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Research Article

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Cardiac Autonomic Function with Iron Deficiency Anemia

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Abstract

Objectives: Iron deficiency (ID) and its anemia (IDA) are the most prevalent nutritional deficiency worldwide. Dysfunction of the autonomic nervous system (ANS) is a consequence of anemia regardless to its type. Many studies found ANS dysfunction in adults with ID/IDA. This study evaluated ANS function in children and adolescents with IDA as related studies are scare.

Patients and Methods: This prospective study included 60 children with IDA (boys = 20; girls = 40; age: 14.50 ± 2.04 yrs.). Blood concentrations of hemoglobin, ferritin and iron were determined. ANS function testing were carried twice (at baseline and 3 months after iron therapy). They included measuring of heart rate at rest and its variation (HRV) in response to standing and breathing and blood pressure (BP) changes in response to standing, sustained handgrip and cold.

Results: Manifestations of IDA included excessive fatigue, dizziness, palpitation at rest and headache. Children with IDA had significant changes in resting heart rate, blood pressure and HRV parameters compared to healthy mates indicating sympathetic hyperactivity and reduction in parasympathetic activity. Early, definite and severe ANS dysfunctions were found in 20%, 36.67% and 3.33%, respectively. For children with IDA, significant correlations were found between ferritin levels and HB and iron levels (P = 0.001), HRV to active standing, deep breathing and Valsalva maneuver (P = 0.001) and systolic and diastolic (P = 0.001) and diastolic BPs in response to sustained handgrip and cold (P = 0.001). Ferrous sulfate therapy (6 mg/kg/day) for 3 months resulted in improvement of ANS manifestations with IDA.

Conclusion: ANS dysfunctions are common consequences of IDA in children and can be attributed to the increased need of tissues for oxygen, resulting in sympathetic hyperactivity. Optimal iron therapy can improve ANS consequences of IDA.

Keywords

Iron deficiency anemia, Autonomic nervous system function, Heart rate variation, Iron therapy

Abbreviations

IDA: Iron Deficiency Anemia; **ANS:** Autonomic Nervous System; **HB:** Hemoglobin; **HRV:** Heart Rate Variation

Introduction

Anemia is the most common nutritional deficiency worldwide. The prevalence of anemia has been estimated to range from 45 to 65% in children, half are caused by iron deficiency (ID). Iron deficiency anemia (IDA) is higher in children and adolescents, females and developing countries because of increased iron demands during growth and puberty and limited iron intake in food [1]. Iron had a critical role in oxygen and electron transports and DNA synthesis and in the formation of several cellular proteins and enzymes [2]. In IDA, there is no iron for heme synthesis. In very early stages of ID, depletion of iron occurs from the body tissue stores (liver, spleen and bone marrow). With progression of ID, there will be a decrease in body concentrations of hemosiderin, ferritin and apotransferrin, an iron transport protein, resulting in reduction in serum iron, increase in total iron binding capacity (TIBC), reduction in transferrin saturation and increase in transferrin receptors. This stage is known as pre-anemic or latent ID [3]. Impairment of hemoglobin (HB) synthesis occurs with reduction of transferrin saturation level below 15-20%. In severe IDA (HB < 8 g/dL), the size of red blood cells (RBCs) becomes small (microcytic) and have reduced amount of HB (hypochromic). The lack of iron stain in bone marrow indicates that serum ferritin level is below 12µg/L which is diagnostic for IDA [4].

Studies have shown that the function of autonomic nervous system (ANS) may be compromised with anemia regardless to its type (e.g. thalassemia, megaloblastic anemia due to vitamin B12 or folate deficiency, sickle cell trait, IDA, etc.) [5-14]. In stressful situations, ANS provides a rapid response to control the function of wide range of cardiac and non-cardiac body systems. The physiological change which occurs in IDA is a compensatory enhancement in cardiac output, preload, heart rate and stroke volume and a reduction in the afterload [15]. Cardiovascular autonomic manifestations of IDA include irritability, palpitation at rest, breathlessness, headache, fatigue, impaired muscular performance, abnormalities of muscle metabolism, exercise intolerance, increase sensitivity to cold, tachycardia or arrhythmias and postural dizziness, faintness and syncope, manifestations of orthostatic hypotension. In adults with IDA, objective testing of autonomic functions showed increase in resting heart rate HR and its variability (HRV) and variations in blood pressure (BP) with active standing, deep breathing and sustained forced expiratory effort against a closed glottis and its response to instant standing, sustained handgrip and cold [8-14].

Studies found that severe IDA carries are a risk factor for arrhythmias, myocardial ischemia and heart failure, life-threatening conditions [16, 17].

Aim of the Study

Studies which evaluated ANS function in children with IDA are few compared to adults. This is a prospective study aimed to evaluate (1) ANS dysfunction (frequency, types, manifestations and severity) (if present) and their correlation to demographic, clinical and hematologic findings, and (2) the effect of iron therapy on ANS dysfunction.

