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Tumor Necrosis Factor alpha, N-terminal Pro Brain Natriuretic Peptide and Interleukin-6 Correlations with Doppler Tissue Imaging findings in Major Thalassemia Patients

Noor Mohammad Noori [1], *Alireza Teimouri [1], Maryam Nakhaee Moghadam [1], Hossein Bagheri [2]

[1]Children and Adolescents Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences and Health Services, Zahedan, Iran.

[2]English Department, Medical School, Zahedan University of Medical Sciences and Health Services, Zahedan, Iran.

Abstract

Background

In respect to abnormalities in thalassemia patients and the effects of TNF_α, IL_6, and NT-pro-BNP on echocardiography findings the study aimed to investigate TNF_α, NT-pro-BNP and IL_6 correlations with Doppler tissue imaging findings in major thalassemia.

Materials and Methods: This case-control study was performed on 112 healthy children and 112 patients with thalassemia in Ali Asghar hospital, Zahedan, Iran, in 2017. Healthy children were selected from those that referred to the pediatric clinics for routine check-up. Fasting blood samples were taken to measure TNF-α, NT-pro-BNP and IL_6 serums. Those thalassemia patients who had regular transfusions of hemoglobin higher than 10 g/dl and 48-72 h after packed red blood cell transfusion and those who were asymptomatic were entered to the study. Participants underwent echocardiography by pediatric cardiologist in Doppler method.

***Results*:** NT-pro-BNP, TNF_α and IL_6 were lower in controls (P<0.001). Height, weight and BMI were higher in controls (P<0.001). Right ET, left ET, IVSD, PWD, PWS, EF, FS, left ET', left ICT', left IRT', right ET', right IRT', right S', right Peak A', VE', VA', left and right MPI', LVMI were different in case and control (P<0.05). Right Peak A (P=0.049), PWD (P=0.032), Left MPI' (P=0.02) and LVMI (P=0.005) were correlated with IL_6. IVSD (P=0.01), LVDS (P=0.01), EF and FS (P<0.001), left ET' (P=0.02), left ICT' (P<0.001), right ET' (P=0.001), right S' (P=0.002), left MPI' (P<0.010) were correlated with NT-pro-BNP.

Conclusion

NT-pro-BNP, TNF_α, IL_6 were higher in thalassemia patients and NT- pro-BNP was correlated with some of the echocardiography findings. This suggests that NT-pro-BNP would be the best biomarker for evaluation of cardiac functions in thalassemia patients.

Key Words: Children, Tumor necrosis factor-α, NT-pro-BNP, Interleukin-6, Thalassemia.

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Corresponding Author:

Alireza Teimouri: M.Phil, Ph.D in Demography, Children and Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran.

Email: Alirezateimouri260@gmail.com

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1- INTRODUCTION

Thalassemia is blood disorders are inherited from parents and are characterized by abnormal hemoglobin. Newborn babies with thalassemia account for 60,000 to 100,000 cases every year globally (1, 2). It occurs in Mediterranean Sea area and extends in a line to regions in the Far East and has now become a global health problem because of migration (3, 4). Iran is located in Mediterranean belt and has approximately 25,000 thalassemia patients and three million carriers. In Iran, the Persian Gulf and the Caspian Sea areas have a prevalence of more than 10% and Sistan and Baluchistan (SB) province has 4 to 10% (1). The province of SB has thalassemia gene frequency in high numbers of patients who are receiving regular health services such as blood transfusion (1, 3, 5).

In thalassemia, one of the most important organs that is impressed by iron overload is heart (6). Natriuretic Peptides (NPs) have been considered as diagnostic biomarkers for cardiovascular diseases, and they contain B-type natriuretic peptide (BNP), prohormone brain natriuretic peptide (pro BNP). In comparison, N Terminal Pro B Type Natriuretic Peptide (NT-pro BNP), is more stable with longer half-life, about 2 h compared to BNP (7), and it is useful for screening asymptomatic patients who are high risk such as Thalassemia patients (2).

