International Blood Research & Reviews
1(2): 72-86, 2013, Article no. IBRR.2013.002

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Atrial Function and Glutathione in Children with Iron Deficiency Anemia- Tanta-Egypt-2012

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Authors' contribution

Author ME designed the study, author OT performed the echocardiographic examination. Author IB wrote the protocol, author SMH managed the analyses of the study and author MAB managed the literature searches and wrote the manuscript. All authors read and approved the final manuscript.

Research Article

Received 10th June 2013
Accepted 28th July 2013
Published 26th August 2013

ABSTRACT

Aims: is to correlate the atrial function with the level of oxidative stress marker (Glutathione) in children with Iron deficiency anemia (IDA).
Materials and Methods: Thirty children with IDA and 20 healthy children had serum Ferritin, total blood Glutathione level and studied with conventional trans-thoracic 2-D echocardiography, Tissue Doppler (TDI) and Speckle Tracking Strain (STI) analysis.
Study Design: A case–controlled study
Place and Duration of Study: Pediatric Outpatient Clinic; Pediatric Hematology Unit; Pediatric Cardiology Unit; Pediatric Department; Faculty of Medicine; Tanta University Hospital; Egypt. The study was conducted between January; 2012 to December; 2012.
Results: Children with IDA had significantly low Glutathione [4.63 ±3.4 ng/ml] (P =.013) and Ferritin [11.88 ±5.3 ng/ml] (P < .0001) levels than that observed in the control group. There was no significant increase in LA dimension and volume (minimum) [31± 27 ml] (P = .433), by M-mode but there was significant decrease in e/a ratio assessed by tissue Doppler in IDA patients [1.29 ±0.5] than in controls [1.6±0.7] (P = .038). There were significant decrease in LA velocity (P = .02) and increase in RA velocity (P = .04) compared to left atrial and atrial septal velocity and insignificant increase in left atrial velocity compared to atrial septal velocity. There was no significant correlation between

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Glutathione level and echo-Doppler parameters of atrial function (P >.05), but there was significant negative correlation between Hemoglobin% and atrial septal velocity (P < .05).
Conclusion: IDA is associated with diastolic dysfunction. Tissue Doppler and STI were more sensitive than conventional echocardiography in detection of subclinical structural and functional changes due to hemodynamic abnormality in children with IDA.

Keywords: Iron deficiency anemia; glutathione; oxidative stress markers; atrial function; echocardiography.

1. INTRODUCTION

Iron deficiency anemia (IDA) is the most common type of microcytic anemia and it ranks 9th among 26 diseases with highest burden. It occurs mostly when the dietary intake; absorption, or metabolism of iron is improper, and as a result; the hemoglobin, which contains iron, cannot be formed [1]**.** Reduction in red blood cells and hemoglobin decreases the ability of oxygen absorption from the lungs. The resultant hypoxia and the decrease in oxygenation of RBC hemoglobin; destabilizes the hemoglobin and increases the rate of hemoglobin autoxidation [2]. The low hemoglobin observed in IDA and the consumption of oxygen by the tissues decrease the partial pressure of oxygen to increase the RBC oxygen transferred to the tissues [3,4]. The increased oxidative stress in IDA is associated also with decrease in antioxidants as selenium and zinc especially if accompanied with pica [5]. As a result, there will be an imbalance between the free radicals and the antioxidant molecules inducing a significant oxidative stress that can play an important role in the pathogenesis of IDA with the possible presence of higher oxidative stress in IDA [6].

There is a compensatory physiologic increase in cardiac output in IDA to maintain adequate oxygen delivery. This increased cardiac output is made possible by increasing the blood volume, preload, heart rate, and stroke volume, along with a decrease in after load. If left untreated and prolonged, severe IDA can induce serious problems by causing secondary organ dysfunction or damage [7]. Chronic severe IDA may have deleterious effects on the heart causing cardiac remodeling, cardiomegaly, arrhythmia, left ventricular (LV) hypertrophy and dysfunction or even overt heart failure [8].

