Section: Physiology

Original Research Article

ANALYSIS OF SERUM FERRITIN AMONG FEMALES WITH IRON DEFICIENCY ANEMIA AND CORRELATION WITH HEART RATE VARIABILITY

Nirmala Devi S[1], Aruna P[2], Vidhya A[3]

[1]Assistant Professor, Department of Physiology, Kanyakumari Government Medical College, Tamil Nadu, India
[2]Associate Professor, Department of Physiology, Government Dharmapuri Medical College, Tamil Nadu, India
[3]Assistant Professor, Department of Physiology, Stanley Medical College, Tamil Nadu, India

Received : 17/06/2024
Received in revised form : 13/08/2024
Accepted : 29/08/2024

Keywords:
Iron deficiency anemia, Serum ferritin, Heart rate variability.

Corresponding Author:
Dr. Nirmala Devi S,
Email: snirmaladevi77@gmail.com

DOI: 10.47009/jamp.2024.6.4.175

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (4); 896-903

Abstract

Background: Iron deficiency anemia (IDA) is a prevalent nutritional disorder affecting millions globally, particularly women and children. IDA is associated with significant cardiovascular risks, including altered autonomic function, which can be assessed through heart rate variability (HRV) analysis. This study aims to evaluate the correlation between serum ferritin levels and HRV parameters in females with IDA. **Materials and Methods:** A cross-sectional case-control study was conducted at Madras Medical College, Chennai, from April 2017 to March 2018, involving 80 female participants aged 18-45 years. The study group included 40 females with clinically diagnosed IDA, and the control group comprised 40 healthy females. Serum ferritin levels were measured using chemiluminescent immunoassay (CLIA), while HRV was assessed using the Medicaid Physiopac 8-channel HRV recorder. Key HRV parameters such as Mean RR, Mean HR, SDNN, RMSSD, LF, HF, and LF/HF ratio were analyzed and compared between the groups. **Result:** The study found that females with IDA had significantly higher mean heart rates (87.23±7.63) and lower Mean RR intervals (704.80±115.69) compared to controls. HRV analysis showed a significant decrease in parasympathetic indicators, including SDNN (39.39±8.37) and RMSSD (33.43±6.12), and an increase in sympathetic indicators such as LF (56.49±11.63) and LF/HF ratio (1.57±0.94). Serum ferritin levels were significantly lower in the IDA group (8.70±2.11 ng/mL) and showed a strong negative correlation with mean HR, LF, and LF/HF ratio, and a positive correlation with Mean RR, SDNN, RMSSD, and HF. **Conclusion:** The study concludes that IDA in females is associated with significant autonomic imbalance, characterized by reduced parasympathetic activity and increased sympathetic dominance. HRV analysis serves as a sensitive tool to detect early autonomic dysfunction in IDA, allowing timely intervention to prevent cardiovascular complications. Serum ferritin is an effective marker for diagnosing iron depletion and planning appropriate treatment strategies.

INTRODUCTION

Iron deficiency anemia (IDA) ranks among the most common nutritional deficiencies globally, affecting populations across both developed and developing nations. This disorder is particularly prevalent among children and women, with regions like South Asia and India experiencing especially high rates—reaching up to 88% in pregnant women and 74% in non-pregnant women.[1] The prevalence of IDA varies widely across different age groups, genders, and socioeconomic classes, with estimates suggesting that nearly three-quarters of the global population may be iron deficient.[2] According to the World Health Organization (WHO), approximately 2 billion individuals worldwide are affected by IDA, with prevalence rates of 8% in developed nations and 36% in developing regions (WHO 2007).

Iron deficiency has far-reaching consequences, not only compromising overall health but also significantly impacting cardiovascular function. It is associated with increased risks for various heart conditions, such as cardiomyopathy, chronic mitral regurgitation, congestive heart failure, and myocardial infarction. These conditions are often linked to reduced heart rate variability (HRV) and a higher risk of mortality.[3-6]

IDA arises when the body's iron reserves are depleted, leading to an insufficient supply of iron to tissues, including red blood cells. This condition most

ISSN (O): 2687-5365; ISSN (P): 2753-6556

commonly affects vulnerable groups such as growing children, pregnant and lactating women, and adolescents. In cases of IDA, levels of hemosiderin and ferritin decrease, which limits the supply of iron to Apo transferrin. This, in turn, reduces transferrin saturation and increases transferrin receptor levels. The WHO defines IDA as having a hemoglobin level below 12 g/dL and a serum ferritin level below 12 ng/mL.

Data from the NHANES III survey indicate a higher prevalence of IDA among children and women, especially adolescent girls and women of childbearing age (12-49 years). Factors contributing to IDA include menstrual blood loss, insufficient dietary iron, and challenges with iron bioavailability. Enhancers like ascorbic acid can improve non-heme iron absorption, while inhibitors such as phytates and calcium can reduce it. High-risk groups for IDA include pregnant women, women of reproductive age, young children under 5, adolescents, and the elderly.