In the early phases of the cardiac involvement, the level of NT-pro-BNP increases before an increase in diastolic pressure and there is a strong relationship between plasma levels of NT-pro-BNP and iron overload (8, 9). A significant relationship between NT-pro-BNP and some diastolic dysfunctions has been observed (9), and approved that an increase can be used as a tool for primary detection of cardiac hemosiderosis (1). Cytokines have great immunologic actions and are considered as important catabolic factors and it seems that cytokines such as tumor necrosis factor alpha (TNF-α), and interleukin IL-6 are important mediators of the cachectic process in cardiac patients and are secreted secondary to several chronic diseases. In addition, they play an important role in cardiac cachexia and exacerbate prognosis (5). In sickle cell anemia, TNF- α has been linked as a risk factor for the occurrence of painful crises, as well as participating in the occlusion of the microcirculation (7). Moreover, pro-resorptive levels of TNF-α and IL-6 are elevated in patients with thalassemia.

Several studies have demonstrated that there is an association between NT-Pro-BNP (1), TNF _α (5), IL_6 (5, 7), and echocardiography findings in thalassemia, congenital heart defect (CHD), and sickle cell anemia patients (2). Tissue Doppler Echocardiography (TDE) has become an established arm of diagnostic ultrasound examination and uses frequency shift of ultrasound waves to calculate myocardial velocity. Two techniques are used to assess myocardial function: pulsed TDE and color-coded TDE. It has the advantage of increased spatial resolution and ability to evaluate multiple structures in a single view (10). The usefulness of TDE in detection of myocardial iron overload has been reported (11).

Kanazirev et al., found that in asymptomatic individuals' diastolic dysfunctions by TDE are proper and significant tools in the prognostic development of symptomatic iron-induced cardiac disease (11). Regarding abnormalities in thalassemia patients such as cardiac involvement and the association with TNF-α, IL-6 and NT-pro-BNP the present study aimed to investigate the possible relationship between these cytokines and NT-pro-BNP with DTE findings in thalassemia patients compared to healthy children.

2- MATERIALS AND METHODS

2-1. Study design

This case-control study was performed on 224 participants aged 7 to 18 years consisted of 112 healthy children as controls and 112 patients with thalassemia as case group in pediatric cardiac center in collaboration with Center for Specific Diseases in Ali Asghar hospital, Zahedan city, Sistan and Baluchestan province, Iran, during the year 2017. Controls were chosen from those healthy children that referred to the clinics for routine check-up.

2-2. Criteria

Those thalassemia patients who had regular transfusions of hemoglobin higher than 10 g/dl and 48-72 h after packed red blood cell transfusion were entered to the study. Exclusion criteria in both groups of participants were valvular heart disease, rhythm and structural abnormality, active infection, systemic inflammatory diseases, renal insufficiency, and these criteria were considered for both groups.

2-3. Echocardiography measurements

Major proceedings on patients were checking medical history, physical examination, chest X-ray and echocardiography which was performed by one pediatric cardiologist. Echocardiography was performed on patients by the same pediatric cardiologist using My lab 60 with transducer 3, 8 [made in Italy). In order to achieve high precision in echocardiography findings, measurement were repeated for 3 cycles and the average was considered. Echocardiogram was performed on participants without them holding their breath, and view was taken in the mitral valve surface in parasternal position (1). Echocardiography findings were as follows: LVDD: left ventricular end-diastolic dimension, Peak E: early mitral and tricuspid valve flow velocity, Peak A: late mitral and tricuspid valve flow velocity, ET: ejection time (for Aorta and Pulmonary), PWD: 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, LVMI: left ventricular mass index, were measured using conventional echocardiography of the left side and estimated from three cardiac cycles. Myocardial performance index (MPI), isovolumic relaxation time (IRT), isovolumic contraction time (ICT) of both sides, were measured with echocardiography (12). 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): the time of interval between the end and the start of trans-mitral and trans-tricuspid flow.