As left atrium (LA) has an important role in the overall cardiovascular performance; it may be affected in IDA. LA serves as a reservoir distended by the inflow volume from pulmonary veins during ventricular contraction and isovolumic relaxation and acts as a conduit during the early ventricular diastole. It may work also as a contractile chamber during late ventricular diastoles [9].

In IDA, the LV changes will be reflected also on LA; so that, LA changes may indicate LV changes. Studying the LA cavity by conventional echocardiography through measuring LA volumes by the two dimensional (2 D) and of the left atrium blood flow by pulsed Doppler mitral inflow or pulmonary vein flow parameters; substantially advanced our understanding of LA function in normal and diseased heart. Analysis of LA function by color tissue Doppler imaging (TDI) and speckle tracking (STI) is still not fully explored. The analysis of atrial deformation could have a significant incremental value by integrating the diagnostic findings of myocardial color TDI and STI functional analysis [10].

The relative strength of the association of inflammation and oxidative stress markers with atrial function in IDA remains unclear [11]. The fears of adverse cardiovascular effects of IDA, and the associated effects of oxidative stress that could be linked to Iron deficiency were enough to stimulate us to investigate the cardiac effects of Iron deficiency and relate it to oxidative stress. So; the aim of this study was to investigate the correlation of the atrial function studied by Color TDI and STI with the level of oxidative stress marker (Glutathione) in children with IDA.

2. MATERIALS AND METHODS

The study was conducted as a case–control analysis of 30 consecutive children with iron deficiency anemia (IDA) who attended to Pediatric Outpatient Clinics, Pediatric Department; Tanta University Hospital; Egypt. An age- and sex-matched group of 20 healthy children was studied as a control group. ***Inclusion criteria:*** children with clinically and laboratory recently diagnosed IDA without associated congenital or acquired systemic diseases and age between 1 year and 12 years. ***Exclusion Criteria:*** other causes of anemia (other than Iron deficiency anemia), congenital or acquired heart diseases, other systemic diseases or medications that could affect the cardiac functions. The study was conducted between January; 2012 to December; 2012 and approved by the Institutional Review Board; Faculty of Medicine; Tanta University; Egypt. All the parents of the included children signed a written informed consent before enrolment into the study.

All children were evaluated by a pediatric hematologist and a pediatric cardiologist. A complete history taking, as well as thorough clinical examination, chest X-ray, ECG, complete blood pictures, CRP, hemoglobin electrophoresis, serum Ferritin, and total blood Glutathione level were done. All children had echocardiographic examination by conventional trans-thoracic 2-D echocardiography, TDI and STI analysis. To avoid intra-observer variability, two examinations were performed by the same operator for each patient within 1 week and we considered the average results.

2.1 Echocardiographic Examination

Echocardiographic examination was done using A Vivid 7 ultrasound system (GE Vingmed Ultrasound, Horten, Norway). Data acquisition was performed with a 3.5-MHz transducer at a depth of 16 cm from the standard cardiac views. M-mode and two-dimensional (2-D) images were obtained and stored in cine loop format from 3 consecutive beats. M-mode and 2-D echocardiography - *to assess left ventricular (LV) and atrial internal dimensions, LV ejection fraction, and fractional shortening* - were done according to the recommendation of the American Society of Echocardiography [12]. All children were examined in a semi-supine, left lateral position.

