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The Correlation of Ferritin and Leptin Serum Levels with Cardiac Involvement in Thalassemia Patients Compared to Controls

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Abstract

Background
Regarding abnormalities in thalassemia major (TM) patients and the effects of leptin and ferritin on heart, aimed to investigate the possible relationship between leptin, ferritin and cardiac involvement in TM patients compared to controls.

Materials and Methods
In total 141 individuals entered to the present case-control study that consisted of 66 TM patients matched in age and sex with 75 healthy controls. The case group selected from those patients attending to the clinic of Ali Asghar Hospital, Zahedan-Iran. From participants, 5ml blood takes by a nurse and was isolated from serum samples in order to analyze ferritin and leptin levels. Major proceedings in patients were checking medical history, physical examination, chest X-ray and Electrocardiogram (ECG) that performed before echocardiography by unique cardiologist. The participants subjected to conventional examination for both right and left heart functions.

Results
Leptin level ($p=0.026$), height, weight and body mass index (BMI) were lower in patients ($p<0.001$). Ferritin levels were 4,625.515±3,782.618 and 49.387±25.386ng/ml in patients and controls, respectively ($p<0.001$). Serum Leptin and ferritin levels were similar in different age groups of patients ($p>0.05$). In left heart, LAd was higher in case (2.44± 0.42) significantly ($p<0.001$). QT ($p<0.001$), QTc ($p<0.001$), S in V_1 ($p=0.030$), and Heart rate ($P<0.001$) were higher in TM patient. In patients, QTc ($p=0.009$), and QTcd ($p=0.002$) correlated with leptin, and IVSS ($p=0.013$), PWD ($p=0.048$), and in right heart, peak E ($p=0.002$), and peak A ($p=0.034$) were correlated with ferritin.

Conclusion
From the present study concluded that PWD and IVSS in left heart and Peak A and Peak E in right heart correlated with ferritin. QTc and QTcd correlated with leptin.

Key Words: Cardiac findings, Children, Ferritin, Leptin, Beta- Thalassemia.

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1- INTRODUCTION

Thalassemia major is a common and genetic disorder that causes severe anemia in early childhood. Every year about 60,000 to 100,000 individuals are burn with thalassemia major around the world (1, 2). This disease is common in the Mediterranean region, the Middle East, and other areas in the world and known as a severe disease. Iran in Middle East with different parts has approximately 25,000 thalassemia patients and three million carriers. Two Persian Gulf and the Caspian Sea regions have more than 10% and Sistan and Baluchestan province has 4 to 10% (3, 4). Leptin is a polypeptide of 146 amino acids that issued widely in different tissues. This hormone is multi-functional, leads to increase energy levels, and affects angiogenesis, inflammation, and hematopoiesis (5). Leptin acts on the hypothalamic receptors and influences the expression of different neuropeptides that regulate energy balance by decreasing food intake and increasing energy expenditure and sympathetic tone in response to normal weight gain (6).

Ferritin is a blood cell protein that contains of Iron. It is the best and the most common test specially in beta thalassemia major. The test is easy to perform compared with other tests for iron overload (7). Due to its variable nature, measuring ferritin level should be done 2-3 times a year and the mean level should be used to measure the level of iron overload in thalassemia patients. Iron is stored routinely which can cause harm various body organs. Iron may deposit in all layers of the heart but the deposits are more likely to occur in the external layer (epicardium) than the internal layer (endocardium). Deposition of iron in myocardium can cause both hypertrophy and dilated heart chambers (8). Mechanisms responsible for raising iron levels consist of blood transfusion, increased iron intake through intestinal absorption, peripheral hemolysis and damaged red blood cells in the first decade of life (7). One of the most common methods to measure the size of heart chambers, systolic and diastolic volume, structure, and function of valves, calculating pulmonary arterial pressure is conventional echocardiography that is produced as an appropriate non-invasive diagnostic method (9). In using this method, several studies have demonstrated that there is an association between ferritin (5, 7, 10), and leptin levels (5) with echo findings in thalassemia patients. In addition, has been reported that the measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle (QT) and its derivation are good parameters of electrocardiography (ECG) that associated with echocardiographic measurements such as left ventricular mass index (LVMI) in patients with thalassemia compared to controls (1). Therefore, regarding highlighted proofs concerning the correlation of ferritin and leptin levels with cardiac functions (3-10), the authors encouraged to investigate the possible correlation of leptin and ferritin serum levels with echo findings in thalassemia patients compared to controls in Sistan and Baluchestan province of Iran.

2- MATERIALS AND METHODS

2-1. Methodology

This case-control study performed on 141 participants consisted of 75 controls (children who referred to hospital for checkup), and 66 patients with thalassemia in pediatric cardiac center in collaboration of center for specific diseases in Ali Asghar Hospital, Zahedan city, Sistan and Baluchestan province (South East of Iran) during the year of 2017.