Materials and Methods

This study included 60 children with IDA (boys = 20; girls = 40) and had age ranged from 11 and 18 years (mean: 14.50 ± 2.04 yrs.). It also included 40 healthy children recruited from school mates matched for age range: 10-18; mean: 15.35 ± 2.28 yrs., P = 0.365), sex (boys = 12; girls = 28) and socioeconomic status as controls for statistical comparisons. Criteria for diagnosis of IDA (in children ≥ 5 years old) were (1) Low HB concentration by at least 2 standard deviations below healthy mates, and (2) Serum ferritin below 15 µg/L [18]. Classification of IDA was as follow: mild (HB: 11 - 11.9 g/dl), moderate (HB: 8 - 10.9 g/dl) and severe (HB: < 8.0 g/dl). Patients were recruited over a period of one year (June 2018 to July 2019) from Pediatric Neurology and Hematology clinics of Assiut and Al Azhar Universities Hospitals, Assiut, Egypt.

Exclusion criteria: (1) children with other forms of anemia (e.g. vitamin B12 or folic acid deficiency, thalassemia major, sickle cell disease) or malnutrition, (2) children with known metabolic problems or other medical diseases as cardiovascular disease (e.g. cardiac syncope, arrhythmias, hypertension, structural heart disease, heart failure, acute myocardial infarction) and respiratory disease, diabetes mellitus, thyroid disease, chronic infections/inflammations, autoimmune disorders, renal dysfunction, etc., (3) malignancy, (4) acute bleeding, (5) medications known to affects HR (e.g. anti-arrhythmic drugs, beta-blockers, digitalis, vasopressors, tricyclic antidepressant, etc.), and (6) intake of iron supplementation shortly before participation of the study.

Methods

Medical and neurological histories and examinations, cardiac ANS functions' testing, echocardiography and laboratory investigations were performed to all children included in the study.

Cardiac ANS function testing

Testing was performed early in the morning after breakfast by two hours (to avoid postprandial circulatory collapse) in a quiet relaxed atmosphere. Measurements were recorded after the ascertainment of identical two consecutive (5 minutes apart) HR and BP readings (i.e. reached basal values). Parasympathetic ANS activity was assessed as follow [19]: **(1) Heart rate monitoring during rest and HRV with active conditions:** HR was monitored in a resting supine position for 15 minutes. HRVs were determined after active standing (30:15 ratio), deep breathing and Valsalva maneuver using conventional 12 lead channel electrocardiography (ECG). In normal children, resting HR is ≤ 100 beats/minute (bpm). After standing quickly within 3-4 seconds, there is immediate shortening of RR interval with its maximum around the 15th beat (tachycardia) followed by a relative RR

interval prolongation with its maximum around the 30th beat (bradycardia). The 30:15 ratio was calculated by dividing the longest (~ 30th beat) over the shortest (~ 15th beat) RR interval. Normal 30:15 HRV ratio is ≥ 1.04. With deep breathing at 6 breaths/minutes, HRV was determined as the difference between maximum or highest (with deep inspiration) and minimum or lowest (with deep expiration) HRs per minute. Normal HRV in response to deep breathing is ≥ 15 bpm. Valsalva maneuver was done by asking the child to blow into the tubing of mercury sphygmomanometer and raise the mercury column to 40 mm Hg and maintain it at that level for ≥ 10-15 seconds. HRV in response to Valsalva maneuver (Valsalva ratio) was calculated by dividing the longest (just after Valsalva or during release) over the shortest (during strain for the following 30 seconds) RR intervals. Normal Valsalva ratio is ≥ 1.21. Resting HR of more than100 bpm was considered abnormal. For HRV: In response to active standing, 30:15 ratio was considered borderline with values ranged from 1.01 to 1.03 and markedly abnormal with values of ≤ 1. In response to deep breathing, HRV was considered borderline with values ranged from 11 to 14 bpm and definitely abnormal with values ≤ 10 bpm. In response to Valsalva maneuver, HRV was considered borderline with values ranged from 1.11 to 1.20 and abnormal with values of < 1.10. **(2) BP monitoring at rest and during instant standing:** BP was monitored in a resting supine position for at least 5 minutes and after standing for 3 minutes. Orthostatic hypotension (OH) was diagnosed in presence of a drop of systolic and diastolic BPs by ≥ 20 and ≥ 10 mmHg, respectively. The orthostatic test was carried out thrice and the average value was considered. Repetition of orthostatic testing was carried after an interval of at least 30 seconds. **Sympathetic ANS activity was assessed as follow: (1) BP response to sustained handgrip (isometric exercise testing):** The child's hand grip was maintained at 30% of maximum grip (determined by a dynamometer) for 3 minute and the BP was simultaneously recorded from non-exercising arm. The procedure was repeated thrice and the average increase in DBP of ≥ 16 mmHg was interpreted as normal, borderline with increase by values ranged from 11 to 15 mmHg and abnormal with increase by 10 mmHg or less. **(2) BP response to cold (Cold pressor testing):** The child was asked to insert one hand in iced water (9 °C) for 1 minute and BP was measured from the other arm just after removal of the hand from water. The procedure was repeated thrice and the maximum increase in diastolic BP was recorded. The normal response is the increase in diastolic BP by > 10 mmHg. It was considered abnormal if there was no increase in BP.