LA maximum anterior-posterior (A-P) diameter was measured in the parasternal long-axis views. LA volumes were measured in the apical 4- and 2-chamber views using a biplane area-length method, and were indexed to body surface area. The ***maximum*** LA volume (pre-A volume) was measured before mitral valve opening while the ***minimum*** LA volume was measured after atrial contraction (Post A volume). LA contractile function was estimated by the LA active emptying fraction (%), calculated by the following equation:

$$[(\text{Maximum LA volume} - \text{Minimum LA volume}) / \text{Maximum LA volume}] \times 100\%]$$

Left ventricular ejection fraction (LVEF) was measured by *Teichholz* formula based on short axis measurements of the left ventricle (LV) inner diameter by M-mode [13]. The LV end-diastolic diameter was obtained from the M-mode images of the parasternal long-axis view. The LV diastolic function was evaluated by the mitral inflow pattern obtained by pulsed-wave Doppler echocardiography. The mitral and tricuspid Doppler signals were recorded in the apical four-chamber view, with the Doppler sample volume placed at the tip of the mitral and tricuspid valve [14].

2.2 Tissue Doppler Imaging (TDI)

Tissue Doppler was performed using the same machine and probe at a depth of 16 cm in the parasternal and apical views (standard long-axis and two- and four-chamber images). Using pulsed-wave angle-corrected color-coded TDI filters, the baseline was adjusted to a low velocity range (-20 to 20 centimeters per second) and Doppler frame rates varied between 80 and 115 frames/s depending on the sector width of the range of interest with minimal gain setting to minimize background noise and to obtain the highest quality images. The sample volume was placed within the myocardium equidistant from the endocardial and epicardial borders. From the apical four-chamber planes, using pulsed-wave TDI, the myocardial velocity curves of septal mitral valve annulus, lateral mitral valve annulus, and lateral tricuspid valve annulus were recorded. The electrocardiogram was connected and traced simultaneously to define the timing of cardiac cycle events. The beginning of QRS complex was used as a reference point [15]. The TDI data were analyzed by an experienced observer blinded to the clinical data. Sample volumes were placed at the basal level in the septum and lateral wall (using the four chamber images) to derive velocity graphs. The systolic wave (S) reflects the systolic function of either right or left ventricle. The early /atrial (e/a) ratio of tricuspid and mitral valve annulus reflects the diastolic function of the right and left ventricle, respectively. Isometric contraction time (ICT) was defined as the time duration between the beginnings of QRS complex in the electrocardiogram to the beginning of TDI systolic wave. The isometric relaxation time (IRT) was defined as the interval between the end of systolic wave and the beginning of the early wave.

For assessment of left atrial and appendage function by TDI; spectral pulse TDI was applied to generate a myocardial velocity curve to assess a regional LA function. To measure the velocity at LA, right atrium (RA), inter-atrial septum (IAS) mid wall; a small sample volume was placed at an atrial segment of interest, about 2 mm for measuring velocity by TDI, because of its thin-walled structure. Peak atrial contraction in late diastole (a-wave velocity) was measured as a traditional parameter of atrial function. Real time color Doppler was superimposed on grey scale with a frame rate > 110 fps. Special attention was paid to the Doppler velocity range to avoid aliasing, briefly, the velocity measured at three points in RA, LA and IAS. The mean peak velocity of atrial contraction was measured in each segment following the "p" wave on the ECG and two consecutive beats were averaged. At least 10 cardiac cycles were recorded at a speed of 100 millimeters per second and the images were stored electronically. TDI measurements were indexed for children's heart size [16].

2.3 Speckle Tracking Imaging (STI)

For speckle tracking analysis of LA chamber, standard grayscale 2-D images were acquired in the 4-chamber and 2-chamber apical views with a stable ECG recording using acoustic-tracking software (Echo Pac, GE, allowing off-line semi-automated analysis of speckle-based strain) to measure global systolic LA myocardial strain with the frame rates of 50-80

Hz [17,18,19]. Particular attention was given to obtain an adequate gray scale image, allowing reliable delineation of myocardial tissue and extracardiac structures. Three consecutive heart cycles were recorded and averaged. LA endocardial surface is manually traced in both 4-chamber and 2-chamber views by a point and-click approach. An epicardial surface tracing was then automatically generated by the system. Peak atrial longitudinal strain (PALS) was measured at the end of the reservoir phase. The time to peak longitudinal strain (TPLS) was also measured as the average of all 12 segments (global TPLS) and by separately averaging values observed in the two apical views (4-chamber and 2-chamber average TPLS). The LA stiffness (Stiffness strain) was estimated by calculating the ratio of E/e' to LA peak strain [20].