The sample volume was thereafter relocated to the left ventricular outflow tract just below the aortic valve (apical five-chamber view), so as to measure (b): which is the left ventricular ejection time. The right ventricular outflow velocity pattern was also 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/m2) = LVM / 2.7 (g/m2). All the parameters in the above formula were measured in the M-mode view and in diastole and were utilized for left ventricular mass evaluation (1). Doppler tissue echocardiography (DTI) was another method that was performed from the apical four-chamber view and a 3 mm pulsed Doppler sample volume was placed at the

level of lateral mitral annulus. Myocardial velocity profiles of the lateral tricuspid annulus and lateral mitral annulus were obtained by placing the sample volume at the junction of the tricuspid annulus and the right ventricle (RV) free wall and at the junction of the mitral annulus and LV posterior wall, respectively. With this modality, the recorded values were the early (E) and late (A) diastolic mitral and tricuspid annular velocities, and the ratio of E/A. Right ventricle and left ventricle myocardial performance index (MPI) was obtained by dividing the sum of isovolumic relaxation time (IRT) and isovolumetric contraction time (ICT) by the ejection time (ET) (MPI = (ICT + IRT)/ET) (**Figure.1**).

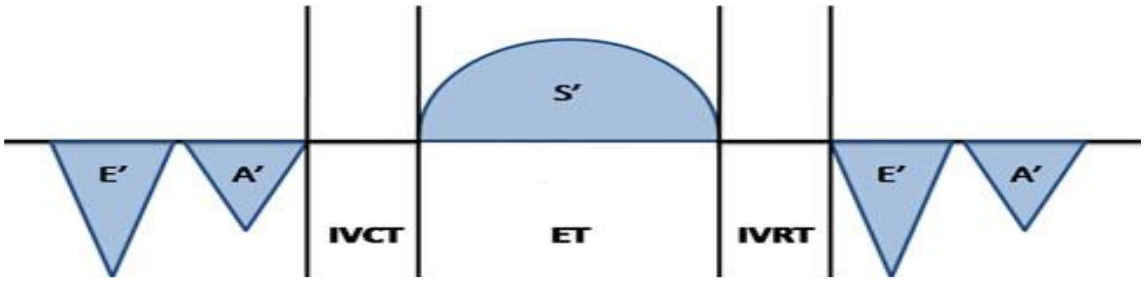

Fig.1: Diagram of Doppler Tissue Echocardiography waves: S', systolic wave; E', early diastolic wave; A', late diastolic wave (12).

Left and right S': Systolic myocardial velocity above the baseline in pulmonic vein, mitral and tricuspid Left and Right E': early diastolic myocardial relaxation velocity below the baseline in pulmonic vein, mitral and tricuspid. Left and Right A': myocardial velocity associated with atrial contraction in pulmonic vein, mitral and tricuspid.

2-3. NT-pro-BNP, TNF- α and Interlukin_6 measurements

To measure NT-pro-BNP, TNF- α and IL_6, 3 milliliters (ml) of blood was taken from all participants at 8.00 AM after an overnight fasting. The samples were centrifuged at 3,000 rpm/min for 10 min at 5 °C. Separated serum of NT-pro –BNP, TNF- α and IL_6 were kept in -70cc refrigerator until measuring. The samples were transferred to the biochemistry library of Zahedan University of Medical Sciences with considering the cold chain circumstances and then 250 microns isolated serum samples were selected for measuring the levels of NT-pro-BNP and as well as TNF- α and Interlukin_6 by ELISA kit (USA).

2-4. Anthropomorphic measurements

Children's height and weight were measured. The recumbent length for children under 2 years was graded using a flat wooden table and weight measurements for children under 2 years were performed by using Mika balance weights with difference of 100gr. Then BMI was calculated with (Kg / m^2) (weight in kilograms divided by height to the power of 2) formula.

2-5. Ethical Approval

Consent form was obtained from the participants or their guardians after the study approval. The study was approved as a project proposed to the Children and Adolescent Health Research Center by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (ID number: 6798).