2-2. Criteria

All thalassemia patients who had regular transfusions to maintain pre-transfusion hemoglobin with the levels higher than 10

g/dL entered to the study and one and a half times children considered for the controls. Exclusion criteria were valvular disease, rhythm and structural abnormality, active infection, systemic inflammatory diseases, renal insufficiency, and these criteria considered for both groups. In addition, from 99 healthy children, during the clinical examination 24 children refused the study because of long process.

2-2. Echocardiography measurements

Major proceedings in patients were checking medical history, physical examination, chest X-ray and Electrocardiogram (ECG) that performed before echocardiography in which performed by unique cardiologist. ECG findings were as follow: QT: a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. QTc: QT / √ RR. NFQTc: (new formula for QTc in which is equal to 2qt/ (1+RR). qTd: the difference between the maximum and minimum QT values. QTcd: the difference between the maximum and minimum QTc values. NFQTcd: (new formula for QTcd in which is equal to 2qtd/ (1+RR). RV_5: (R wave in V_5) the amplitude of R wave in left Precordial. Sv_1: (S wave in V1) the amplitude of S wave in right Precordial lead. All cases and controls underwent simultaneous 12-leads standard (made in Japan) (3). Echocardiography was performed 48-72 hours after packed red blood cell transfusion on patients by same pediatric cardiologist with using of My lab 60 with transducer 3, 8 (made in Italy).

For having high precision in information echocardiography repeated 3 cycles in 2D, M-Mode, Doppler method and the average was considered. Echocardiogram applied in participants without breath holding, and view taken in the mitral valve surface in parasternal position. LVDD: left ventricular end-diastolic dimension, AT: Acceleration time, DT: deceleration time, Peak E: early mitral and tricuspid valve flow velocity, Peak A: late mitral and tricuspid valve flow velocity, LAd: Diameter of LA in Diastole, Aod: Diameter of Aorta in Diastole, LAs: Diameter of LA in Systole, Aos: Diameter of Aorta in Systole, Et: ejection time (for Aorta and Pulmonary), PWDD: posterior wall dimension in diastole, IVSD: interventricular septal dimension in Diastole, IVSS: interventricular septal dimension in systole, EF: ejection fraction, FS: fractional shortening, LVM: left ventricular mass, RWT: relative wall thickness, LVMI: left ventricular mass index, measured by conventional echocardiography and myocardial performance index (MPI), isovolumic relaxation time (IRT), isovolumic contraction time (ICT) of both sides, were measured with pulsed Doppler echocardiography (11).

The sample volume was positioned at the tips of the tricuspid and mitral valve leaflets in the apical four-chamber view to enable the measurement of (a): that is the time of interval between the end and the start of trans-mitral and trans-tricuspid flow. The sample volume thereafter relocated to the left ventricular outflow tract just below the aortic valve (apical five-chamber view), to measure b: which is the left ventricular ejection time. The right ventricular outflow velocity pattern recorded from the parasternal short-axis view with the Doppler sample volume positioned just distal to the pulmonary valve for the measurement of (b). Myocardial Performance Index (Tei Index) was calculated as: a-b/b = IRT + ICT/ET. The left ventricular mass index (LVMI), was also calculated by the following formula: LVM (g) = 0.8 (1.04 [LVEDD + PWTD + IVSTD)^3 - LVEDD 3]) + 0.6. And LVMI (g/m^2) = LVM / 2.7 (g/m^2). All the parameters in the above formula measured in the M-mode view and in diastole and utilized for left ventricular

mass evaluation (2). Relative Wall Thickness (RWT), was calculated as 2 times PWD divided by the LVEDD (3).

2-3. Ferritin and leptin measurements

From participants, 5ml blood takes by a nurse at 8:00 am. Samples were centrifuged at 3000 g for 10 minutes at 5 ° C. Separated serum kept at -70 fridges until measurement time of ferritin and leptin. Finally, under the cold chain, the samples transferred to the Biochemistry Lab of Zahedan University of Medical Sciences (ZaUMS). Then, 250 micron was isolated from serum samples in order to analyze ferritin by ELISA method/kit (USA), and the other serum samples used for evaluation of leptin level by ELISA method Kit (Canada).

2-4. Anthropometric measurements

The height and weight of children were performed by standard equipment by an experienced expert. The recumbent length for children under 2 years were graded using a flat wooden table and weight measurements for children under 2 years were performed by using balance weights Mika with difference of 100gr and then BMI calculated [Weight (Kg) / Height (m^2)].

2-5. Ethical Approval

Consent form obtained from the participants or their guardians after the study approval. The study approved as a project proposed (ID-code: 7230) to the Children and Adolescent Health Research Center by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran.