IDA progresses in stages, beginning with the depletion of iron stores (evidenced by low serum ferritin), followed by a decrease in transport iron (referred to as latent iron deficiency), and ultimately leading to a significant reduction in hemoglobin production. This results in microcytic, hypochromic anemia. Clinically, IDA manifests as pallor, fatigue, glossitis, koilonychia, and, in children, symptoms such as irritability and pica. If left untreated, IDA can lead to serious complications, including heart failure and death. HRV is an important measure of autonomic function and can be used to predict the risk of sudden cardiac arrest and arrhythmias in patients with IDA.[7-10]

Heart rate variability (HRV) is the beat-to-beat variation in the RR intervals of the cardiac cycle, reflecting the intrinsic activity of the sinoatrial (SA) node modulated by the autonomic nervous system. HRV, measured in milliseconds, serves as a non-invasive method to assess the balance between sympathetic and parasympathetic influences on the heart. Typically, vagal tone predominates over sympathetic tone, keeping the resting heart rate below 100 beats per minute. HRV is influenced by various internal and external stimuli and can be evaluated through cardiac autonomic function tests, such as Resting HRV, which is a sensitive and specific tool. High HRV indicates a well-balanced autonomic function, while low HRV suggests impaired autonomic regulation and reduced adaptability.[11-13]

There is a direct correlation between the amount of iron stored in the body and serum ferritin levels, making serum ferritin a key marker for diagnosing both iron deficiency and iron overload. A serum ferritin concentration of 1 µg/L corresponds to 8-10 mg of stored iron (Finch, Huebers 1982). The normal range for serum ferritin is 15-300 µg/L, with levels below 12 µg/L indicating a lack of iron stores.

However, serum ferritin levels can be nonspecifically elevated in inflammatory conditions, neoplastic disorders, and liver diseases, as it acts as an acute phase reactant. To ensure accuracy in our study, we excluded patients with conditions that could elevate serum ferritin levels.

Serum ferritin estimation is the most sensitive and specific test for diagnosing iron deficiency anemia (IDA), as it decreases before anemia becomes apparent. A serum ferritin level below 12 µg/L is highly specific for IDA.[14]

Several methods exist for determining serum ferritin. Among those, the immunoradiometric assay is the most sensitive.[15] Phlebotomy studies demonstrate that serum ferritin levels drop when iron stores are depleted. While other parameters like hemoglobin, packed cell volume (PCV), mean red cell volume, and red blood cell free protoporphyrin are useful for diagnosing IDA, they only become abnormal in the later stages due to the long lifespan of red blood cells. Numerous studies have explored the relationship between iron levels, heart disease, and cancer.

In this study, serum ferritin is used as a diagnostic marker for IDA, with an aim to correlate serum ferritin levels with heart rate variability (HRV) parameters.

Aim & Objective

- To estimate the serum ferritin among female patients with iron deficiency anemia and normal female population.
- To correlate the heart rate variability with serum ferritin levels of female patients with iron deficiency anemia.

MATERIALS AND METHODS

This case control study was conducted at the Institute of Physiology & Experimental Medicine, Department of Hematology, and Institute of Internal Medicine, Madras Medical College, Chennai, from April 2017 to March 2018. The study population included females aged 18-45 years, with and without iron deficiency anemia, visiting RGGGH, Chennai. Inclusion criteria for the anemic group were hemoglobin levels <12 gm%, serum ferritin <12 ng/mL, and Complete Blood Count parameters (MCV, MCH, MCHC, RDW) showing values lower than the normal range, with a peripheral smear indicating microcytic hypochromic anemia. Exclusion criteria included other forms of anemia, structural heart diseases, diabetes, hypertension, renal failure, thyroid disorders, pregnancy, chronic infections or inflammations, radiation exposure, drug intake, autoimmune disorders, and malignancy. The sample size was calculated based on the previous study results using Openepi software and found to be 80. About 40 in each group. The institutional human ethics committee approval was obtained.

One group is those with iron deficiency anemia and another group is normal female population without iron deficiency anemia. After explaining the study and getting informed consent from them, the study was conducted. Data was collected using a semi structured questionnaire. Investigations were done

ISSN (O): 2687-5365; ISSN (P): 2753-6556

for patients which includes complete blood count and heart rate variability.

Under universal sterile precautions, 5 ml of venous blood was collected for serum ferritin estimation, complete blood count (CBC), and peripheral smear (PS) analysis. The serum was separated by centrifuging the blood sample at 3000 rpm for 10 seconds within 30 minutes of collection, and stored at -20°C. Once sufficient samples were collected, serum ferritin levels were measured using the chemiluminescent immunoassay (CLIA) method, conducted in the biochemistry laboratory at Rajiv Gandhi Government Hospital. CBC and PS were performed using a fully automated analyzer in the clinical pathology department. The CLIA method, based on a colorimetric enzymatic reaction, was used to determine serum ferritin, with normal levels ranging from 12-291 ng/mL. The automated Cobas analyzer was calibrated before sample analysis, and results were retrieved from the computer after the assay was completed.