2-6. Statistical Analysis

Statistical software of SPSS version 20.0 for windows was used for all statistical analyses. Data were reported as mean ± standard deviation (SD) for continuous

variables or percentage for categorical variables. Differences in baseline characteristics between the two groups were evaluated with Mann-Whitney U test for all continuous variables, all categorical variables were compared using chi-square test. All p-values were two-sided and the results were considered statistically significant at the level of $p<0.05$.

3- RESULTS

The study aimed to assess NT-pro-BNP, TNF_α and IL_6 correlations with echocardiography findings in thalassemia patients. In the results, due to normality analysis of Kolmogorov-Smirnov test, the null hypothesis stated that the data followed free distribution because of the p-value that was less than 0.0.05 in the majority of variables except a few (left Peak E and left Peak E' with p-value lower than [0.05]), especially considering all participants. Participants' sex distribution was similar between case and controls (Chi-square=0.029 and P=0.866) (**Table.1**). **Table.2** showed that controls and patients were matched by age ($p>0.05$). The table also showed that NT-pro-BNP (119.55±199.84 vs. 606.21±993.34), TNF_ (23.18±13.43 vs 39.72±18.54) and IL_6 (3.01±1.00 vs 23.49±13.84) were lower in controls significantly compared to patients ($P<0.001$). Height, weight and BMI were significantly higher in controls compared to patients ($P<0.001$). Amongst echocardiography findings, Right ET ($P<0.001$), Left ET ($P<0.001$), IVSD ($P<0.001$), PWD ($P=0.029$), PWS (0.014), EF ($P<0.001$), FS($P<0.001$), left ET' ($P<0.001$), left ICT' ($P=0.041$), Left IRT' ($P<0.001$), right ET' ($P=0.004$), right IRT' ($P=0.004$), right S' ($P<0.001$), right Peak A' ($P=0.049$), VE'($P=0.010$), VA' ($P=0.011$), left and right MPI' ($P<0.001$), LVMI ($P=0.004$) were different between case and control significantly.

Table-1: Sex distribution of participants in patients and controls.

Gender	Statistics	Groups of participants		Chi-square	P-value
		Control	Case		
Female	Number (%)	37(50.74%)	36(49.26%)	0.029	0.866
Male	Number (%)	33(49.30%)	34(50.70%)		

Table-2: Case-control comparison of the interested variables in the study.

Variables	Participants	Mean	SD	Mean Rank	Sum of Ranks	M-W U	P- value
Age	Control	11.49	4.04	70.4	4928	2443	0.977
	Case	11.27	3.92	70.6	4942		
NT-pro-BNP	Control	119.55	199.84	50.54	3538	1053	<0.001
	Case	606.21	993.34	90.46	6332		
TNF	Control	23.18	13.43	52.06	3644.5	1159.5	<0.001
	Case	39.72	18.54	88.94	6225.5		
Interlukin	Control	3.01	1	38.42	2689.5	204.5	<0.001
	Case	23.49	13.84	102.58	7180.5		
Height	Control	155.04	14.32	91.41	6399	986	<0.001
	Case	134.07	18.83	49.59	3471		
Weight	Control	44.7	12.92	90.56	6339	1046	<0.001
	Case	30.27	11.25	50.44	3531		