2-6. Statistical Analysis

Data analyzed by SPSS version 18.0 (SPSS Inc, Chicago, IL, USA**).** Data reported as mean ± standard deviation (SD) for continuous variables or percentage for categorical variables. Differences between the two groups were evaluated with t-tests and the correlation examined by Pearson test. The level of significant considered as $p < 0.05$.

3- RESULTS

The participants in the study were 75 and 66 in healthy and patients, respectively. Sex distribution was similar in groups (r=0.075, p= 0.373) (**Table.1**) (*please see the table in the end of paper*). Means' age were 11.424±3.938 and 11.267±4.041 year-old (p>0.05) for case and controls, respectively. Serum leptin levels of the patients and controls were 3.739±6.254 and 7.260±11.293 ng/ml, respectively (p=0.026). Height, weight and BMI were lower in patients significantly (p<0.001). Serum ferritin levels of the patients and controls were 4625.515±3782.618 and 49.387±25.386 ng/ml, respectively (p<0.001) (**Table.2**) (*please see the table in the end of paper*).

Table.3 showed the sex comparison on mean of some variables in patients and controls separately. In patients, females' age was higher than males' age (p=0.906). Females' serum leptin levels was higher than males in patients group (p=0.005), and observed same trends for controls. Height, weight and BMI were lower in males (p>0.05) and in thalassemia patients, males and females' serum ferritin levels was similar (p>0.05) (*please see the table in the end of paper*).

Table.4 showed some variables means comparison in different age groups of patients, controls and combined population. Serum Leptin and ferritin levels were similar in different age groups of patients, controls and combined population (p>0.05). In the patients population BMI were significantly different in age groups (p<0.001). Elder patients had higher BMI values (*please see the table in the end of paper*). **Table.5** showed the echocardiography findings of conventional left and right heart and electrocardiogram (ECG) parameters

comparison between case and control. In left heart, LAd was higher in cases (2.44± 0.42) compared to controls (2.22± 0.37) significantly (p<0.001) (*please see the table in the end of paper*).

Other significant findings were Aos (p= 0.010), LAs (p<0.001), Et (p=0.040), IVSD (p<0.001), PWD (p<0.001), IVSS (p=0.036), LVDS (p=0.040), PWS (p<0.001), EF (p<0.001), FS (p<0.001), LAs/Aos (p<0.001), LAd/ Aod (p<0.001), Simpson EF (p=0.043), MPI (p<0.001), LVM (p=0.001) and LVMI (p=0.001). In right heart the findings of ET (p=0.001), and Peak E/A (p=0.001) were significant, both were higher in controls and MPI (p<0.001) which was higher in case group; and ECG parameters of QT (p<0.001), QTc (p<0.001), SV_1 (p=0.030), and Heart Rate (P<0.001) were significant and all were higher in case.

Table.6 showed the Leptin level correlation with echocardiography and ECG findings in all participants and patients group. Regarding all participants the analysis showed that all the right and the left echo findings and ECG parameters were not correlated significantly with serum leptin level (p>0.05) (*please see the table in the end of paper*). But considering the patients group, observed that a few parameters were correlated significantly. These parameters belonged to ECG parameters and were QTc (r= 0.247, p=0.009), and QTcd (r= 0.283, p=0.002).

Table.7 showed the ferritin level correlation with left and right heart echocardiography and ECG findings in all participants and patients group (*please see the table in the end of paper*). Regarding all participants, DT (r= 0.164 , p=0.013), Et (r=- 0.143, p=0.031), IVSD (r= 0.346, p<0.001), LVDD (r= 0.168, p=0.011), PWD (r= 0.192, p=0.004), IVSS (r= 0.262, p=0.002), LAAo (r= 0.197 , p=0.019), LAAO.s (r= 0.268, p=0.001), Peak E/A (r=- 0.160, p=0.016), MPI (r= 0.316, p<0.001), BMI (r= -0.153, p=0.021), LVMI (r= 0.274, p=0.001), LVM(r= 0.274, p=0.001), Simpson IVSD (r= 0.180, p=0.007), Simpson EF (r= -0.202, p=0.002), FS mod (=- 0.350 , p<0.001), and EF (r= -0.317, p<0.001) in left heart, and A (r= 0.134, p=0.045), Peak A (r= 0.159, p=0.017), ET (r= -0.143, p=0.031), and MPI (r= 0.336, p<0.001). In right heart were significantly correlated with ferritin level. Amongst the ECG parameters, QT(r= 0.386, p<0.001), and QTc (r= 0.255, p<0.001) were significant all these variable in the significant levels were for participants. Regarding only the patients, the correlation of ferritin and echo findings observed for a few right and left heart. IVSS (r= 0.306, p=0.013), PWD (r= 0.187, p=0.048), and in right heart, peak E (r= 0.294, p=0.002), and peak A (r= 0.200, p=0.034).

