–0.40 Hz) is influenced by respiratory frequency and thought to reflect parasympathetic tone. The LF/HF ratio is an index of sympatho-vagal balance and thus of autonomic status. LF and HF may also be measured in normalized units (n.u.) which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in n.u. emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system [5, 6-9].

HRV has been studied for several years, with an increasing interest in understanding its mechanism and its clinical utility in various diseases. Changes in HRV have previously been described as early indicators of chronic heart failure [10], lethal arrhythmia [11], cardiac arrest and sudden death [12], hypertension [13] and ischemic heart disease [4, 14], thus reflecting the vital role of the autonomic nervous system in maintaining health. Data from these studies shows that decreased HRV may be associated with higher cardiac risk. The Framingham study has also reported that low HRV in healthy subjects is a risk factor for adverse cardiac events [6].

Association of changes in HRV indices with various types of anemia like iron deficiency anemia [15, 16], vitamin B<sub>12</sub> deficiency [17, 18], beta-thalassemia [19] etc. has been studied previously but very few studies are available which evaluated the association of various HRV parameters with hemoglobin concentration. In this context the present study was aimed to find out and determine the strength of correlation of hemoglobin levels with various HRV indices of young adult males.

## **MATERIAL AND METHODS**

The present study was conducted in the Upgraded Department of Physiology, S.M.S. Medical College; Jaipur from June 2014 to May 2015 between 9:00 AM to 11:00 AM involving sixty, life style matched, young adult males aged 20-30 years, recruited from the employees and medical undergraduates of S.M.S. Medical College, Jaipur.

After obtaining a prior ethical clearance from institutional ethical committee and informed written consent from the subjects, medical history assessment and routine clinical examinations was performed before HRV recording to exclude any kind of disorder. Smokers, alcoholics, subjects with diabetes mellitus, hypertension, heart diseases, or any other illnesses or on medications (anticholinergics, beta-blockers etc.) known to affect the HRV and non-cooperative subjects were excluded from the study.

## **Hemoglobin Estimation:**

Sahli's method was used for hemoglobin estimation [20]. Hemoglobin tube was filled with 0.1 N hydro-chloric acid up to 2 g marking. 20µL capillary blood sample was collected under aseptic precautions in Sahli's pipette and then added to the hemoglobin tube containing 0.1 N hydro-chloric acid. It was then thoroughly mixed with the stirrer and left for 10 minutes for acid hematin formation. The resulting dark brown fluid was then diluted with distilled water until a match with the brown glass standard was attained. Results were read as g/dL marked on the hemoglobin graduated tube. Subjects with hemoglobin concentration below 13 g/dL were considered anemic [2].

OneTouch® SelectSimple™ blood glucose monitoring system (LifeScan Inc.) was used for estimation of blood glucose to exclude diabetics [21]. Subjects with random blood sugar level more than 140 mg/dL were excluded from the study [22]. Height, weight, BMI and blood pressure were also recorded using standard instruments and procedures.

## **Heart rate variability Assessment:**

For accurate measurement of HRV the subjects were instructed to wear loose and comfortable clothes and to avoid coffee, nicotine or alcohol preceding 24 hours and food preceding two hours prior to the recording. The room temperature was maintained at 25°C and the subjects were instructed to breathe quietly with closed eyes and to avoid talking, coughing, sleeping and moving hands, legs or body during the entire recording period. All standard limb leads were applied and after 15 minutes of rest in a quiet environment electrocardiogram (E.C.G.) was recorded in the supine posture for 5 minutes by RMS ECG 101 (DECG 1/63041/ ADBXB) instrument. The lead with upright 'R' wave was selected and the analogue signal was converted to digital signal by National Instrument Software NI-DAQ Version 8.0. The recordings were visually examined and manually edited if required and the analysis of HRV in time and frequency domain was done by HRV Software Version 1.1 provided by the Autonomic Function Lab, AIIMS, New Delhi.

## **Statistical analysis:**

Numerical data were expressed as mean ± SD and analyzed using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. The variables were first assessed for normality using the Kolmogorov Smirnov test and then various HRV parameters and hemoglobin concentration were correlated using Pearson's correlation co-efficient. Statistical significance was assigned at  $p < 0.05$ .