The ANS testing results were also classified as: **(1) Normal:** defined by the presence of normal tests' results or presence of only one borderline test's result. **(2) Early ANS dysfunction:** defined by the presence of abnormal one out of the three HR test or by the presence of 2 borderline HR testing's results. **(3) Definite ANS dysfunction:** defined by the presence of 2 or more abnormal HR testing's results. **(4) Severe ANS dysfunction:** defined by the presence of 2 or more abnormal HR tests' results and one or more abnormal or borderline BP testing's results. **(5) Atypical ANS dysfunction:** defined by the presence of any other combination of abnormal HR and BP testing results.

Laboratory investigations

Venous blood samples were collected into polypropylene tubes containing EDTA as anticoagulants and stored at -20 °C. Serum samples were diluted with deionized water (0.5:4.5 v/v). Complete blood count (CBC) and serum concentrations of creatinine, alanine (ALT) and aspartate (AST) aminotransferase activities were measured. Serum iron was determined using chromazurol B (C. I. 43830) in the presence of cetyltrimethyl-ammonium bromide (CTMA) with an absorption maximum at 630 nm and a molar coefficient of extinction of 1.68×10^5 1 $mol^{-1}.cm^{-1}$ [20]. Serum ferritin was measured by electrochemiluminescence immunoassay (a sandwich principle) using Elecsys Ferritin kits (Variable Name: LBXFER) [Cobas e601 analyzers] (Roche Diagnostics, Indianapolis, IN). Re-evaluation of children with IDA (clinical, autonomic testing and hematological laboratory investigations) was done after 3 months of ferrous sulfate in a dose of 6 mg/kg/day.

The protocol of the study was approved by the local Ethical Committees of the Faculties of Medicine, Assiut and Al Azhar Universities (ID#: AUFM_315/2018), Assiut, Egypt. Written informed consent was obtained from each patient/parent to participate in the study.

Statistical Analyses

SPSS 16.0 for windows was used for analysis of data. The distribution of data was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics were presented with means ± standard error of mean (SEM). Comparative statistics were done using Student's *t*-test and Chi-square test. Spearman's rank correlation coefficient was used to test the correlation between variables. In children with IDA, paired *t* test was used to compare results before and after iron therapy. The significance level was set at probability value less than 0.05.

Results

We found that 70% (n = 42) of children had IDA of moderate severity and only 13.33% (n = 8) had severe anemia. Symptoms of IDA included excessive fatigue, dizziness, palpitation at rest and headache. Children with anemia had normal echocardiography. In children with anemia, early ANS dysfunction was found in 20% (n = 12), definite in 36.67% (n = 22) and severe in 3.33% (n = 2) (Table 1). Compared to healthy children, children with anemia had higher resting HR (P = 0.001), lower mean systolic and diastolic BPs (P = 0.05) and reduced 30:15 (P = 0.001) and Valsalva (P = 0.05) ratios and increased diastolic BP in response to sustained handgrip (P = 0.001) and cold (P = 0.001). No gender difference was found in ANS tests' results. Tachycardia was found in 56.67%. Abnormal 30:15 and Valsalva ratios were found in 36.67% of children with moderate/severe anemia (Table 2). For children with IDA, significant correlations were observed between

Table 1: Demographic, clinical and laboratory characteristics of the studied children.

Variable	Patients (n = 60)	Controls (n = 40)	P-value
Age; years	11-18 (14.50 ± 0.20)	10-18 (15.35 ± 0.23)	0.336
Gender			
Male	20 (33.33%)	12 (30%)	0.303
Female	40 (66.67%)	28 (70%)	0.426
BMI; kg/m^2	20-28 (26.20 ± 0.23)	18-27 (25.36 ± 0.26)	0.432
HB; g/dl	7.00-11.00 (9.86 ± 0.50)	12.00-14.00 (12.26 ± 0.24)	0.01
RBCs; million/L	1.88-4.50 (3.64 ± 0.15)	2.65-6.20 (4.88 ± 0.24)	0.08
HCT; %	26-33 (32.42 ± 0.16)	35-46 (39.33 ± 0.11)	0.01
MCV; femtolitres	55-78 (70.57 ± 0.36)	82-95 (88.63 ± 0.34)	0.01
MCH; pg	20-27 (24.83 ± 0.12)	27-31 (29.56 ± 0.11)	0.02
MCHC; g/dl	26.-33 (30.94 ± 0.13)	31.5-35 (34.69 ± 0.15)	0.03
Serum iron; µg/dL	10-40 (18.29 ± 0.05)	45-120 (57.52 ± 1.10)	0.001
Serum ferritin; µg/L	5.-45 (10.88 ± 0.12)	50-150 (58.83 ± 0.88)	0.001
Severity of IDA; n (%)			
Mild	10 (16.67%)	-	-
Moderate	42 (70%)	-	-
Severe	8 (13.33%)	-	-