BMI	Control	18.23	3	84.3	5901	1484	<0.001
	Case	16.14	2.49	56.7	3969		
Left Peak E	Control	102.09	17.91	71.51	5006	2379	0.767
	Case	99.7	19.98	69.49	4864		
Left Peak A	Control	52.42	8.84	68.09	4766	2281	0.481
	Case	54.86	12.43	72.91	5104		
Right Peak E	Control	70.13	11.28	72.54	5077.5	2307.5	0.552
	Case	70.22	13.85	68.46	4792.5		
Right Peak A	Control	49.18	12.73	64.5	4515	2030	0.080
	Case	51.37	11.14	76.5	5355		
Right ET	Control	288.15	28.97	90.68	6347.5	1037.5	<0.001
	Case	260.39	21.16	50.32	3522.5		
Left ET	Control	287.76	21.99	97.94	6855.5	529.5	<0.001
	Case	248.14	26.76	43.06	3014.5		
IVSD	Control	0.66	0.11	56.32	3942.5	1457.5	<0.001
	Case	0.75	0.13	84.68	5927.5		
LVDD	Control	3.9	0.4	68.09	4766.5	2281.5	0.482
	Case	3.92	0.73	72.91	5103.5		
PWD	Control	0.36	0.05	63.06	4414	1929	0.029
	Case	0.38	0.09	77.94	5456		
IVSS	Control	0.83	0.15	64.78	4534.5	2049.5	0.094
	Case	0.88	0.14	76.22	5335.5		
LVDS	Control	2.1	0.29	66.57	4660	2175	0.252
	Case	2.21	0.52	74.43	5210		
PWS	Control	0.36	0.05	62.11	4347.5	1862.5	0.014
	Case	0.39	0.08	78.89	5522.5		
EF	Control	73.88	18.69	94.66	6626.5	758.5	<0.001
	Case	42.07	34.51	46.34	3243.5		
FS	Control	42.87	13.83	95.01	6651	734	<0.001
	Case	22.7	18.93	45.99	3219		
LVM	Control	51.19	15.59	66.59	4661.5	2176.5	0.254
	Case	55.4	22.29	74.41	5208.5		
Left ET'	Control	361.84	86.78	91.41	6399	986	<0.001
	Case	263.03	52.87	49.59	3471		
Left ICT'	Control	94.33	15.41	77.49	5424	1961	0.041
	Case	89.69	28.98	63.51	4446		
Left IRT'	Control	70.51	12.29	47.79	3345	860	<0.001
	Case	96.03	38.38	93.21	6525		
Left S'	Control	8.95	2.02	71.76	5023	2362	0.714
	Case	8.69	1.57	69.24	4847		
Left Peak E'	Control	14.65	2.52	71.54	5007.5	2377.5	0.762
	Case	14.57	3.26	69.46	4862.5		

Left Peak A'	Control	6.42	1.85	67.52	4726.5	2241.5	0.384
	Case	6.67	1.49	73.48	5143.5		
Right ET'	Control	280.03	58.98	80.4	5628	1757	0.004
	Case	255.89	38.81	60.6	4242		
Right ICT'	Control	75.84	13.93	69.6	4872	2387	0.791
	Case	77.98	18.57	71.4	4998		
Right IRT'	Control	96.14	21.69	60.84	4258.5	1773.5	0.004
	Case	107.61	22.57	80.16	5611.5		
Right S'	Control	64.51	20.47	81.87	5731	1654	0.001
	Case	42.81	41.33	59.13	4139		
Right Peak E'	Control	14.58	2.51	67.99	4759	2274	0.463
	Case	14.63	2.98	73.01	5111		
Right Peak A'	Control	6.69	2.14	63.75	4462.5	1977.5	0.049
	Case	7.75	2.99	77.25	5407.5		
VS'	Control	10.18	2.12	76.24	5337	2048	0.094
	Case	9.62	1.63	64.76	4533		
VE'	Control	17.03	2.19	79.31	5551.5	1833.5	0.010
	Case	15.92	2.71	61.69	4318.5		
VA'	Control	8.87	2.84	79.21	5545	1840	0.011
	Case	7.78	1.63	61.79	4325		
Left MPI '	Control	0.48	0.11	45.07	3155	670	<0.001
	Case	0.74	0.25	95.93	6715		
Right MPI'	Control	0.63	0.12	57.45	4021.5	1536.5	<0.001
	Case	0.74	0.18	83.55	5848.5		
LVMI	Control	23.64	7.42	60.71	4250	1765	0.004
	Case	28.85	11.85	80.29	5620		
Left A/A’	Control	9.79	7.64	70.41	4928.5	2443.5	0.978
	Case	8.84	4.68	70.59	4941.5		
Left E/E’	Control	7.32	2.9	69.25	4847.5	2362.5	0.715
	Case	7.25	2.62	71.75	5022.5		
Right E/E’	Control	5.03	1.72	72.56	5079.5	2305.5	0.547
	Case	5.12	2.07	68.44	4790.5		
Right A/A’	Control	8.94	8.68	74.32	5202.5	2182.5	0.265
	Case	7.91	6.45	66.68	4667.5		