Heart rate variability (HRV) of the selected subjects was recorded using the Medicaid Physiopac 8-channel HRV recorder, equipped with in-built software for data analysis. After explaining the procedure and obtaining consent, participants were instructed to ensure good sleep, have breakfast 2 hours prior, avoid caffeine, nicotine, and alcohol, wear loose clothing, remove accessories, and relax. The recording was conducted between 10 am and 12 noon in a quiet, temperature-controlled room. Participants rested in a supine position for 10-15 minutes before electrode placement. Limb leads were attached after cleaning the areas with spirit, and the ECG was recorded for 5 minutes using lead II. The data was analyzed using short-term HRV analysis software, with artifact screening, and key parameters like Mean RR, Mean HR, SDNN, RMSSD, Low Frequency, High Frequency, and LF/HF ratio were estimated through power spectral analysis using Fast Fourier Transformation.

RESULTS

In our study, 40 patients who were clinically diagnosed of Iron deficiency anemia were tested for assessment of their autonomic functions by using the Heart rate variability analysis and the estimated values are compared with that of 40 clinically healthy individuals.

The mean age of the study group was 32.1±7.7, where the control group were of mean age of 29.18±7.32 and the female Patients with Iron deficiency anemia were of mean age of 35.1 ±6.98. The mean value of weight for the control group and the female patients with Iron deficiency anemia were 49.7 ±11.1 and 164.12 ±11.7 respectively. The mean value of BMI for the control group and the female patients with Iron deficiency anemia were 21.4 ±2.5 and 22.3 ±1.9 respectively. The mean value of Hemoglobin for the control group and the female patients with Iron deficiency anemia were 12.76 ±0.64 and 9.46 ±1.49 respectively. [Table 1]

The blood pressure parameters, systolic and diastolic blood pressure as well as the heart rate at the resting levels were compared between the control group and female patients with Iron deficiency anemia. A very highly significant increase in mean heart rate (85.38±5.8, $p<0.001$) is observed in female patients with iron deficiency anemia when compared to that of the control group (78.28±5.26). This could be mainly due to sympathetic overactivity in patients with Iron deficiency anemia. The resting systolic blood pressure in female patients with Iron deficiency anemia showed a significantly lower value (103.55±9.47, $p<0.001$) compared to that of the control group (112.95±9.40). The decrease in systolic blood pressure could be due to anemia. The resting diastolic blood pressure in female patients with Iron deficiency anemia showed a significantly lower value (69.60±6.87, $p<0.014$) compared to that of the control group (73.10±5.43). Diastolic blood pressure reflects the peripheral resistance, here in this study the decrease in DBP indicates the decrease in afterload due to adaptability changes that occur in anemia. [Table 2]

Serum ferritin reflects the iron in the storage pool. The serum ferritin levels were significantly lesser in female patients with iron deficiency anemia (8.70±2.11, $p<0.001$) compared to that of the control group (121.85±65.40). Decrease in serum ferritin levels indicates iron stores are depleted in iron deficiency anemia. [Table 3]

The mean Heart rate in female patients with Iron deficiency anemia was found to be significantly increased (87.23±7.63, $p<0.001$) when compared to that of the controls (77.20±6.19). Increased mean heart rate of HRV analysis in Iron deficiency anemia indicates the possibility of sympathetic overactivity. The mean RR interval in female patients with Iron deficiency anemia was found to be significantly decreased (704.80±115.69, $p<0.001$) when compared to that of the controls (804.68±84.46). Mean RR interval (ms) of HRV analysis reflects inter beat intervals. Decreased mean RR interval in Iron deficiency anemia indicates the possibility of reduced vagal tone. The mean SDNN in female patients with Iron deficiency anemia was found to be significantly decreased (39.39±8.37, $p<0.012$) when compared to that of the controls (44.14±8.14). Mean SDNN of HRV analysis reflects parasympathetic function. Decreased mean SDNN in patients with Iron deficiency anemia indicates the possibility of reduced parasympathetic activity. The mean RMSSD in female patients with Iron deficiency anemia was found to be significantly decreased (33.43±6.12, $p<0.001$) when compared to that of the controls (39.22±6.37). Mean RMSSD of HRV analysis reflects parasympathetic function. Decreased mean RMSSD in patients with Iron deficiency anemia indicates the possibility of reduced parasympathetic tone. The mean LF in female patients with Iron deficiency anemia was found to be significantly

ISSN (O): 2687-5365; ISSN (P): 2753-6556

increased (56.49±11.63, p<0.001) when compared to that of the controls (44.8±8.28). Mean LF of HRV analysis reflects sympathetic function. Increased mean LF in patients with Iron deficiency anemia indicates the possibility of sympathetic over activity. The mean HF in female patients with Iron deficiency anemia was found to be significantly decreased (42.46±12.53, p<0.001) when compared to that of the controls (54.92±7.97). Mean HF of HRV analysis reflects parasympathetic function. Decreased mean HF in patients with Iron deficiency anemia indicates the possibility of reduced parasympathetic tone. The mean LF/HF ratio in female patients with Iron deficiency anemia was found to be significantly increased (1.57 ±0.94, p<0.001) when compared to that of the controls (0.86 ±0.33). Mean LF/HF ratio of HRV analysis reflects sympathovagal balance. Increased mean LF/HF ratio in patients with Iron deficiency anemia indicates sympathetic overactivity. [Table 4]

Table 1: Comparison of socio-demographic details between the control group and patients with Iron deficiency anemia.