Data are expressed as Mean ± Standard Error of Mean (SEM), number (%). HB: Hemoglobin; IDA: Iron Deficiency Anemia; RBC: Red Blood Cell; HCT: Hematocrite; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration. Reference ranges for target population (10 -18 years): HB: 11.9-16.9 g/dL; RBCs: 3.80-5.70 million/L, HCT%: 35-50%, MCV: 79.9-98.0 fL, MCH: 27-31 pg; MCHC: 31.5-35 g/dl; serum iron: 50 -150 µg/dl; serum ferritin: 12-150 µg/L [31].

ferritin levels with HB and iron levels (P = 0.001), HRV to active standing, deep breathing and Valsalva maneuver (P = 0.001), systolic and diastolic BPs (P = 0.001) and diastolic BP in response to sustained handgrip and cold (P = 0.001). For controls, a significant correlation was observed between ferritin with iron levels (P = 0.001) (Figure 1). Treatment with ferrous sulfate for 3 months resulted in significant increase in HB level (P = 0.05), improvement of IDA symptoms and resting HR (P = 0.03), BP changes with active standing (P = 0.5), 30:15 and Valsalva ratios (P = 0.05) and BP response to sustained hand grip (P = 0.05) (Table 3).

Table 2: Results of cardiovascular ANS function tests in the studied children.

Variable	Patients (n = 60)	Controls (n = 40)	P-value
HR and its variability (HRV)			
Resting HR; bpm	105-125 (110.5 ± 0.68)	60-100 (80.28 ± 0.65)	0.001
***Number; %**	34 (56.67%)	3 (7.5%)	0.0001
Mild IDA	2 (5.88%)	-	-
Moderate IDA	24 (70.59%)	-	-
Severe IDA	8 (23.53%)	-	-
30:15 ratio	0.5-1.01 (0.82 ± 0.04)	1.26-1.54 (1.34 ± 0.03)	0.001
***Number;** %	22 (36.67%)	0	-
Mild IDA	1 (4.55%)	-	
Moderate IDA	13 (59.09%)	-	
Severe IDA	8 (36.36%)	-	
In response to deep breathing	8-25 (15.25 ± 0.84)	20-35 (30.05 ± 0.56)	0.001
***Number; %**	22 (36.67%)	0	-
Mild IDA	2 (9.1%)	-	
Moderate IDA	12 (54.55%)	-	
Severe IDA	8 (36.36%)	-	
Valsalva ratio	0.8-1.2 (1.01 ± 0.04)	1.21-1.54 (1.36 ± 0.03)	0.01
***Number; %**	22 (36.67%)	0	-
Mild IDA	2 (9.1%)	-	
Moderate IDA	12 (54.55%)	-	
Severe IDA	8 (36.36%)	-	
BP			
BP changes in response to standing (OH); mmHg			
Systolic	80-110 (100.50 ± 0.80)	100.-130 (110.30 ± 0.63)	0.05
Diastolic	50-70 (60.30 ± 0.50)	60.-80 (78.56 ± 0.52)	0.05
***Number; %**	4 (6.67%)	3 (7.5%)	0.455
Mild IDA	-	-	
Moderate IDA	2 (50%)	-	
Severe IDA	2 (50%)		
Change in response to handgrip; mmHg	15-30 (26.72 ± 0.85)	15 -0 (18.36 ± 0.40)	0.01
*Number; %	0	0	-
Change in response to cold (Cold Pressor); mmHg	15-25 (22.67 ± 0.68)	10-30 (12.24 ± 0.77)	0.01
***Number; %**	0	0	-

Data are expressed as Mean ± Standard Error of Mean (SEM), HR: Heart Rate; HRV: Heart Rate Variation, BP: Blood Pressure, *Number of patients with abnormal ANS function.

Discussion

Children and adolescents are one of the most vulnerable populations to ID/IDA due to increase need for iron for puberty and growth [1]. ANS dysfunction is a consequence of different types of anemia [5-7]. IDA is an independent risk for arrhythmias, cardiac events and diseases and child's mortality [16, 17]. Therefore, it is important to identify the manifestations, frequency and extent of ANS dysfunctions with IDA for prevention and treatment implications.

In this study, ferritin and iron levels were used to diagnose IDA in addition to HB because serum ferritin is considered the most sensitive and specific lab marker for diagnosing IDA. Serum ferritin decreases even before the appearance of anemia, correlates with the individual's body iron stores, and is not affected by recent iron intake [3, 4].