4- DISCUSSION

The present study performed on 141 participants that formed of 75 controls and 66 TM patients. Girls were a little more than boys in both case and control groups. In case and control comparison, height, weight, BMI and leptin levels were more in controls while ferritin increased in patients. In patients, leptin was higher in females. Age group comparison of the patients showed that the levels of BMI were higher in the age group of 15-18 years, and it shows that elder children tend to be overweight or obese. Shaharamian et al. (12) resulted that leptin increased in controls and ferritin increased in patients. They also found that leptin had a significant correlation with the gender. Khalilian et al. (7) conducted a study on a number of thalassemia patients and found level of 2,419.13+177.65 for ferritin serum, consisted with, Ashena et al. (10) who performed approximately same study, so that both of these studies had lower level of ferritin than present study. Aessopos et al. (13) showed that overall mean ferritin levels was 1,657±1,477ng/ml

which was lower than what we found in the present study (2,419.13±1,772.65ng/ml) that could be the result of less frequent blood transfusions and misusing of iron-chelation therapy. Choobine et al. (14) resulted that the serum leptin level was higher than the present study in patients; and in overall the results of comparison between case and control was similar. Al-Naama et al. (15) presented that, leptin was meaningful lower in thalassemia patients and found a significant and direct correlation between leptin and BMI. They revealed that BIM was lower and ferritin was higher in thalassemia patients. They also found that male patients had lower level of leptin compared to their counterparts similar with the results that observed in Greece (16), and Iran (5, 14), and similar with the present study that showed males had a non-significant increase in leptin levels.

The present study showed that leptin levels was lower in higher age, but not significantly and this result was not confirmed Choobineet al.'s study (14) that revealed a significant correlation between age and leptin levels. This dissimilarity probably could be due to the different of age patients in study. The cause of higher leptin level in girls compared to boys considered more adipose tissue in girls and difference in sex hormones that diminished iron overload in girls. The most probable reason for such difference is the toxic effects of iron on cell membranes and proteins in thalassemic, since free iron causes peroxidative damage in lipid membrane and proteins with creating free radicals. Thus, in iron overload following the destructions of adipocyte and then leptin level decreased. Furthermore, the replacement of red bowel movement (BM) with yellow BM, which contains adipocytes, can be a cause of this decrease (14). From the analysis of the present study revealed that in left heart, LAd, Aos, LAs, ET, IVSD, PWD, IVSS, LVDS, PWS, LAs/Aos, LAd/Aod, MPI, LVM, and LVMI were higher in TM patients and Aos, EF, FS, ET, peak E/A, and Simpson EF in controls. In right heart the findings of ET and Peak E/A was significant, both were higher in controls and MPI which was higher in case group. The parameters of ECG such as QT, QTc, SV_1 and heart rate were significant and all were higher in cases. ECG findings of QTc and QTcd correlated with leptin in thalassemia patients and this pattern for ferritin was for PWD, IVSS in left; and peak E and Peak A were correlated with ferritin in right heart. Noori et al., conducted several studies (2, 3, 11) to compare echocardiography findings in thalassemia and healthy children with different main goals. Noori et al. (2) resulted that left heart findings of EF, FS, MPI were different in case and controls in which were similar with the other studies by Noori et al. (3, 11) as well with the present study. EF and FS were higher and MPI was lower in controls compared to patients in all Noori et al. studies similar to the present study.

The result of the LVMI was lowering in control (2) similar with the present study. LVDD was lower (3) and higher (2) in control when in the present study it was higher in control. Right ejection time was lower in case resulted by Noori et al. (3, 11) similar with the present but in another study they found similarity in case and control (2). LAd/Aod and LAs/Aos were lower in controls in the study conducted by Noori et al. (3), and the results were similar with the present study. Noori et al. presented that MPI and Peak E/A was different between case and control in the right heart that all were similar with the present study (2, 3, 11). Khalilian et al. (7) found that the left ventricular systolic dysfunction approximately was similar with our finding. In their study the evaluation of the relationship between ferritin level and ejection fraction

displayed no significant relationship when we found a significant correlation for two findings of PWD and IVSS in left heart, peak E and Peak A in right heart. The difference between these two studies was likely due to the number of echocardiography findings and age of participants. Shivanna et al. (17), conducted a study on thalassemia patients and found that the majority of echocardiography findings in their study were correlated with ferritin. Other reports did not find any significant relationship of serum ferritin concentration with systolic and diastolic indices (10). Tanner et al. (19) conducted a study by T2 MRI and concluded that thalassemia patients who were treated with monotherapy (deferoxamine) had more occurrences of LV dysfunction and reported Iron overload as a main cause in these. However, Bosi et al. (20) found a weak but significant correlation between left ventricular ejection fraction and serum ferritin concentration, where patients with a high ferritin concentration (>2,500ng/ml) had a lower ejection fraction than patients with a low level. In our study found that EF and FS were dissimilar but Simpson EF was similar with Bosi et al. (20) results.