2.4 Estimation of Glutathione Level

The blood was collected without using an anticoagulant such as Heparin, Citrate, or Edta allowing blood to clot for 30 minutes at 25 c. Then; the blood was centrifuged at 2,000 xg for 15 minutes at 4℃ and the serum was separated and stored at -20℃. Glutathione was estimated using a carefully optimized enzymatic recycling method using Glutathione Reductase so that the measured Glutathione reflects total Glutathione.

2.5 Data Collection and Statistical Analysis

The demographic data including age, sex and weight; were collected from the patients' medical record, history taking and clinical examinations. Data pertaining to all the previously mentioned investigating tools were collected, processed in a microcomputer and entered into the Microsoft Excel Data-base. The data were analyzed separately for the predominant echocardiographic parameters using SPSS V.16; Chicago, SPSS Inc; USA. The power level of the number of cases in the study was more than 90%. Data are presented as mean (± standard deviation) values. The two-way analysis of variance with repeated measures and chi-square test by SPSS V.16 were used to identify statistically significant differences in the different parameters among the groups. For all analyses, a statistical significance of *P*-value less than 0.05 was used. Wilcoxon's signed-rank test was used to assess the normality of distributions of the data. The Bonferroni correction/adjustment procedure was performed to avoid ''significance'' due to chance only, in multiple comparisons with echocardiographic parameters. Correlation between variables was evaluated using Pearson's correlation coefficient.

3. RESULTS

The study included 30 children with IDA and 20 normal children as a control group. There were no significant differences in age, sex and body weight between the patients and the control group ($P > .05$). The age of the patient group had a mean of 5.31±3.68 years with male to female ratio of 2.7:1 and mean weight of 13.30±8.7 kg. Hemoglobin% and serum Ferritin were significantly low in the patient group than that in the control group ($P < .0001$). There was also a significant reduction of Glutathione level in the patient group than that in the control children ($P = .013$) [Table 1].

Table 1. Demographic data and laboratory results among patients and control children

		Patient group (n=30)	Control (n=20)	t	*P*-Value
Age (year)	Mean	5.31	6.5	0.148	0.883
	SD±	±3.68	±3.2		
Sex Male: Female)	Mean	2.7:1	2.2:1		
	SD±				
Body weight (Kg):	Mean	13.30	14.77	0.616	0.541
	SD±	8.71	±7.48		
Blood Hb%	Mean	7.26	13.5	- 10.4	< 0.0001
	SD±	1.67	2.3		
Serum Ferritin level (ng/ml)	Mean	11.88	70.8	12.8	< 0.0001
	SD±	5.37	20		
Glutathione level (um)	Mean	4.63	7.90	2.6	= 0.013
	SD±	3.46	4.77		

In Table 2 the M-mode showed insignificant increase in LA dimension in children with IDA than control children. However, there was significant increase in both the maximum LA volume (*P* =.003) and LA emptying fraction% in children with IDA than the control group (*P* = .031). At same time there was insignificant increase in minimum LA volume in children with IDA than the control group (*P*= .29). There was no significant difference as regard to E/A ratio assessed by conventional Doppler between IDA patients and control group (*P*=.77). However, there was significant difference in e/a ratio assessed by TDI between IDA patients and control group (*P*=.038) that indicates presence of diastolic dysfunction in IDA patients. There was also a significant decrease between e/a ratio assessed by TDI and significant increase in E/e ratio between IDA patient in comparison to control group (*P*= .037) indicating more sensitivity of TDI in detection of diastolic dysfunction than conventional pulsed Doppler. Table 2 showed also a significant decrease in LA velocity (*P*=.019) and increase in RA (*P*=.041) and AS (*P*=.019) velocities in patients with IDA in comparison to control group ($p<0.05$).There was significant increase in RA velocity compared to LA and atrial septal velocity (*P*=.0001) and insignificant increase in LA velocity compared to atrial septal velocity (*P*>0.05) in patients with IDA. There were significant increase in RA velocity compared to LA velocity (*P* <.001), significant increase in RA velocity compared to atrial septal velocity (*P* <.001) and insignificant increase in LA velocity compared to atrial septal velocity (*P* = .07) in control group.