Variables	Control group, n=40		IDA patients, n=40	
	Mean	SD	Mean	SD
Age (years)	29.18	7.32	35.1	6.98
Height (cm)	161.32	12.5	*164.12	11.7
Weight (Kg)	49.7	11.1	*51.4	9.8
BMI	21.4	2.5	*22.3	1.9
Hemoglobin (gm/dl)	12.76	0.64	*9.46	1.49

*p value<0.05 is considered as statistically significant. IDA = patients with Iron Deficiency Anemia. BMI= Body Mass Index.

Table 2: Comparison of the Resting heart rate, resting systolic blood pressure and resting diastolic blood pressure between the control group and female patients with iron deficiency anemia

Variables	Control group, n=40		IDA patients, n=40	
	Mean	SD	Mean	SD
Resting Heart rate (beats/min)	78.28	5.26	***85.38	5.83
Resting SBP (mm/Hg)	112.95	9.4	***103.55	9.47
Resting DBP (mm/Hg)	73.1	5.43	*69.6	6.87

*p<0.05 which is considered statistically significant. ** p value < 0.01 was considered as highly significant. SBP=systolic blood pressure, DBP=diastolic blood pressure.

Table 3: Comparison of the serum ferritin levels between the control group and female patients with iron deficiency anemia.

Variable	Study group	N	Mean	SD
Serum Ferritin (ng/ml)	Control	40	121.85	65.40
	IDA	40	***8.70	2.11

*p<0.05 which is considered statistically significant. **p value < 0.01 was considered as highly significant. IDA = patients with Iron Deficiency anemia.

Table 4: Comparison of the heart rate variability factors between the control group and female patients with iron deficiency anemia

Variable	Study group	N	Mean	SD
Mean Heart Rate (beats/min)	Control	40	77.20	6.19
	IDA	40	***87.23	7.63
Mean RR interval (ms)	Control	40	804.68	84.46
	IDA	40	** *704.80	115.69
Mean SDNN (ms)	Control	40	44.14	8.14
	IDA	40	**39.39	8.37
Mean RMSSD (ms)	Control	40	39.22	6.37
	IDA	40	***33.43	6.12
Mean LF (nu)	Control	40	44.8	8.28
	IDA	40	***56.49	11.63
Mean HF (nu)	Control	40	54.92	7.97
	IDA	40	* * * 42.46	12.53
Mean LF/HF ratio	Control	40	0.86	0.33
	IDA	40	** *1.57	0.94

*p<0.05 which is considered statistically significant. **p value < 0.01 was considered as highly significant. IDA = patients with Iron Deficiency anemia.

Table 5: Correlation between the Serum ferritin levels and variables of HRV analysis.

Dependent variable	Variables	R2 value	95% C.I.		p
			Lower	Upper	
Serum Ferritin levels	Mean Heart rate per min	-0.446	-0.43	-0.69	<0.001
	Mean RR (ms)	0.342	0.31	0.56	<0.001
	SDNN (ms)	0.277	0.24	0.48	0.01
	RMSSD (ms)	0.276	0.24	0.48	0.01

ISSN (O): 2687-5365; ISSN (P): 2753-6556

	LF (nu)	-0.467	-0.45	-0.72	<0.001
	HF (nu)	0.471	0.46	0.72	<0.001
	LF/HF ratio	-0.376	-0.35	-0.61	<0.001

A highly significant negative correlation($p<0.001$) was found between the serum ferritin levels and the mean heart rate, mean LF and mean LF/HF ratio. A significant positive correlation($p<0.01$) was observed between the serum ferritin levels and the mean RR, mean SDNN, RMSSD and mean HF. [Table 5]

DISCUSSION

Iron deficiency anemia has been found to be associated with increased cardiovascular morbidity and mortality. It affects cardiac function. Decreased Heart rate variability (HRV) has been found to be associated with increased mortality and morbidity in several heart disorders like myocardial infarction, cardiomyopathy, congestive heart failure and chronic mitral regurgitation.[16-19]

The physiological change which occurs in anemia is a compensatory increase in cardiac output, preload, heart rate, stroke volume associated with a decrease in the afterload. This increase in sympathetic activity causes palpitation, tachycardia in iron deficiency anemic patients and Progression of iron deficiency anemia results in cardiomyopathy.[20]

The mechanism behind this pathogenesis has not been fully understood so far. But there are many advancing research trails related to this that have led to the formation of new hypothesis. This study was primarily aimed to analyze the cardiac autonomic function in Iron deficiency anemia by doing Heart rate variability analysis. Forty iron deficiency female subjects and forty age and gender matched normal controls were included in our study. The study group were selected between the age group of 18 to 45 years females.

The mean age of the control group was 35.10 ± 6.98 and the mean age of female patients with Iron deficiency anemia was 29.18 ± 7.32. The difference was not significant in relation to age. This was consistent with the earlier studies. The mean height of the control group was 161.32±12.5 and the mean height of female patients with Iron deficiency anemia was 164.12 ±11.7.The mean weight of the control group was 49.7±11.1 and mean weight of female patients with Iron deficiency anemia was 51.4±9.8.The mean BMI of the control group was 21.4±2.5 and the mean BMI of female patients with Iron deficiency anemia was 22.3±1.9 .The difference was found to be significant in relation to height, weight and BMI .