In this study, we used battery subsets that are sensitive to detect changes in sympathetic and parasympathetic ANS functions. They showed that significant number of children with IDA had tachycardia (56.67%) and HRV to active standing, deep breathing and Valsalva maneuver (36.67%) indicating sympathetic hyperactivity and weak parasympathetic activity. Sympathetic hyperactivity was also evidenced by increase in diastolic BP in response to handgrip and cold. There were significant correlations between serum ferritin levels and

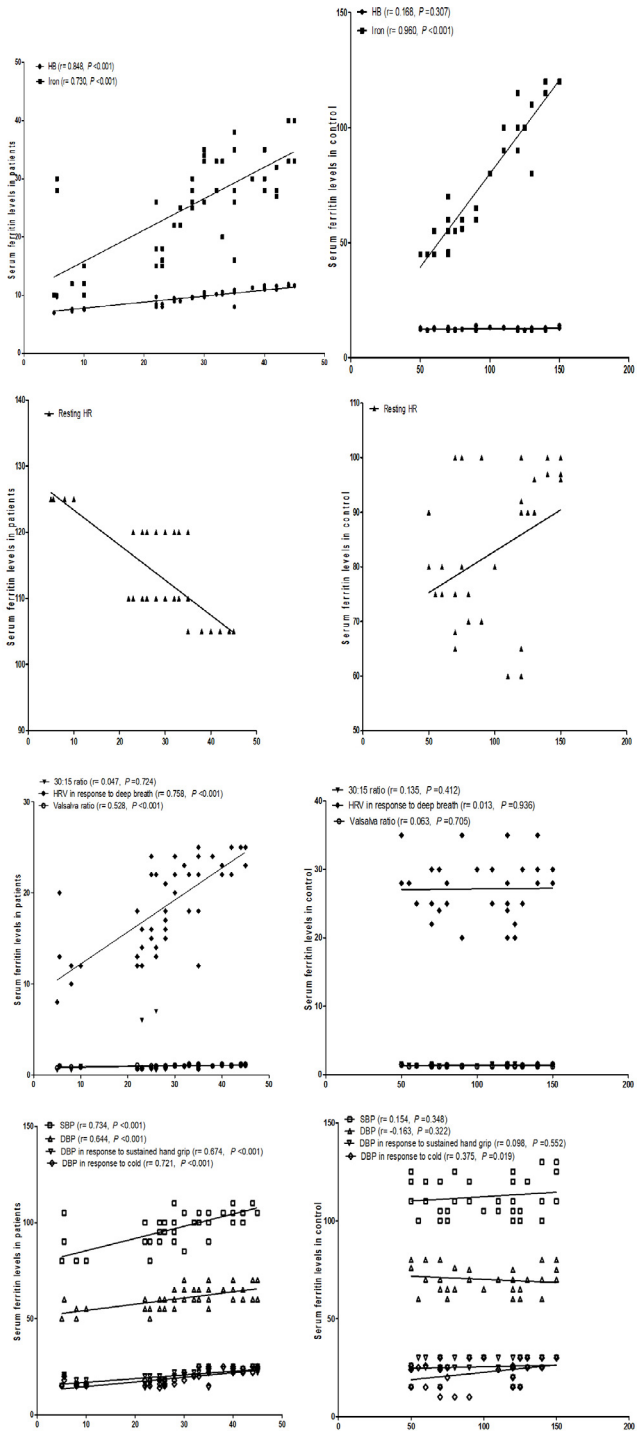

Figure 1: Correlation analyses between ferritin concentrations and laboratory and ANS findings in the studied groups.

ANS dysfunction. Results of this study are in accordance to majority of studies in adults which showed reduction of HRV in response to various stimuli (e.g. standing, breathing, etc) [8-14]. HRV is the oscillations in RR interval between two consecutive QRS complexes recorded by ECG. It reflects the fluctuations in central oscillators (as sinus node), efferent neural pathway (i.e. sympathovagal activities) and humoral factors in response to external and internal conditions. It has been established that high HRV reflects well-functioning

Table 3: Results of laboratory and ANS function testing before and after iron treatment.

Variable	Patients before treatment (n = 60)	Patients after treatment (n = 60)	P-value
HB; g/dl	9.86 ± 0.50	11.20 ± 0.36	0.05
Resting HR; bpm	110.5 ± 0.68	88.30 ± 0.48	0.03
BP response to standing; mmHg			
Systolic BP	0.82 ± 0.04	110.25 ± 0.38	0.05
Diastolic BP	60.30 ± 0.48	70.62 ± 0.44	0.05
30:15 ratio	0.82 ± 0.04	1.12 ± 0.01	0.01
Valsalva ratio	1.01 ± 0.04	1.11 ± 0.02	0.05
BP response to sustained handgrip; mmHg	26.72 ± 0.85	20.46 ± 0.25	0.05

Data was expressed as Mean ± Standard Error of Mean (SEM). ANS: Autonomic Nervous System; HB: Hemoglobin; HRV: Heart Rate Variation; BP: Blood Pressure.