Table 3 showed insignificant decrease in PALS (*P* = .072) and increase in TPALS (*P* = .77) in IDA patient in comparison to control group. However; there was significant increase in LA stiffness in IDA patient in comparison to control group (*P* = .001).

The study showed also insignificant correlation between Glutathione level as a marker of oxidant stress and echo-Doppler parameters for assessment of atrial function (Table 4).

Table 2. Conventional and Tissue Doppler Findings in both patients and control groups

		Patient group (n=30)	Control (n=20)	t	P-Value
LA Dimension by M-mode (cm)	Mean	2.42	2.26	0.793	0.433
	SD±	0.55	0.54		
Maximum LA volume (ml)	Mean	483.93	42.49	3.088	0.003*
	SD±	58.48	15.46		
Minimum LA volume (ml)	Mean	30.83	20.16	1.060	0.297
	SD±	27.31	13.02		
LA emptying fraction%	Mean	67.07	60.25	2.228	0.031*
	SD±	9.60	11.69		
E/A ratio Mitral	Mean	1.52	1.50	0.288	0.775
	SD±	0.21	0.23		
e/a ratio Mitral	Mean	1.29	1.67	2.11	0.0381
	SD±	0.48	0.68		
E/e ratio Mitral	Mean	8.17	6.90	2.146	0.037*
	SD±	2.55	0.45		
S wave (cm/sec)	Mean	4.2	6.6	4.159	0.001*
	SD±	0.016	0.009		
Right atrium velocity (cm/s) (I)	Mean	9.55	7.57	2.106	0.041*
	SD±	3.30	3.02		
Left atrium velocity (cm/s) (II)	Mean	3.84	5.36	2.420	0.019*
	SD±	2.37	1.75		
Atrial septal velocity (cm/s) (III)	Mean	5.47	3.88	2.419	0.019*
	SD±	2.35	2.06		
F-test	35.328	11.964			
P	0.0001*	0.0001*			
Scheffe test	I vs II, P=0.0001*		I vs II, P=0.020*		
	I vs III, P=0.0001*		I vs III, =0.0001*		
	II vs III= P=0.071		II vs III= =0.158		

Table 3. Speckle Tracking [LA peak longitudinal strain (PALS); Time to peak atrial longitudinal strain (TPLS); and LA stiffness] of the studied patients with iron deficiency anemia and the control group

		Patient group (n=30)	Control (n=20)	t	P-Value
LA peak longitudinal strain (PALS) %	Mean	51.08	58.21	1.841	0.072
	SD±	13.92	12.18		
Time to peak longitudinal strain (TPLS) (second)	Mean	407.61	402.25	0.294	0.770
	SD±	70.10	47.52		
LA stiffness (mmHg)	Mean	0.17	0.11	3.423	0.001*
	SD±	0.07	0.03		

Table (4). Correlation between oxidative stress marker (Glutathione) and echo-Doppler parameters for assessment of LA function among the studied patients with IDA and the control group