Borgna-Pignatti et al. (21) in an echocardiographic study showed that cardiovascular prognosis was acceptable when serum ferritin was <2,500ng/ml in thalassemia patients and illustrated a negative correlation between serum ferritin and left ventricular ejection fraction. Khalilian et al. (7) demonstrated that the serum ferritin <2,500ng/ml was safe in patients with thalassemia major, but Derchi et al. (22) reported that this data highlighted the importance of careful evaluation of cardiac functional status in patients. Heart diseases are the base case for prognosis and estimating the survival in major thalassemia patients, in this regards, myocardia iron deposition seems to be a major development trigger cardiac involvements in these patients (23). Therefore, the prognosis improved by intensifying blood transfusions and iron-chelation therapy as well as proper treatments. Hyder et al. (24) confirmed that early detection of the associated complications in thalassemia patients was immensely beneficial in reducing the burden of the disease through preventive measures and regular assessment of cardiac functions that may help to improve the quality of life for these patients and may reduce the morbidity and mortality to a great extent and in final advised to be performed echocardiogram annually. The majority of patients with thalassemia major have left ventricular dysfunction, mainly, due to chronic anemia, iron overload and poor compliance with chelation therapy, but intermedia thalassemia patients was due to higher cardiac output, splenectomy, and greater intravascular hemolysis to generate pulmonary hypertensions (25). Anderson et al. (26) confirmed that heart failure due to iron overload is often reversible with intravenous iron chelation with desferrioxamine.

4-1. Limitation of study

The study limitation was lack of proper corporation by participants especially controls.

5- CONCLUSIONS

From the present study concluded that a low numbers of the echocardiography findings of PWD and IVSS in Left heart and Peak A and Peak E in right heart correlated with ferritin. Also, concluded that none of the echocardiography findings correlated with leptin. Amongst ECG parameters, QTc and QTcd were correlated with leptin. Furthermore, additional research is required to clarify the biochemical and physiological changes in children with thalassemia that affect the levels of serum leptin and ferritin.

6- ABBREVIATIONS

Simpson EF: EF was calculated in the apical chamber; LVDD: left ventricular end-diastolic dimension; a: is the time of interval between the end and the start of trans-mitral and trans-tricuspid flow; At: Acceleration time; Dt: deceleration time; Peak E: early mitral and tricuspid valve flow velocity; PeakA: late mitral and tricuspid valve flow velocity; LAd: Diameter of LA in Diastole; Aod: Diameter of Aorta in Diastole; Las: Diameter of LA in Systole; Aos: Diameter of Aorta in Systole; Et: ejection Time (for Aorta and Pulmonary); PWDD: posterior wall dimension in diastole; IVSD: interventricular septal dimension in Diastole; IVSS: interventricular septal dimension in systole; EF: ejection fraction; FS: fractional shortening; LVM: left ventricular mass; RWT: relative wall thickness; MPI: myocardial performance index; LVMI: left ventricular mass index; ICT: isovolumic contraction time; IRT: Isovolumic relaxation time; BMI: Body Mass Index.

7- CONFLICT OF INTEREST: None.

8- ACKNOWLEDGMENTS

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9- AUTHORS CONTRIBUTION

Noori designed the study, Teimouri analyzed data, Noori, Birjandi and Teimouri wrote the primary version of manuscript. All Authors are agreeing for the present manuscript publication.

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Table-1: The sex distribution in case and control groups

Groups	Gender		Total	P-value
	Female	Male		
Control	42	33	75	0.373
	56.0%	44.0%	100.0%	
Case	32	34	66	
	48.5%	51.5%	100.0%	
Total	74	67	141	
	52.5%	47.5%	100.0%	

Table-2: The comparison of major indices of the study in case and control groups

Variables	Groups	Mean	SD	T- value	P- value
Age, year	Control	11.267	4.041	-0.234	0.815
	Case	11.424	3.938		
Leptin, ng/ml	Control	7.260	11.293	2.247	0.026
	Case	3.739	6.254		
Height, cm	Control	155.160	13.912	8.241	<0.001
	Case	132.667	18.411		
Weight, kg	Control	44.827	12.692	7.913	<0.001
	Case	29.212	10.436		
Ferritin, ng/ml	Control	49.387	25.386	-10.482	<0.001
	Case	4625.515	3782.618		
BMI	Control	18.277	3.056	5.122	<0.001
	Case	15.942	2.228		

BMI: Body mass index; SD: Standard deviation.