The mean resting heart rate of control group was 78.28 ± 5.26 and the mean resting heart rate of female patients with Iron deficiency anemia was 85.38±5.83. An elevated level of sympathetic activity and decreased parasympathetic tone was seen in iron deficiency anemia patients as per results of the study done by Tuncer et al.[21] Our study showed there was increase in the mean heart rate in female patients with Iron deficiency anemia. The Possible reason for this could be the increase in the sympathetic activity of the iron deficiency anemia patients.

The mean resting systolic blood pressure in control group was 112.95± 9.40 and the mean resting systolic blood pressure in female patients with iron deficiency anemia was 103.55±9.47. Our study showed decreased systolic blood pressure in iron deficiency anemia female patients when compared to controls. Anemia leads to many adverse effects, and this is observed in many pathological conditions like end stage renal failure and myocardial infarction. But how the presence of anemia progresses to heart failure is being evaluated recently only.[22-24] Studies show anemia patients with systolic dysfunction when not treated can progress to heart failure.[22,23,25-28]

The mean resting diastolic blood pressure in control group was 73.10±5.43 and the mean resting diastolic blood pressure in female patients with Iron deficiency anemia was 69.60±6.87. Our study showed decreased diastolic blood pressure in cases when compared to controls. This was consistent with the findings observed in the study done by Deepu Nair et al.[29]

There are many explanations regarding mechanisms concerned with the relationship between anemia and diastolic dysfunction. Presence of anemia for prolonged period results in adaptation and this causes increase in heart rate, cardiac index, stroke volume and plasma volume.[30,31] All these changes of increased sympathetic activity and ionotropic activity leads to additional stress on myocardium.[32]

The mean hemoglobin in control group was 12.76 ±0.64 and the mean hemoglobin in female patients with Iron deficiency anemia was 9.46±1.49. Our study showed that there was decreased hemoglobin in female patients with Iron deficiency anemia when compared to controls. The hormonal, metabolic changes due to anemia also cause direct myocardial damage and also through salt, water retention indirectly exerts strain on the myocardium. This forms the basis of the hypothesis that anemic adaptability causes ventricular remodeling, diastolic dysfunction and systolic dysfunction.

The mean serum ferritin in control group was 121.85±65.40 and the mean serum ferritin in female patients with Iron deficiency anemia was 8.70±2.11 Female patients with Iron deficiency anemia showed decreased serum ferritin levels when compared to controls. This is due to the decrease in the iron stores in iron deficiency anemia. Studies show that normal ferritin can show variations related to age and sex. Serum ferritin level in women shows lower values until menopause and values shows increase thereafter.[33]

Heart Rate Variability (HRV) analysis is considered as one of the effective, non- invasive tools to assess the function of the Autonomic nervous system. There occurs an increased risk of adverse cardiac events

ISSN (O): 2687-5365; ISSN (P): 2753-6556

when there is alteration in resting HRV. Analysis of the resting heart rate variability by doing 5 minutes ECG recording using the HRV analysis among the study groups showed the following results.

Mean HR, Mean RR, SDNN, RMSSD were the variables taken for analysis in our study as prescribed by the Task force. Our findings showed decreased HRV in female patients with Iron deficiency anemia. Decreased HRV suggests either increased sympathetic tone or decreased parasympathetic tone. The Mean HR was significantly increased in female patients with Iron deficiency anemia (87.23±7.63) when compared to controls (77.20±6.19). This shows tachycardia in Iron deficiency anemia patients. This finding was consistent with the findings observed in the studies done by Tuncer et al.[21] The Mean RR among the study groups were measured which shows decreased mean RR interval (704.80±115.69) in the female patients with Iron deficiency anemia when compared with controls (804.68±84.46). Similar findings were also observed in studies done by Yokusoglu et al.[34]

In our study female patients with Iron deficiency anemia showed decreased mean values of SDNN (39.39±8.14) and RMSSD (33.43±6.12) when compared with the normal controls SDNN (44.14±8.14) and RMSSD (39.22±6.37). This was also consistent with the findings of Farhana rahman et al.[35] Similar finding was also observed in studies done by Yokusoglu et al.[34]

Results from studies done by Shetty KP et al,[36] and Tuncer et al,[21] showed there is no difference in the HRV parameters in iron deficiency anemia patients and normal subjects except mean HR. Lufti et al,[37] reported no significant difference in values of SDNN and RMSSD in Iron deficiency anemia and healthy controls.

But our study results show decreased SDNN and RMSSD which indicates that parasympathetic activity is decreased in iron deficiency anemia when compared with normal subjects. The SDNN and RMSSD were considered as sensitive indicators of parasympathetic function and thereby a significant low value indicates reduced vagal activity in Iron deficiency anemia patients.