## **RESULTS**

Table No. 1 shows mean ± SD of various general characteristics of subjects viz. age, height,

weight, BMI, systolic and diastolic blood pressure, random blood sugar and hemoglobin concentration. The systolic blood pressure ( $118.18 \pm 4.60$  mm of Hg), diastolic blood pressure ( $77.13 \pm 4.16$  mm of Hg) and random blood sugar ( $90.03 \pm 10.45$  mg/dL) were within the normal limits. The mean hemoglobin concentration ( $12.32 \pm 1.12$  g/dL) was below normal limit.

In the present study all the time domain parameters namely mean heart rate (MHR) ( $r=0.142$ ;  $p>0.05$ ), SDNN ( $r=0.233$ ;  $p>0.05$ ) and RMSSD ( $r=0.176$ ;  $p>0.05$ ) correlated positively with the

hemoglobin concentration, except Mean NN ( $r= -0.126$ ;  $p>0.05$ ) which showed negative correlation, but none of them showed statistically significant correlation with hemoglobin concentration (Table No. 2). In the frequency domain analysis of HRV all the variables showed positive correlation with hemoglobin concentration except the LF norm which was negatively correlated as shown in Table No. 2. Only Total Power ( $r=0.267$ ;  $p<0.05$ ) and HF power ( $r=0.283$ ;  $p<0.05$ ) showed statistically significant positive correlation with the hemoglobin concentration out of all (Figure 1 & 2).

**Table-1: Characteristics of studied subjects**

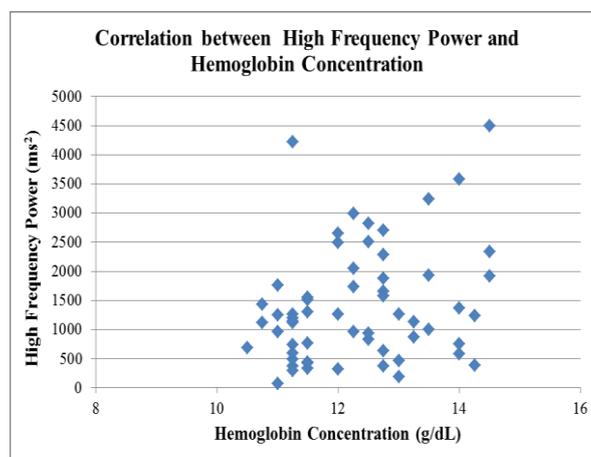
Parameter	Mean $\pm$ SD (n=60)
Age (Years)	20.83 $\pm$ 2.19
Height (meter)	1.71 $\pm$ 0.07
Weight (Kg)	63.83 $\pm$ 11.80
Body Mass Index (Kg/m <sup>2</sup> )	21.79 $\pm$ 3.44
Systolic blood pressure (mm of Hg)	118.18 $\pm$ 4.60
Diastolic blood pressure (mm of Hg)	77.13 $\pm$ 4.16
Random blood sugar (mg/dL)	90.03 $\pm$ 10.45
Hemoglobin concentration (g/dL)	12.32 $\pm$ 1.12

n= No. of subjects

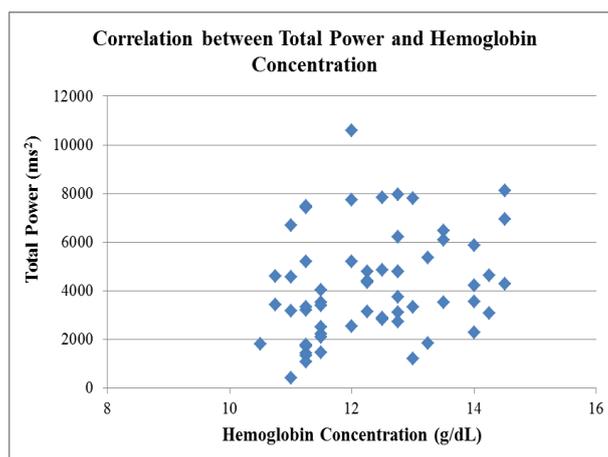
**Table-2: Correlation between Hemoglobin concentration and Heart rate variability indices**