ANS and decreased HRV reflects decrease in the capacity of ANS for adaptation. HRV is considered as a predictor tool to diagnose arrhythmias and sudden cardiac arrest [21]. Findings of various studies indicated the predominance of sympathetic and weakness of parasympathetic activities with IDA. Evidence for the latter is also supported by the findings of Jibhkate et al. [14] who observed reduction in HR relative to initial HR in 40% of patients with IDA in response to Valsalva maneuver, which reflects the complex hemodynamic results for activation of sympathetic as well as parasympathetic neurons. However, the authors' observation of abnormal postural tachycardia syndrome (POTS) along with normal atropine response indicated dysfunction of afferent limb of parasympathetic reflex arch.

Dysfunction of ANS with IDA have also been observed in studies of HRV using other techniques (e.g. 24 h Holter monitoring, Polyrite-D, etc.). Yokusoglu et al. [10] evaluated HRV indices in 43 adults with IDA. The authors found increase in the mean HR, impairment in global Standard deviation (SD) of NN intervals (SDNN) and SD of the average NN intervals for each 5-minute segment (SDANN), indices of sympathetic hyperactivity. They also observed a decrease in a root mean square of successive differences (rMSSD) and the percentage of differences between adjacent normal RR intervals > 50 ms (PNN50), indices of decrease in parasympathetic activity. Rahman et al. [12] evaluated HRV in 100 adult females with IDA using 4 active channels, Polyrite-D. Compared to controls, they observed higher low frequency (LF) power and LF values in normalized units and higher ratio between LF to high frequency (HF) band of spectral analysis of HRV (LF/HF), indices of cardiac sympathetic hyperactivity. They also observed lower total power, HF power and in normalized units, indices of decrease in parasympathetic activity. Furthermore, other studies reported POTS in patients with IDA. Jarjour and Jarjour [22] observed very frequent POTS in females with lower ferritin levels compared to healthy females which have been attributed to parasympathetic dysfunction. Furthermore,

studies indicated that anemia is a risk for progression of heart failure. They suggested that the associated increase in sympathetic activity and adrenergic stimulation may accelerate ventricular remodeling [23]. Groenveld et al. [16] in their systematic review and meta-analysis showed that 37.2% of patients with heart failure were anemic, and the latter was independently associated with an increased risk of mortality in both systolic and diastolic heart failures.

In contrast, some investigators found no difference between HRV in response to deep breathing and Valsalva maneuver in patients with IDA and healthy subjects (despite the presence of tachycardia) [11] and even in presence of moderate/severe IDA [13]. Kapoor et al. [24] showed increased HR but no change in resting systolic BP in mild, moderate or severe anemic cases. Sönmezler et al. [25] found evidence of reduced sympathetic function in children with IDA. The authors found delayed palmar sympathetic skin response (SSR) latency with IDA compared to control subjects. SSR is a polysynaptic reflex and its final efferent pathway (C fibers) involves pre- and post-ganglionic sympathetic sudomotor fibers that activate the sweat glands through sympathetic outflow.

Studies suggested several possibilities for the mechanisms of ANS dysfunction due to IDA. Gradual onset of anemia may allow compensatory mechanisms to take place. They include: (1) A short circulatory time (i.e. increase in resting HR) occurs as a physiological compensatory mechanism to reduction in HB concentration and consequent tissue hypoxia. It has been suggested that hypoxia induced by anemia may result in mitochondrial respiratory chain inhibition or potassium channel suppression which result in increase in intracellular calcium. The latter results in activation of carotid bodies and sympathetic (adrenergic) hyperactivity, tachycardia and increase in cardiac output and systemic vascular resistance (venous and arterial) [16, 23]. This may explain the significant increase in diastolic BP in response to sustained handgrip or exposure to cold observed with IDA compared to pre-testing levels and healthy children. Studies indicated that sustained hand grip increases the concentration of local metabolic wastes [e.g. lactic acid and adenosine] and metaboreceptors' afferent fibers discharge, and initiates a potent reflex sympathetic hyperactivity, vasoconstriction and increase in BP [26, 27]. (2) Studies observed increase the shift of HB oxygen dissociation curve to the right and increase in oxygen extraction of anemic blood by the tissues [28], anaerobic metabolism and local accumulation of local metabolites (e.g. lactic acid) resulting in stimulation of chemoreceptors and increase in non-catecholamine humoral mediator (e.g. adrenal medullary hormones, serotonin and others). These events result in decrease in peripheral resistance, vasodilatation and consequent tachycardia [29]. Some suggested that local hypoxia initiates gene expression secondary to production of factor- 1α which results in vasodilatation and consequently increases the cardiac output [15]. This may explain that the increased susceptibility of patients of IDA to develop orthostatic hypotension and syncope. Glick et al. [15] observed increase in HR in un-anesthetized dog with induced anemia and suggested the following: (a) increase the rhythmicity of the sinoatrial node as a result of increase in the right atrial pressure by increasing the tension of the atrial wall, (b) changes in tissue partial pressure of oxygen produced by anemia may result in local metabolic changes, and (c) non-catecholamine humoral substances released during anemia.