M-W U: Mann-Whitney U, SD: Standard Deviation, Peak E: Early mitral and tricuspid valve flow velocity, Peak A: Late mitral and tricuspid valve flow velocity, ET: Ejection time (for Aorta and Pulmonary), IVSD: Interventricular septal dimension in diastole, LVDD: Left ventricular end-diastolic dimension, PWD: Posterior wall dimension in diastole, IVSS: Interventricular septal dimension in systole, LVDS: Left ventricular diameter at systole, PWS: Posterior wall dimension in systole, EF: Ejection fraction, FS: Fractional shortening, LVM: Left ventricular mass, ICT: Isovolumetric contraction time, ICT: Isovolumetric contraction time, S': Systolic myocardial velocity above the baseline in pulmonic vein, mitral and tricuspid, E' : early diastolic myocardial relaxation velocity below the baseline in pulmonic vein, mitral and tricuspid. A': myocardial velocity associated with atrial contraction in pulmonic vein, mitral and tricuspid, MPI: Myocardial Performance Index (Tei Index), BMI: Body Mass Index, LVMI: Left Ventricular Mass Index.

Table.3 showed the correlation between Doppler finding and major variables of the study. Left Peak E was correlated with BMI (P<0.001). Right Peak A had correlation with IL_6 (P=0.049). Left ET correlated with height (P=0.001) and weight (P=0.008). IVSD correlated with NT-pro-BNP (P=0.01), height (P<0.001), weight (P<0.001), and age (P<0.001). LVDD correlated with height (P<0.001), weight (P<0.001), age (P<0.001) and BMI (P=0.024). PWD correlated with IL_6 (P=0.032), height (P=0.032), age (P=0.001). LVDS correlated with NT-Pro-BNP (P=0.01), height (P=0.005), weight (P=0.009), age (P=0.001), and BMI (P=0.038). PWS was correlated with height (P=0.045), age (P=0.002). EF and FS were correlated with NT-pro- BNP (P<0.001), height (P<0.001), weight (P<0.001), age (P<0.001), and BMI (P<0.001). Left ET' was correlated with NT-pro-BNP (P=0.02), weight (P=0.024), age (P=0.001), and BMI (P=0.016). Left ICT' was correlated with NT-pro- BNP (P<0.001), height (P<0.001), weight (P<0.001), and age (P<0.001).

Right ET' was correlated NT-pro- BNP (P=0.001), height (P=0.003), weight (P<0.001), age (P=0.001) and BMI (P=0.004). Right ICT' was correlated with age (P=0.036). Right IRT' correlated with age (P<0.001). Right S' was correlated with NT-pro- BNP (P=0.002), height (P=0.001), weight (P=0.001) and age (P=0.001). VE' was correlated with height (P=0.04). VA' was correlated with IL_6 (P=0.002). Left MPI' was correlated with NT-pro- BNP (P<0.010), height (P=0.003), weight (P=0.02), and age (P<0.001). Left MPI' correlated with IL_6 (P=0.02), height (P<0.001), weight (P=0.003) and age (P<0.001). LVMI correlated with IL_6 (P=0.005), height (P<0.001), weight (P<0.001), age (P<0.001) and BMI (P=0.025). Left E/E' was correlated with BMI (P=0.009) (*Please see the table.3 in the end of paper*).