Table-3: The comparison of major indices of the of participants in case and control groups according to the gender

Case group						Control group			
Variables	Gender	Mean	SD	T value	P value	Mean	SD	T- value	P- value
Age, year	Female	11.38	3.53	0.119	0.906	11.238	3.95	-0.069	0.945
	Male	11.3	4.45			11.303	4.22		
Leptin, ng/ml	Female	7.723	11.21	2.868	0.005	9.643	12.88	2.109	0.038
	Male	3.281	6.22			4.228	8.09		
Height, cm	Female	146.91	18.10	1.449	0.15	155.833	13.91	0.47	0.639
	Male	142.12	21.11			154.303	14.09		
Weight, kg	Female	39.01	14.06	1.334	0.184	45.476	13.13	0.497	0.62
	Male	35.87	13.91			44	12.27		
Ferritin, ng/ml	Female	1879.3	2929.99	-1.13	0.26	48.643	24.34	-0.284	0.777
	Male	2536.12	3937.73			50.333	27.01		
BMI	Female	17.39	3.10	0.877	0.382	18.381	3.23	0.331	0.741
	Male	16.96	2.74			18.144	2.87		

BMI: Body mass index; SD: Standard deviation.

Table-4: The serum levels of leptin, ferritin, and BMI of participants in case and control groups according to the age

All Participants						Control Participants				Thalassemia Participants			
Variables	Age groups	Mean	SD	F- value	P- value	Mean	SD	F- value	P- value	Mean	SD	F- value	P- value
Leptin, ng/ml	<10	6.99	11.65	1.425	0.244	9.14	13.16	1.264	0.289	3.93	8.37	0.185	0.832
	10-14	4.27	6.09			5.76	8.8			3.23	2.8		
	15-18	4.42	7.35			4.35	8.05			4.52	6.57		
	Total	5.61	9.42			7.26	11.29			3.74	6.25		
Ferritin, ng/ml	<10	1643.51	2991.75	2.325	0.102	52.23	24.12	1.918	0.154	3916.79	3619.28	0.85	0.432
	10-14	3047.46	4111.44			52.58	32.45			5154.96	4253.14		
	15-18	2112.81	3104.51			38.5	15.55			5130	2856.62		
	Total	2191.4	3448.85			49.39	25.39			4625.52	3782.62		
BMI	<10	16.75	3.31	2.353	0.099	18.38	3.16	0.156	0.856	14.42	1.81	19.076	<0.001
	10-14	17.24	1.89			17.94	2.31			16.76	1.39		
	15-18	18.18	3.21			18.42	3.68			17.82	2.5		
	Total	17.18	2.93			18.28	3.06			15.94	2.23		

BMI: Body mass index; SD: Standard deviation.

Table-5: Left and Right conventional echocardiography and ECG comparison in case and control groups

Echo findings	Groups	Mean	SD	T- value	P- value	Echo findings	Mean	SD	T- value	P- value
Echocardiography findings in Left						Simpson LVDD (cm)	57.1	21.79	-0.73	0.469
							60.07	26.8		
a (ms)	Control	419	36.2	0.84	0.4	Simpson LVSD (cm)	29.4	12.5	-1.65	0.101
	Case	413	50				33.5	16.83		
AT (ms)	Control	59.6	7.71	-1.16	0.25	Simpson EF (%)	48.84	8.98	2.038	0.043
	Case	61.3	9.59				45.59	9.95		
DT (ms)	Control	135	21.2	-0.81	0.42	LMPI	0.46	0.16	-7.77	<0.001
	Case	138	23.7				0.69	0.20		
Peak E (cm/s)	Control	102	21.7	0.75	0.46	LVM (g)	40.91	13.20	-3.25	0.001
	Case	99.6	15.1				50.26	20.58		
Peak A (cm/s)	Control	52.1	9.22	-1.82	0.07	LVMI(g/m2)	15.15	4.89	-3.25	0.001
	Case	55.4	12.2				18.62	7.62		
Aod (cm)	Control	2.04	0.34	0.96	0.34	RWT	0.18	0.03	-1.87	0.064
	Case	1.99	0.33				0.22	0.15		
LAd (cm)	Control	2.22	0.37	-3.19	0	Echocardiography findings in Right heart and Electrocardiography				
	Case	2.44	0.42							
Aos (cm)	Control	1.96	0.32	2.85	0.01	a (ms)	431.21	35.396	-1.58	0.115
	Case	1.8	0.35				441.58	42.266		
LAs (cm)	Control	1.49	0.25	-3.45	0	AT (ms)	62.59	8.979	-0.82	0.412
	Case	1.66	0.3				64.02	11.578		
ET (ms)	Control	254	31.2	2.05	0.04	DT (ms)	131.88	23.527	-0.08	0.935
	Case	245	23.1				132.2	22.376		
IVSD (cm)	Control	0.66	0.11	-4.22	<0.001	Peak E (cm/s)	69.8	11.287	-0.27	0.789
	Case	0.75	0.13				70.371	14.035		
LVDD (cm)	Control	3.88	0.43	-0.46	0.64	PeakA (cm/s)	49.255	12.439	-1.11	0.268
	Case	3.93	0.73				51.492	11.305		