LF, HF and LF/HF ratio were the variables taken for analysis in our study as prescribed by the Task force. A significant variation was observed in the frequency domain variables among the study groups. The LF values in normalized units(nu) which is an indicator of sympathetic tone was significantly higher in female patients with Iron deficiency anemia (56.49±11.63) when compared with normal controls. (44.81±8.28). The HF values in normalized units(nu) which is an indicator of parasympathetic tone was found to be significantly lower in female patients with Iron deficiency anemia (42.46±12.53) when compared with the control (54.92±7.97). As the HF power indicates the vagal activity, our study finding suggest that there is decreased parasympathetic activity in female patients with Iron deficiency anemia. Our study findings were consistent with findings of Lufti et al.[37] His study showed VLF, LF, HF, TP were positively correlated with hemoglobin concentration. HRV parameters were found to be decreased. Study by Gehlot Pinkesh et al shows that there was no statistically significant relation between hemoglobin and HRV in anemia.[38]

LF/HF ratio was increased in iron deficiency individuals (1.57±0.94) when compared with the controls (0.86±0.33) due to sympathovagal imbalance. This ratio signifies the overall balance between sympathetic and parasympathetic system. In addition, sympathetic stimulation enhances erythropoiesis. Studies of Biaggioni et al support this hypothesis. Sympathetic stimulation in anemia stimulates erythropoiesis. These cases with autonomic dysfunction showed good response to erythropoietin therapy.[39]

Therefore, in our study it was found that parasympathetic activity decreases and sympathetic activity increases in patients with iron deficiency anemia.

The possible reason is, in iron deficiency anemia due to decreased hemoglobin concentration the oxygen carrying capacity of blood is decreased which leads to hypoxia. This hypoxia will be sensed through the carotid bodies which influences the cardiovascular centers leading to increase in the sympathetic activity.[40] The mechanism causing carotid body activation is supposed to be either due to hypoxia related mitochondrial respiratory chain inhibition or due to potassium channel suppression that causes intracellular calcium accumulation.[41]

This type of anemic hypoxia stimulates the adrenergic nervous system. Stimulation of this leads to cardiovascular response like tachycardia and increased cardiac output. These changes will try to compensate for the decrease in the oxygen content of the blood. Moreover, the activation of the adrenergic system can be known by estimating the concentration of plasma and urine epinephrine,[42] which is not done in our study.

These patients have low basal parasympathetic outflow and as a compensatory mechanism there is an increase in the heart rate. So these changes in HRV has been taken as a sensitive indicator of health deterioration due to autonomic changes.[43]

Hence HRV is used as an early and better qualitative and quantitative method to detect autonomic impairment. High HRV indicates well-functioning of the autonomic nervous system and on the other hand when HRV is reduced it acts as a risk indicator to know the adverse complications in the patients suffering from wide range of diseases.[44] So in our study HRV has been used to know the cardiovascular mortality in female patients with Iron deficiency anemia. There are studies which have reported that supplementation of iron can improve the dysregulated autonomic nervous system reflexes.[45]

So, our study has been aimed to diagnose Heart rate variability by HRV analysis as early as possible to avoid the morbidity and mortality due to cardiovascular changes by assessing the autonomic

ISSN (O): 2687-5365; ISSN (P): 2753-6556

imbalance, so that by supplementing iron therapy it is possible to prevent the cardiovascular complications in these patients.

Serum ferritin levels were correlated with Mean heart rate, Mean RR, SDNN, RMSSD, LF, HF, LF/HF ratio. A highly significant negative correlation ($p<0.001$) was found between the serum ferritin levels and the mean heart rate, mean LF and mean LF/HF ratio. A significant positive correlation ($p<0.01$) was observed between the serum ferritin levels and the mean RR, mean SDNN, RMSSD and mean HF. This finding was consistent with findings in the study done by Daniel wallman et al. This study showed a decrease in serum ferritin that led to the change in HRV parameters which reflected autonomic dysfunction. The mechanism which was proposed was decreased iron stores initiated carotid body reflex, and this reflex activity increased due to decreased blood flow.[46] Dysfunction of sympathetic system affected the vascular tone. These individuals are prone to develop syncope when they stand from lying posture.

CONCLUSION

The sympathetic and parasympathetic activity and the functional status of the heart were evaluated in iron deficiency anemia female patients using Resting Heart rate variability. This study concludes that there is an autonomic imbalance as evidenced by decrease in SDNN, RMSSD which were indicators of parasympathetic and increase in LF power (nu) showing the sympathetic activity. The ratio between LF and HF was increased in iron deficiency anemia individuals which showed sympathetic dominance of autonomic nervous system activity. So, by using HRV analysis as a sensitive and non-invasive tool, treatment can be started at the earliest and thus we can prevent the complications in iron deficiency anemia. Serum ferritin is used as a marker to diagnose iron store depletion at the earliest, so that treatment modality can be planned effectively in order to prevent cardiovascular complications.

Limitations

Our study has involved a smaller sample size. It is necessary to apply it to a general population using larger sample size to get better outcome of the study. The duration of the disease and autonomic dysfunction was not correlated in our study.

The study has evaluated only the resting autonomic activity and not the response of the autonomic nervous system to various external stimuli or lab stressors, which is a drawback for the study.

One of the mechanisms suggested for autonomic dysfunction is elevated circulating nor epinephrine levels and urine norepinephrine concentration. Hence our study needs to be substantiated by measurements of the catecholamine, norepinephrine levels which is not done in our study.