Variables	Patients with iron deficiency anemia (n=30)		The control group (n=20)	
	r	P	r	P
LA dimension by M-mode (Cm)	0.207	0.273	0. 218	0.545
Maximum LA volume (ml)	0.196	0.299	-0.249	0.289
Minimum LA volume (ml)	0.246	0.217	0.209	0.238
LA emptying fraction%	0.230	0.222	0.133	0.588
E/A	0.097	0.686	0.337	0.146
e/a	0.001	0.999	0.104	0.671
E/e	0.004	0.983	0.259	0.284
Right atrium velocity (cm/s)	0.075	0.696	-0.148	0.546
Left atrium velocity (cm/s)	0.106	0.577	-0.187	0.444
Atrial septal velocity (cm/s)	0.132	0.488	0.032	0.897
LA peak longitudinal strain (PALS) (m/second)	0.007	0.973	0.175	0.461
Time to peak longitudinal strain (TPLS) (sec)	0.358	0.072	0.339	0.144
LA stiffness (mmHg)	-0.084	0.684	-0.060	0.800

On the other hand; Table 5 showed significant negative correlation between hemoglobin% and atrial septal velocity, while there was no correlation between it and other echo-Doppler parameters for assessment of atrial function. When correlating LA volume (maximum) to LA velocity; we found no significant correlation between the two parameters (P >.05) (Table 6).

Table 5. Correlation between Blood Hemoglobin% and echo-Doppler parameters for assessment of atrial function among the studied patients with iron deficiency anemia and the control group

Variables	Patients with iron deficiency anemia (n=30)	
	r	P
LA dimension (Cm)	-0.227	> 0.05
Maximum LA volume(ml)	-0.281	> 0.05
Minimum LA volume(ml)	0.198	> 0.05
LA emptying fraction%	0.237	> 0.05
E/A	0.191	> 0.05
e/a	0.233	> 0.05
E/e	0.369	> 0.05
Right atrium velocity (cm/s)	-0.308	> 0.05
Left atrium velocity(cm/s)	-0.188	> 0.05
Atrial septal velocity(cm/s)	-0.361	< 0.05*
LA peak longitudinal strain (PALS) %	-0.124	> 0.05
Time to peak longitudinal strain (TPLS) (second)	-0.130	> 0.05
LA stiffness (mmHg)	-0.170	> 0.05

Table 6. Correlation between LA volume maximum and LA velocity among the studied patients with iron deficiency anemia and the control group

Variables	LA volume maximum (ml)	
	Patients with iron deficiency anemia (n=30)	Control group (n=20)
	r P	r P
LA velocity (cm/s)	-0.224 0.234	0.359 0.131

4. DISCUSSION

Iron deficiency and IDA are major public health problems and are considered as the most common nutritional deficiency worldwide [21]. They present with a spectrum of oxidative stress and altered antioxidant activity [22]. Numerous published observations showed that severe iron deficiency can produce cardiac dysfunction and even overt heart failure; one of the possible mechanisms is the associated oxidative stress that is usually related to the severe anemia. However; Glutathione (GSH) plays important roles in antioxidant defense [23].