	Parameters	Correlation coefficient (r)	p-value
<b>Time Domain</b>	MHR (bpm)	0.142	0.281
	Mean NN (ms)	-0.126	0.339
	SDNN (ms)	0.233	0.074
	RMSSD (ms)	0.176	0.179
<b>Frequency Domain</b>	TP (ms <sup>2</sup> )	0.267	0.039*
	VLF (ms <sup>2</sup> )	0.147	0.264
	LF (ms <sup>2</sup> )	0.164	0.210
	HF(ms <sup>2</sup> )	0.283	0.029*
	LF Norm (n.u.)	-0.054	0.681
	HF Norm (n.u.)	0.054	0.681
	LF/HF ratio	0.063	0.632

\* Significant ( $p<0.05$ )



**Fig-1: Correlation between High Frequency Power and Hemoglobin concentration**



**Fig-2: Correlation between Total Power and Hemoglobin concentration**

## DISCUSSION

The most important findings of the present study were that total power and HF bands of power spectral density of HRV showed significant positive correlation with hemoglobin concentration in the young adult males. (Table No. 2). This finding may provide a valuable insight regarding the altered cardiac autonomic modulations in anemic patients, which lead to the deranged HRV patterns. Results of this study corroborate with the findings of Lutfi MF in which TP and HF band of power spectral density of HRV correlated positively with hemoglobin concentrations [23].

Significant correlation of HF band of power with hemoglobin concentration has also been reported in a study done on non-transfusion dependent thalassemia patients [24]. Rutjanaprom W et al also found a significant correlation between haemoglobin concentration and HRV in patients of beta-thalassemia major, which supports the results of the present study [19]. In contrast to these findings, study of Agrawal K et al, revealed no significant correlations between HRV and blood parameters indicating, mild and moderate anemia may not have effect on cardiac autonomic modulation [25].

Anemia is one of several independent risk factors for adverse cardiovascular outcomes in the general population [3]. In many of the previous studies, HRV has been found to be decreased in anemic patients [5, 15-19]. The study results of Gehi A et al have proved that in anemic patients with stable coronary heart disease each 1 g/dL decrease in hemoglobin was associated with increased odds of having low HRV, which puts the anemics at greater cardiac risk [4]. Conversely hemoglobin normalization by administering epoetin in non-diabetic patients with stage 4 chronic kidney disease and renal anemia have shown improvements in frequency domain variables, which may subsequently reduce the cardiac risk [26]. Study of

Biaggioni I et al also revealed similar findings, in which severe autonomic failure showed good response to erythropoietin therapy [27].

In patients with iron deficiency anemia it has been observed that parasympathetic activity decreases, whereas sympathetic activity increases and this autonomic imbalance lead to altered electrophysiological activity of the heart [15]. The increase in sympathetic activity is due to the decreased levels of hemoglobin leading to hypoxia, which is sensed through carotid bodies [28].

Anemic patients also have lower basal parasympathetic outflow to increase the heart rate as a compensatory mechanism [29]. This autonomic imbalance has also been reported in patients of megaloblastic anemia and sickle cell trait [18, 30]. The significant correlation of HF with hemoglobin concentration in the present study may also provide supporting evidence in this regard.

Thus the findings of this study reveal the altered status of cardiac autonomic modulations with changing levels of hemoglobin, suggesting a greater cardiac risk in anemic patients. In this context HRV may sub-serve not only as a simple and non-invasive quantitative marker of cardiac autonomic modulations in anemic patients, but also as an adjunct to the prevailing diagnostic modalities to predict the cardiac risk due to altered autonomic balance.

## CONCLUSION

Combining current results with previous studies, it can be concluded that altered levels of hemoglobin concentrations lead to alteration of overall status of heart rate variability along with cardiac parasympathetic activity, subsequently putting anemic subjects with low hemoglobin at greater cardiac risk.

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