PWD (cm)	Control	0.35	0.05	-2.95	0	ET (ms)	260.87	26.226	3.501	0.001
	Case	0.39	0.09				246.97	19.992		
IVSS (cm)	Control	0.83	0.15	-2.12	0.036	PeakE/A	1.484	0.313	3.285	0.001
	Case	0.88	0.14				1.342	0.336		
LVDS (cm)	Control	2.08	0.29	-2.12	0.04	MPI	0.51	0.14	-7.70	<0.001
	Case	2.23	0.5				0.71	0.16		
PWS (cm)	Control	0.35	0.05	-3.1	0	QT (s)	.347	.020	-7.393	<0.001
	Case	0.39	0.08				.374	.034		
EF (%)	Control	78.65	18.1	11.66	<0.001	QTc (s)	.441	.043	-4.853	<0.001
	Case	68.58	34.1				.476	.063		
FS (%)	Control	46.88	13.46	15.12	<0.001	QTCd (s)	50.351	9.534	-0.837	0.403
	Case	35.86	18.27				51.371	8.769		
Las / Aos	Control	0.777	0.151	-6.219	<0.001	RV5(mm)	8.368	2.835	-0.420	0.675
	Case	0.937	0.154				8.518	2.500		
LAd / Aod	Control	1.104	0.202	-4.171	<0.001	SV1(mm)	4.702	2.306	-2.184	0.030
	Case	1.233	0.157				5.451	2.829		
Peak E/A	Control	2.02	0.46	2.91	0.004	HR Beat in min	88.857	21.628	3.623	<0.001
	Case	1.84	0.46				98.974	20.335		

Simpson EF: EF was calculated in the apical chamber; LVDD: left ventricular end-diastolic dimension, a: is the time of interval between the end and the start of trans-mitral and trans-tricuspid flow; At: Acceleration time; Dt: deceleration time; Peak E: early mitral and tricuspid valve flow velocity; PeakA: late mitral and tricuspid valve flow velocity; LAd: Diameter of LA in Diastole; Aod: Diameter of Aorta in Diastole; Las: Diameter of LA in Systole; Aos: Diameter of Aorta in Systole; Et: ejection Time (for Aorta and Pulmonary); PWDD: posterior wall dimension in diastole; IVSD: interventricular septal dimension in Diastole; IVSS: interventricular septal dimension in systole; EF: ejection fraction; FS: fractional shortening; LVM: left ventricular mass; RWT: relative wall thickness; MPI: myocardial performance index, LVMI**:** left ventricular mass index; ICT: Isovolumic contraction time; IRT: Isovolumic relaxation time.

Table-6: The correlation of Leptin level and echocardiography findings in right, in left and ECG in study groups

All participants			Thalassemia		All participants			Thalassemia	
Variables	Pearson Correlation	P-value	Pearson Correlation	P-value	Variables	Pearson Correlation	P-value	Pearson Correlation	P-value
Echocardiography findings in Left Heart					FS (%)	0.053	0.535	0.119	0.340
A (ms)	0.003	0.96	0.038	0.689					
AT (ms)	0.03	0.659	-0.013	0.888	LVM (g)	-0.095	0.264	0.89	0.476
DT (ms)	0.04	0.553	0.105	0.269	Simpson LVDD (cm)	-0.05	0.456	-0.002	0.981
Peak E(cm/s)	0.064	0.342	0.00	0.998	Simpson LVSD (cm)	-0.069	0.301	0.119	0.21
Peak A (cm/s)	0.047	0.486	0.059	0.536	Simpson EF (%)	0.045	0.497	-0.167	0.078
Aod (cm)	-0.056	0.399	-0.053	0.581	EF (%)	0.158	0.061	0.129	0.301
LAd (cm)	-0.065	0.327	-0.065	0.498	Echocardiography findings in Right Heart				
Aos (cm)	-0.058	0.386	-0.059	0.537	A (ms)	-0.03	0.652	0.072	0.45
Las (cm)	-0.071	0.291	-0.078	0.42	AT (ms)	-0.043	0.52	0.081	0.398
Et (ms)	-0.074	0.271	0.046	0.632	DT (ms)	-0.009	0.895	0.025	0.791
IVSD (cm)	-.131	0.123	0.099	0.428	Peak E (cm/s)	0.005	0.942	0.021	0.83
LVDD(cm)	-0.079	0.234	0.065	0.499	Peak A(cm/s)	0.092	0.17	0.167	0.078
PWD(cm)	-0.095	0.155	0.019	0.838	ET(ms)	-0.096	0.15	-0.024	0.805
Ivss (cm)	-0.060	0.478	0.66	0.598	MPI	0.044	0.602	0.148	0.236
Lvds(cm)	-0.131	0.123	0.099	0.428	Peak E / A	-0.084	0.211	-0.171	0.071
Pws (cm)	-0.063	0.343	-0.055	0.565	Electrocardiography				
LAd / Aod	-0.024	0.778	0.019	0.881	QT (s)	-0.071	0.291	0.004	0.964
Las / Aos	-0.075	0.379	-0.033	0.795	QTc (s)	0.006	0.934	.247**	0.009
Peak E / A	-0.019	0.782	-0.063	0.508	QTCd (s)	0.063	0.345	.283**	0.002
MPI	-0.003	0.968	-0.019	0.0882	RV5(mm)	0.028	0.672	-0.095	0.32
BMI	0.006	0.929	0.016	0.867	Sv1(mm)	0.033	0.625	0.145	0.128
LVMl (g/m^2)	-0.095	0.264	0.089	0.476	HR beats in min	0.011	0.865	0.092	0.336