Moreover, blood gas analysis was not done for anemic hypoxia. Serum ferritin assay has to be combined with other iron studies like total iron binding capacity, and transferrin saturation for better outcome, because serum ferritin is also an acute phase reactant that shows elevated levels in inflammatory conditions.

REFERENCES

1. Iron Deficiency Anaemia: Assessment, prevention and control.pdf [Internet]. [cited 2018 Jul 9]. Available from: http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf
2. Litchmann MA, Beutler E. Williams Hematology. Seventh. Mc Graw Hill Medical;
3. Turner LR, Premo DA, Gibbs BJ, Hearthway ML, Motsko M, Sappington A, et al. Adaptations to iron deficiency: cardiac functional responsiveness to norepinephrine, arterial remodeling, and the effect of beta-blockade on cardiac hypertrophy. BMC Physiol. 2002 Jan 9;2:1.
4. Sarnak MJ, Tighiouart H, Manjunath G, MacLeod B, Griffith J, Salem D, et al. Anemia as a risk factor for cardiovascular disease in the atherosclerosis risk in communities (aric) study. J Am Coll Cardiol. 2002 Jul 3;40(1):27.
5. Gehi A, Ix J, Shlipak M, Pipkin SS, Whooley MA. Relation of Anemia to Low Heart Rate Variability in Patients With Coronary Heart Disease (from the Heart and Soul Study). Am J Cardiol. 2005 Jun 15;95(12):1474–7.
6. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J. 1996 Mar;17(3):354–81.
7. Adaman JW Dan L . Anemia and polycythemia. Section-10 Hematological Alterations. In Harrison's Principles of Internal Medicine 16th ed. Vol. 1, editors Kasper DL Fauci AS Longo DL Braunwald E Hauser SL Jameson JL. 2005; pp 334-336 McGraw Hill, N. Y.
8. Agarwal K N. Iron and brain: neurotransmitter receptors and magnetic response spectroscopy. Brit J Nutr 2001; 85 Suppl 2 S147-150.
9. Indicators for Assessment of Anemia and Iron Deficiency Prof. K N AgarwalHealth Care & Research Association for Adolescents;D-115, Sector-36 NOIDA, Gautam Budha Nagar, U.P., INDIAe-mail: adolcare@hotmail.com
10. Hegde N, Rich MW, Gayomali C. The cardiomyopathy of iron deficiency. Tex Heart Inst J. 2006; 33: 340-4.
11. Levy MN, Schwartz PJ, Armonk N. Vagal control of the heart : experimental basis and clinical implications. Futura;
12. Barett KE, Barmann SM. Ganong's Review of Medical Physiology. 24th Edition.
13. Genovely H, Pfeifer MA. RR-variation: the autonomic test of choice in diabetes. Diabetes Metab Rev. 1988 May;4(3):255–71.
14. Essentials of haemotology shirish m kawthalkar first edition pg81
15. Varley' s practical clinical biochemistry 6th edition pg 629
16. Stein PK, Domitrovich PP, Kleiger RE, CASR investigators. Including patients with diabetes mellitus or coronary artery bypass grafting decreases the association between heart rate variability and mortality after myocardial infarction. Am Heart J. 2004; 147: 309-16.
17. Karcz M, Chojnowska L, Zareba W, Ruzyllo W. Prognostic significance of heart rate variability in dilated cardiomyopathy. Int J Cardiol. 2003; 87: 75-81.
18. Stein KM, Borer JS, Hochreiter C, Okin PM, Herrold EM, Devereux RB, et al. Prognostic value and physiological correlates of heart rate variability in chronic severe mitral regurgitation. Circulation. 1993; 88: 127-35
19. Anand IS, Kuskowski MA, Rector TS, Florea VG, Glazer RD, Hester A, et al. Anemia and change in hemoglobin over time related to mortality and morbidity in patients with chronic heart failure: results from Val-HeFT. Circulation. 2005; 112: 1121-7.
20. Lee GR. Iron deficiency and iron-deficiency anemia. In: Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN. (eds.).