In this study, we observed improvement of ANS dysfunction with iron therapy [10]. This have clinical implications which include: (1) encouraging the use of pharmacological iron supplements (e.g. ferrous sulfate), intake of nutrients rich in heme or organic iron (e.g. meat and shellfish) and non-heme iron (e.g. plants) and the use of supplements which facilitate non-heme iron absorption (e.g. zinc, vitamin C, B12 and B9), (2) discouraging the intake of nutrients and substances which inhibit (e.g. phytates, lignins, and calcium) or interfere with non-heme iron (e.g. dairy products) absorption [30], and (3) testing for ANS function before intense physical exercise and general anesthesia for surgical interventions.

Limitations

a) The sample size was small which may induce statistical bias and higher percentage of ANS abnormalities, however, the prospective nature of study confirms the improvement of ANS manifestations with iron therapy.

b) We did not evaluate HRV using a 24 h Holter which is better and more reliable for estimations of RR intervals. This could be explained by the fact that in our locality, a 24 h Holter monitoring is not readily available for research purposes except for hospitalized critical cases.

c) We did not do arterial blood gases (ABG), or measure CO_2 and/or O_2 for children before participation of the study. However, we do not consider this a major limitation because: (a) Under normal circumstances, rebreathing (as deep breathing and Valsalva testing) results in changes in CO_2, O_2 and arterial pH, and (b) Children with known metabolic problems, cardiovascular disease (e.g. cardiac arrhythmias) and respiratory disease, etc., other causes of ABG changes, were not included in the study.

d) Estimation of the plasma and urine concentrations of catecholamine was not done. Their estimation could provide more support for the diagnosis of sympathetic hyperactivity.

Conclusion

Moderate/severe ANS dysfunctions are common consequences of IDA in children. The common manifestations include fatigue, dizziness, and palpitation at rest, headache, tachycardia, and decrease in HRV to instant and active standing, deep breathing and Valsalva maneuver. Clinical, laboratory and physiological improvement of ANS manifestations occurred with iron therapy. IDA may result in tissue hypoxia resulting in pathological excess of sympathetic activity and reduction of parasympathetic activity. IDA has been suggested as a predictor for arrhythmias, heart diseases and sudden cardiac death. Optimal iron therapy can improve ANS manifestations induced by IDA.

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Declaration of Interests

The authors declared no conflict of interests.