4- DISCUSSION

The results of the present study revealed that NT-pro-BNP, TNF-α, IL_6, were higher in case. As an adaptive mechanism of chronic anemia, heart rate is expected to be higher in thalassemia patients. Many studies indicated that NT-pro-BNP is a sensitive biomarker to detect asymptomatic LV dysfunction especially with an important role in diagnostic and prognostic thalassemia implications. These studies proposed an increase of NT-pro-BNP in thalassemia patients compared to controls that is in line with the present study results (1, 3, 13). In this line Noori et al., conducted a study on children and adolescents to find the correlation between NT-pro-BNP and cardiac involvement in patients with thalassemia and resulted that the NT-pro-BNP levels were significantly higher in thalassemia children compared to controls (1). Noori et al. (14), Kremastinos et al. (3), and Ozyoruk et al. (15), reported higher levels of NT-pro-BNP in the patients than controls, all these results were in line with the present study. Aessopos et al., found high levels of NT-Pro BNP in patients with overt heart failure, independent of severity of cardiac insufficiency (6, 16). In this line Kanazirev et al. (11) arrived at the same conclusion of higher level of NT-pro-BNP in thalassemia patients compared to controls. It was hypothesized that a relationship between serum chemokines and inflammatory responses in thalassemia patients may be established because of the presence of active inflammation in these patients. A broad range of cytokines such as, granulocyte-macrophage-colony-stimulating factor (GM-CSF), IL_1, IL_3, IL_6, IL_8 and TNF_α are released by activated endothelium and these have been detected in the plasma of patients with sickle cell disease (SCD) (17). Abnormal IL_6 productions may play a significant

role in a number of diseases, such as autoimmune diseases, chronic inflammation, and lymphoid malignancies (18). The present study showed a low level of IL_6 for the asymptomatic thalassemia patients compared to controls that was in line with Asem Metwally Abo-Shanab et al. (19), Elzubeir et al. (18), Akcalı et al. (20), and El-Rasheidy et al. (21). It was suggested that an increase in TNF_α could be a cause for macrophage activation due to iron overload and the antigenic stimulation induced by chronic transfusion therapy. The activated macrophages were selectively phagocytosing apoptotic erythroid precursors, thereby contributing to ineffective erythropoiesis (22). Garadah et al. (23), and Ragab et al. (24) reported that TNF_α had higher level in patients compared to controls. All these results were similar to the present study.

Noori et al. (1, 25, 26) conducted some studies with the aims of echocardiography findings comparison between thalassemia patients and healthy group. In all studies, Noori et al., concluded that left heart findings of EF, FS and MPI were varied in groups that was consistent with the present study. EF and FS were higher and MPI was lower in controls compared to patients in all Noori et al.'s studies similar to the present study. The result of the LVMI was lower in control (25), consistent with the present study. LVDD was lower (1), and higher (25) in control while in the present study, it was higher in control. Right ejection time was lower in case resulted by Noori et al. (1, 26) in agreement with the present study but in another study they found similarity in case and control (25). Noori et al. presented that MPI and Peak E/A were different between case and control in the right heart that all were similar with the present study (1, 25, 26). Recently Noori et al. (14), performed a study on The Correlation of Ferritin and Leptin Serum Levels with Cardiac Involvement in Thalassemia Patients Compared to Controls and concluded that PWD, PWS, EF, FS and right ET were different in patients and controls which were in line with the present study results. The ratio of E/E' as an important marker of diastolic dysfunction was either normal or increased, especially in cases of restrictive pattern of diastolic dysfunction and correlated with NT-pro-BNP (4). Kremastinos et al. (3, 13) found that NT-pro-BNP was correlated with E/E' in thalassemia patients and concluded that NT-pro-BNP may be an early marker of diastolic dysfunction. Similarly, Garadah et al. (23) reported higher NT-pro-BNP, greater E wave velocity with shorter deceleration time and a greater E/A ratio with a tendency to restrictive diastolic dysfunction in thalassemia patients. They establish a higher E/E' ratio and lower S' and E' values compared to controls. Sarray et al. (27) concluded that IL_6 was correlated with the duration of vaso-occlusive crisis, but TNF_α was not correlated. Compared to the present study, it is observed that IL_6 had significant correlation with LVSD and TNF_α had significant correlation with left MPI in thalassemia patients. Akpinar et al. (28) stated that NT-pro-BNP was correlated with systolic longitudinal myocardial velocities dissimilar with the present study.