Echocardiography is one of the most commonly diagnostic tools used to assess the cardiac functions including atrial function. The conventional echocardiography has several limitations need to overcome with new modalities including TDI and STI techniques [24,25]. The current research studied the negative impact of IDA on the LA function and detected presence of subclinical alteration in atrial myocardium in children with IDA and correlated these changes to Glutathione and hemoglobin blood levels. The current study showed preserved LV systolic cardiac function in children with IDA (Preserved LV EF) by conventional echocardiographic examination. These findings were previously confirmed by Nair et al., 2005 who reported that anemic patients had preserved systolic function measured by LV ejection fraction [26]. However, our study detected impaired systolic function by TDI as indicated by significantly lower S wave in IDA patients than in control group. In the current study; there was diastolic dysfunction detected only by TDI and not by the conventional Doppler. This diastolic dysfunction occurs because iron deficiency promotes metabolic and dystrophic alteration in the myocardium which results in early diastolic dysfunction prior to systolic failure [27]. Iron deficiency may be associated with cardiac hypertrophy, ultrastructural changes in mitochondria and sarcomeres, with impairment of myocardial mitochondrial electron transport and increased release of cytochrome c from mitochondria into cytosol in cardiac muscles [28]. Goncharovan and govorin 2008 previously reported presence of diastolic dysfunction in patients with IDA. They showed a decrease in e/a ratio at mitral annulus measured by TDI. They also reported reversal and improvement of the diastolic dysfunction after iron therapy [29]. The cardiac effects associated with IDA are mostly due to the associated tissue hypoxia; numerous hormonal and metabolic effects that can result in direct myocardial toxicity and ischemia [30]. This tissue hypoxia could affect LV diastolic function earlier than systolic function. Diastolic dysfunction without systolic dysfunction may present at an early stage of myocardial ischemia, and this is consistent with the finding that LV diastolic function is more susceptible to ischemia than systolic function [31]. However, Odemis et al., (2006) reported that iron deficiency in the absence of overt anemia didn't lead to important changes in cardiac function [32].

Enlargement of LA may present even in mild anemia and plays a pivotal role in the prognosis [33]. In our study; there was a significant increase in LA volume and emptying fraction in IDA children than that found in the control group. This could be related to volume overload as a compensatory mechanism to presence of anemia and also due to the possible LV diastolic dysfunction that occurs in such patients. Pritchett et al., 2005 found strong association between LV diastolic dysfunction and the increase in LA volume. They proposed that LA volume was a better index of LA remodeling, and there was a strong association between LA volume and the presence of cardiovascular affection [34]. However, atrial functions determined from atrial volumes and volume-derived indices are load–dependent, time consuming, difficult to reproduce and observer-dependent [35]. We should also consider the possible underestimation of the true atrial dimensions with the conventional 2-D and M-Mode echocardiography.

The atrial and appendage functions can also be impaired in anemia especially if severe. These functions can be assessed better with spectral pulse TDI. In the current study; there was significant increase in the RA wall velocity than both LA and atrial septal velocity. The differences in the velocities between RA and LA wall velocities were previously documented by Zhang, et al.; 2006 who studied 131 healthy persons. They found a higher RA velocity than that measured at LA free wall but the lowest velocity was observed at IAS [36]. In our study; there was also a significant increase in RA and AS velocities and decrease in LA velocity in patients with IDA than in control group. This decrease in LA velocity could be related to the long standing preload (due to hyper dynamic state) and after load (due to LV dysfunction) effects. These loading effects may lead to hypertrophy of atrial muscle which may lead to some degree of fibrosis and hence decrease in the wall velocity. The LA volume is not only a good predictor of LA remodeling; but also the LA strain pattern. Tops et al.; 2006 showed that LA strain at baseline was an independent predictor of LA reverse-remodeling. Anemia is also an important negative determinant of reversing LA remodeling [37].

In our study, there were no significant changes in LA strain parameters (as insignificant decrease in LA peak strain (PALS) and no significant increase in time to peak longitudinal strain (TPLS)) in IDA patients than that seen in healthy children. This could be explained by being unlike velocity; the strain is not load-dependant to such extent. Strain is mainly a measure of regional deformity, which is dependent on the ultra structural components of the atrium, such as the extent of atrial myocyte hypertrophy and amount of interstitial fibrosis [38].

On the other hand, LA stiffness was significantly increased in IDA patients than in the control group. It reflects the structural remodeling and deterioration of the LA function. It is also an indication of LV dysfunction, LA dilatation, and collagen synthesis [39]. This may reflect the presence of subclinical systolic dysfunction of the LA (as indicated by the decreased LA velocity) despite the apparently normal LA systolic function (by high LA emptying fraction %). It reflects also the observed LV systolic dysfunction as indicated by low S wave measured by TDI. The reflected effect of LV dysfunction on the LA function and increased stiffness was explained in a previous study done by D 'Andrea et al., 2007 [40]. The increased LV diastolic pressure with increased stiffness or non-compliance of the LV resulted in increase in LA pressure to maintain adequate LV filling. This was also accompanied by increased atrial wall tension which led to chamber dilatation and stretch of the atrial myocardium. Early LV diastolic dysfunction results in augmentation of LA pump function and increasing LA stiffness and work mismatching. With increasing anemia severity

and progression of LV dysfunction, LA pump function started to be impaired as a result of increased after load imposed on the LA myocardium.