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References

1. Bailey RL, West KP Jr, Black RE. 2015. The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab* 66(Suppl 2): 22-33. https://doi.org/10.1159/000371618
2. Puig S, Ramos-Alonso L, Romero AM, Martínez-Pastor MT. 2017. The elemental role of iron in DNA synthesis and repair. *Metallomics* 9(11): 1483-1500. https://doi.org/10.1039/c7mt00116a
3. Hallberg L, Bengtsson C, Lapidus L, Lindstedt G, Lundberg PA, et al. 1993. Screening foe iron deficiency: an analysis based on bone marrow examinations and serum ferritin determinations in a population sample of women. *Br J Haematol* 85(4): 787-798. https://doi.org/10.1111/j.1365-2141.1993.tb03225.x
4. Means RT. 2013. Iron deficiency anemia. *Clinic Pediatr hematol oncol* 27(2): 101-112. https://doi.org/10.15264/cpho.2020.27.2.101
5. De Chiara B, Crivellaro W, Sara R, Ruffini L, Parolini M, et al. 2005. Early detection of cardiac dysfunction in thalassemic patients by radionuclide angiography and heart rate variability analysis. *Eur J Haematol* 74(6): 517-522. https://doi.org/10.1111/j.1600-0609.2005.00434.x
6. Aytemir K, Aksoyek S, Buyukasık Y, Haznedaroglu I, Atalar E, et al. 2000. Assessment of autonomic nervous system functions in patients with vitamin B12 deficiency by power spectral analysis of heart rate variability. *Pacing Clin Electrophysiol* 23(6): 975-978. https://doi.org/10.1111/j.1540-8159.2000.tb00883.x
7. Connes P, Martin C, Barthelemy JC, Monchanin G, Atchou G, et al. 2006. Nocturnal autonomic nervous system activity impairment in sickle cell trait carriers. *Clin Physiol Funct Imaging* 26(2): 87-91. https://doi.org/10.1111/j.1475-097x.2006.00655.x
8. Singh K, Singh PI. 1994. Autonomic function in chronic severe anemia. *Indian J Med Sci* 48(4): 93-95.
9. Gehi A, Ix J, Shlipak M, Pipkin SS, Whooley MA. 2005. Relation of anemia to low heart rate variability in patients with coronary heart disease (from the Heart and Soul study). *Am J Cardiol* 95(12): 1474-1477. https://doi.org/10.1016/j.amjcard.2005.02.017
10. Yokusoglu M, Nevruz O, Baysan O, Uzun M, Demirkol S, et al. 2007. The altered autonomic nervous system activity in iron deficiency anemia. *Tohoku J Exp Med* 21(4): 397-402. https://doi.org/10.1620/tjem.212.397
11. Tuncer M, Gunes Y, Guntekin U, Gumrukcuoglu HA, Eryonucu B, et al. 2009. Heart rate variability in patients with iron deficiency anemia. *Arq Bras Cardiol* 92(5): 368-371. https://doi.org/10.1590/s0066-782x2009000500011
12. Rahman F, Akhter QS, Akhter FQ, Siddika ST. 2014. Assessment of cardiac autonomic nerve function status in female with iron deficiency anemia. *Bangladesh Med J* 43(3): 125-129. https://doi.org/10.3329/bmj.v43i3.26293
13. Pinkesh G. 2016. Effect of haemoglobin concentration on cardiovascular system by heart rate variability modulations. *Indian J Basic Applied Med Res* 5(2): 855-860.
14. Jibhkate AN, Lath RK. 2019. Assessing severity of involvement of autonomic functions in iron-deficiency anemia patients. *Natl J Physiol Pharm Pharmacol* 9(5): 429-433. https://doi.org/10.5455/njppp.2019.9.0307912032019
15. Glick G, Plauth WH Jr, Braunwalde E, Cook H, Lewis RM. 1964. Role of the autonomic nervous system in the circulatory response to acutely induced anemia in unanesthetized dogs*. *J Clin Invest* 43(11): 2112-2124. https://doi.org/10.1172/jci105085
16. Groenveld HF, Januzzi JL, Damman K, Wijngaarden JV, Hillege HL, et al. 2008. Anemia and mortality in heart failure patients a systematic review and meta-analysis. *J Am Coll Cardiol* 52(10): 818-827. https://doi.org/10.1016/j.jacc.2008.04.061
17. von Haehling S, Jankowska EA, van Veldhuisen DJ, Ponikowski P, Anker SD. 2015. Iron deficiency and cardiovascular disease. *Nat Rev Cardiol* 12(11): 659-669. https://doi.org/10.1038/nrcardio.2015.109
18. 2008. World Health Organization. Iron deficiency anemia assessment, prevention and control.
19. Mathias CJ, Bannister SR. 2013. Autonomic failure. Oxford Medicine Online. https://doi.org/10.1093/med/9780198566342.001.0001
20. Garcic A. 1979. A highly sensitive, simple determination of serum iron using chromazurol B. *Clin Chim Acta* 94(2): 115-119. https://doi.org/10.1016/0009-8981(79)90003-2
21. 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. 1996. *Circulation* 93(5): 1043-1065.
22. Turner LR, Premo DA, Gibbs BJ, Hearthway ML, Motsko M, et al. 2002. Adaptations to iron deficiency: cardiac functional responsiveness to norepinephrine, arterial remodeling, and the effect of beta-blockade on cardiac hypertrophy. *BMC Physiol* 2: 1. https://doi.org/10.1186/1472-6793-2-1
23. Jarjour IT, Jarjour LK. 2008. Low iron storage in children and adolescents with neurally mediated syncope. *J Pediatr* 153(1): 40-44. https://doi.org/10.1016/j.jpeds.2008.01.034
24. Kapoor RK, Singh L, Mehrotra S, Mishra PK, Chandra M. 1999. Demasking of subclinical left ventricular dysfunction in anemic children. *Indian Paediatr* 36(10): 991-998.
25. Sönmezler A, Abuhandan M, Yoldas TK, Oymak Y, Çalık M, et al. 2019. Sympathetic skin response in children with iron deficiency anemia. *IJTSRD* 3(2): 1074-1075. https://doi.org/10.31142/ijtsrd21593
26. Koletsos N, Dipla K, Triantafyllou A, Gkaliagkousi E, Sachpekidis V, et al. 2019. A brief submaximal isometric exercise test 'unmasks' systolic and diastolic masked hypertension. *Hypertens* 37(4): 710-719. https://doi.org/10.1097/hjh.0000000000001943
27. Silverthorn DU, Michael J. 2013. Cold stress and the cold pressor test. *Adv Physiol Educ* 37(1): 93-96. https://doi.org/10.1152/advan.00002.2013
28. Jensen FB. 2004. Red blood cell pH, the Bohr effect and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. *Acta Physiol Scand* 182(3): 215-227. https://doi.org/10.1111/j.1365-201x.2004.01361.x
29. Justus DW, Cornett RW, Hatcher JD. 1957. A humoral influence on cardiovascular adjustments to acute and chronic posthaemorrhagic anemia in dogs. *Circ Res* 5(2): 207-213. https://doi.org/10.1161/01.res.5.2.207
30. Prentice AM, Mendoza YA, Pereira D, Cerami C, Wegmuller R, et al. 2017. Dietary strategies for improving iron status: balancing safety and efficacy. *Nutr Rev* 75(1): 49-60. https://doi.org/10.1093/nutrit/nuw055
31. WHO/UNU/UNICEF Iron Deficiency anemia: Assessment, Prevention and Control, A Guide for Programme Managers, WHO, Geneva, Switzerland, 2001.