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Wintrobe's clinical hematology. 9th ed. Philadelphia: Lea & Fabiger; 1993. p. 808-10
21. Tuncer M , Gunes Y, Guntekin U, Gumrukcuoglu HA, Eryonucu B, Guler N, Dilek I, & Demir C. Heart rate variability in patients with iron deficiency anemia. Arq Bras Cardiol. 2009; 92: 368-371.
22. Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Borenstein J. Anemia is associated withworse symptoms, greater impairment in functional capacity and a significant increase in mortality inpatients with advanced heart failure. J Am Coll Cardiol 2002;39:1780–1786. [PubMed: 12039491]
23. McClellan WM, Flanders WD, Langston RD, Jurkovitz C, Presley R. Anemia and renal insufficiencyare independent risk factors for death among patients with congestive heart failure admitted tocommunity hospitals: a population-based study. J Am Soc Nephrol 2002;13:1928–1936. [PubMed:12089390]Nair et al. Page 5Am J Cardiol. Author manuscript; available in PMC 2009 November 12.NIH-PA
24. Silverberg DS, Wexler D, Blum M, Keren G, Sheps D, Leibovitch E, Brosh D, Laniado S, SchwartzD, Yachnin T, et al. The use of subcutaneous erythropoietin and intravenous iron for the treatment ofthe anemia of severe, resistant congestive heart failure improves cardiac and renal function andfunctional cardiac class, and markedly reduces hospitalizations. J Am Coll Cardiol 2000;35:1737–1744. [PubMed: 10841219]
25. Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associatedwith poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. Circulation2003;107:223–225. [PubMed: 12538418]
26. Kosiborod M, Smith GL, Radford MJ, Foody JM, Krumholz HM. The prognostic importance of anemiain patients with heart failure. Am J Med 2003;114:112–119. [PubMed: 12586230]
27. Al-Ahmad A, Rand WM, Manjunath G, Konstam MA, Salem DN, Levey AS, Sarnak MJ. Reducedkidney function and anemia as risk factors for mortality in patients with left ventricular dysfunction.J Am Coll Cardiol 2001;38:955–962. [PubMed: 11583864]
28. Szachniewicz J, Petruk-Kowalczyk J, Majda J, Kaczmarek A, Reczuch K, Kalra PR, Piepoli MF, AnkerSD, Banasiak W, Ponikowski P. Anaemia is an independent predictor of poor outcome in patients withchronic heart failure. Int J Cardiol 2003;90:303–308. [PubMed: 12957766
29. Association of Anemia With Diastolic Dysfunction AmongPatients With Coronary Artery Disease in the Heart and Soul StudyDeepu Nair, MD, Michael G. Shlipak, MD, MPH, Brad Angeja, MD, Haiing H. Liu, MD, MPH,Nelson B. Schiller, MD, and Mary A. Whooley, MDDepartments of Medicine and Epidemiology and Biostatistics and the Division of Cardiology,University of California, San Francisco, California; and the Section of General Internal Medicine, VAMedical Center, San Francisco, California
30. Duke M, Abelmann WH. The hemodynamic response to chronic anemia. Circulation 1969;39:503–515. [PubMed: 5778251]
31. Varat MA, Adolph RJ, Fowler NO. Cardiovascular effects of anemia. Am Heart J 1972;83:415–426.[PubMed: 4551028
32. Metivier F, Marchais SJ, Guerin AP, Pannier B, London GM. Pathophysiology of anaemia: focus onthe heart and blood vessels. Nephrol Dial Transplant 2000;15:14–18. [PubMed: 11032352]
33. Gibson R. Principles of nutritional assessment, 2nd ed. Oxford, UK, Oxford University Press, 2005.
34. Yokusoglu M, Nervuz O, Baysan O, Uzun M, Demirkol S, Avcu F, Koz C, Cetin T, Hasimi A, Ural AU, & IsikE. Tohoku J Exp Med.2007; 212: 397-402.
35. Time domain measures of heart rate variability to assess cardiac autonomic nerve function in female with iron deficiency anemia. Farhana Rahman1, Qazi Shamima Akhter2, Qazi Farzana Akhter3, Susmita Sinha4, Sybila Ferdousi5
36. Shetty KP, Moodithaya S, Naik A, &Mirajkar A. Autonomic nervous system activity among adolescent girls with iron deficiency anemia. Nat J Bas Med Sci. 2012; 2: 224-229.
37. Dr. Mohamed Faisal Lutfi / International Journal of Pharmaceutical and Biological Research (IJPBR) Vol. 2(5), 2011, 127-131
38. Effect of haemoglobin concentration on cardiovascular system by heartrate variability modulations Gehlot Pinkesh1, Tonpay P.S.2, Siddiqui N.I. 3
39. Biaggioni I, Robertson D, Krantz S, Jones M, Haile V. The anemia of primary autonomic failure and its reversal with recombinant erythropoietin. Ann Intern Med 1994;121:181-6.
40. Schultz HD, & Sun SY. Chemoreflex function in heart failure. Heart Fail Rev. 2000; 5: 45-46.
41. Lahiri, s,roy a..baby s.m.,hoshi,t.,semenza,G.I.and prabhakar,N.R.(2006) oxygen sensing in the body. prog.biophys.mol.biol.,91,249-286
42. FavretF, & Richalet JP. Exercise and hypoxia: The role of the autonomic nervous system. RespPhysiolNeurobiol.2007; 158: 280-286.
43. Vanderlei LC, Pastre CM, Hoshi RA, Carvalho TD, & Godoy MF. Basic notions of heart rate variability and its applicability. Rev Bras Cir Cardiovasc.2009; 24: 205-217.
44. Everengul H, Dursunoglu D, Cobankara V, Polat B, Seleci D, Kabukeu S, Kaftan A, Semiz E, & Kilic M. Heart Rate Variability in Patients with Rheumatoid Arthritis. Rheumatol Int. 2004; 24: 198-202.
45. Orii KE, Kato Z, Osamu F, Funato M, Kubodera U, Inoue R, Shimozawa N, & Kondo N. Changes of autonomic nervous system function in patients with breath- holding spells treated with iron. J Child Neurol. 2002; 17: 337-340.
46. Jacobs, A., Worwood, M., 1975. Ferritin in Serum. New England Journal ofMedicine 292, 951–956.

ISSN (O): 2687-5365; ISSN (P): 2753-6556