In the current study; there was slightly significant decrease in Glutathione level in the patient group than that in the control children denoting an increased oxidative stress among our studied group. Previous studies confirmed the presence of oxidative stress among patients with IDA. Gekova et al., 1982 found reduced Glutathione in the patients with IDA in parallel with the decreased serum Iron and saturation rate of transferrins and enhanced total iron-binding capacity (TIBC) in patients' serum [41]. Yoo et al., 2009 studied the oxidative stress and the imbalance between free radicals and antioxidant molecules and its role in the pathogenesis of iron-deficiency anemia (IDA). They studied 33 patients with IDA and 25 healthy controls using another oxidative stress marker [42]. Their results supported the assumption of presence of higher oxidative stress in IDA; as there was significant decrease in catalase in IDA patient in comparison to control group. A more recent study done by Shet et al., 2012 showed elevated level of Glutathionyl hemoglobin (as a recent potential biomarker of oxidative stress) in patients with IDA than the controls. They found inverse correlations of Glutathionyl hemoglobin with hemoglobin; mean cell volume; serum iron and transferrin saturation [43].

Our study showed no significant correlation between Glutathione level and neither Hb% nor echo parameters. There was also no significant correlation between Hb% and echo parameters except for atrial septal velocity. Absence of correlation between Glutathione and echo finding denotes that the underlying mechanism of cardiac dysfunction may be due to abnormal hemodynamics. Despite lack of correlation between Glutathione level and Hb% observed in our study, some studies showed that decrease level of Glutathione may be an influential factor that can decrease Hb% which may denote the presence of an interaction between the two parameters and vicious circle effect [44,45].

Lack of correlation of most echocardiographic parameter with both Hb% and Glutathione indicates that neither decrease hemoglobin nor Glutathione was the sole mechanism affecting cardiac muscle. So the cardiac dysfunction that occurs in IDA is mulifactorial and does not depend only on a single factor. This assumption was supported with the result of the work of Finch et al., 1976 who showed that the work performance increased to normal when the hemoglobin was corrected, but only after iron therapy. They showed that the iron-deficient rats, had marked impairment in running ability persisted even after hemoglobin was corrected [46].

5. STUDY LIMITATIONS AND CLINICAL IMPLICATIONS

There were number of limitations to our study. First, it is of a single-center nature. Second, the study included relatively few numbers of patients; despite that the statistical power of the study was more than 90%. Also, we did not assess the systolic and diastolic functions of the right ventricle which is an important limitation of our study.

6. CONCLUSION

We concluded that IDA was associated with diastolic dysfunction. There was significant increase in LA volume, decrease in LA longitudinal peak strain and increase in LA stiffness. Assessment of e/a ratio by TDI was more sensitive than E/A ratio assessed by conventional Doppler as it can differentiate the normal and pseudo normal mitral inflow pattern. TDI was

able to detect even subclinical structural and functional alteration of the atrial myocardium. Results in our study denote that atrial dysfunction in IDA patients was due to hemodynamic abnormality. Primary and secondary prevention of IDA should be done in infants, toddlers and children to avoid cardiac complications. Recent echocardiography modalities (tissue Doppler & speckle tracking) are useful in early detection of subclinical myocardial deformation.

CONSENT

The authors declare that all the parents of the included children signed a written informed consent before enrolment into the study.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board; Faculty of Medicine; Tanta University; Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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