Table-7: The correlation of ferritin level and echocardiography findings in right, in left and ECG in case and control groups

All participants			Thalassemia		All participants			Thalassemia	
All	r	P-value	r	P-value	Total	r	P-value	r	P-value
Echocardiography findings in Left Heart					LVM (g)	0.274	0.001	0.164	0.187
a (ms)	0.013	0.846	-0.081	0.397					
AT (ms)	0.08	0.229	0.037	0.7	Simpson LVDD (cm)	0.123	0.066	0.091	0.341
DT (ms)	.164	0.013	0.07	0.466	Simpson LVSD (cm)	.180	0.007	0.117	0.219
Peak E (cm/s)	0.021	0.757	0.114	0.233	Simpson EF (%)	-.202	0.002	-0.078	0.414
Peak A (cm/s)	.210	0.001	0.124	0.193	FS (%)	-0.350	<0.001	0.064	0.612
Aod (cm)	0.099	0.139	0.018	0.849	EF (%)	-0.317	<0.001	0.081	0.519
LAd (cm)	0.11	0.099	-0.003	0.979	Echocardiography findings in Right Heart				
Aos (cm)	0.091	0.175	0.023	0.812	A(ms)	.134	0.045	-0.035	0.713
Las (cm)	0.119	0.075	0.002	0.983	AT(ms)	0.081	0.227	0.038	0.691
Et (ms)	-.143	0.031	0.007	0.939	DT(ms)	0.054	0.422	0.008	0.933
IVSD (cm)	0.346	<0.001	0.227	0.067	Peak E(cm/s)	0.091	0.173	.294**	0.002
LVDD (cm)	.168	0.011	0.144	0.129	Peak A(cm/s)	.159	0.017	.200*	0.034
PWD (cm)	.192	0.004	0.187*	0.048	ET(ms)	-.143	0.031	0.099	0.298
Ivss (cm)	0.262	0.002	0.306	0.013	Peak E/A	-0.101	0.131	0.071	0.459
Lvds (cm)	0.123	0.065	0.015	0.872	MPI	0.336	<0.001	-0.061	0.629
Pws (cm)	0.13	0.051	0.037	0.695	Electrocardiography				
LAd / Aod	0.197	0.019	-0.062	0.621	QT(s)	.386	0	0.168	0.076
Las / Aos	0.268	0.001	-0.094	0.455	QTc(s)	.255	0	0.091	0.339
Peak E / A	-.160	0.016	-0.068	0.473	QTCd (s)	0.049	0.459	0.024	0.798
MPI	0.316	<0.001	-0.104	0.406	RV5(mm)	0.071	0.287	0.105	0.271
BMI	-.153	0.021	0.109	0.251	Sv1(mm)	0.058	0.384	-0.061	0.52
LVMl(g/m^2)	0.274	0.001	0.164	0.187	HR beats in min	-0.127	0.057	0.048	0.612

Simpson EF: EF was calculated in the apical chamber; LVDD: left ventricular end-diastolic dimension; a: is the time of interval between the end and the start of trans-mitral and trans-tricuspid flow; At: Acceleration time; Dt: deceleration time; Peak E: early mitral and tricuspid valve flow velocity; PeakA: late mitral and tricuspid valve flow velocity; LAd: Diameter of LA in Diastole; Aod: Diameter of Aorta in Diastole; Las: Diameter of LA in Systole; Aos: Diameter of Aorta in Systole; Et: ejection Time (for Aorta and Pulmonary); PWDD: posterior wall dimension in diastole; IVSD: interventricular septal dimension in Diastole; IVSS: interventricular septal dimension in systole; EF: ejection fraction; FS: fractional shortening; LVM: left ventricular mass; RWT: relative wall thickness; MPI: myocardial performance index; LVMI: left ventricular mass index; ICT: isovolumic contraction time; IRT: Isovolumic relaxation time; BMI: Body Mass Index; r: Pearson Correlation.