The reason for the difference may be due to the type of thalassemia patients that asymptomatic patients considered in the present study. Noori et al., conducted a study to evaluate the correlation of NT-pro BNP and heart findings in thalassemia patients and found that with using a reference cut point for NT pro BNP, LAd, LVDS and RWT correlated with NT pro BNP levels patients (1). From the study it can be concluded that there is a correlation between Doppler finding and major variables of the study. Right Peak A had correlation with IL_6, IVSD correlated with NT-pro-BNP, PWD correlated with IL_6, LVDS correlated with NT-Pro-BNP,

EF and FS were correlated with NT-pro-BNP, Left ET' was correlated with NT-pro- BNP, Left ICT' was correlated with NT-pro- BNP, Right ET' was correlated NT-pro- BNP, Right S' was correlated with NT-pro- BNP, VA' was correlated with IL_6. Left MPI' was correlated with NT-pro- BNP, Left MPI' correlated with IL_6, LVMI correlated with IL_6. Surprisingly, from the results concerned with correlation analysis it is resulted that TNF_α was not correlated with any of echocardiography findings when NT- pro-BNP was correlated with some of the echocardiography findings compared to IL_6.

4-1. Study Limitations

The study limitations were lack of proper cooperation by participants especially controls and selecting asymptomatic patients amongst thalassemia.

5- CONCLUSIONS

NT-pro BNP, TNF-α, IL_6 were higher in thalassemia patients and they correlated with some of echocardiography findings. From the study it is also concluded that TNF_α was not correlated with any of echocardiography findings when NT- pro-BNP was correlated with some of the echocardiography findings compared to IL_6. The findings of the study suggested that NT-pro BNP would be the best biomarker for evaluation of cardiac functions in thalassemia patients.

6- CONFLICT OF INTEREST: None.

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Table-3: Correlation of NT-pro-BNP, TNF_α, IL_6, and anthropometric indices with echocardiography findings in thalassemia patients.

Variables	Statistics	NT-pro-BNP	TNF_α	Interleukin	Height	Weight	BMI	Variables	NT-pro-BNP	TNF_α	Interleukin	Height	Weight	BMI
Left Peak E	P.C	-0.052	-0.10	-0.142	0.073	-0.111	-.323	Left S'	0.127	0.06	-0.084	0.001	0.025	0.049
	P-value	0.669	0.411	0.24	0.55	0.359	0.006		0.295	0.622	0.491	0.994	0.835	0.684
Left Peak A	P.C	-0.097	-0.027	-0.078	0.082	0.062	0.004	Left Peak E'	-0.002	-0.16	-0.126	0.055	0.099	0.096
	P-value	0.423	0.825	0.523	0.502	0.611	0.976		0.986	0.185	0.297	0.653	0.415	0.427
Right Peak E	P.C	-0.213	-0.135	-0.198	0.094	0.054	-0.012	Left Peak A'	0.228	0.022	-0.007	-0.156	-0.093	0.026
	P-value	0.076	0.264	0.1	0.437	0.655	0.92		0.057	0.858	0.952	0.196	0.445	0.833
Right Peak A	P.C	-0.118	-0.056	-0.236	-0.074	-0.034	0.006	Right ET'	0.446	0.132	0.026	-0.347	-0.374	-.337
	P-value	0.331	0.644	0.049	0.543	0.78	0.962		<0.001	0.277	0.832	0.003	0.001	0.004
Right ET	P.C	-0.108	-0.087	-0.002	0.199	0.15	0.033	Right ICT'	-0.099	0.062	-0.103	0.211	0.171	0.086
	P-value	0.374	0.473	0.987	0.099	0.216	0.787		0.416	0.608	0.395	0.079	0.157	0.479
Left ET	P.C	-0.055	0.04	-0.073	0.394	0.313	0.074	Right IRT'	-0.147	0.132	-0.076	0.173	0.087	-0.009
	P-value	0.649	0.744	0.551	0.001	0.008	0.544		0.226	0.276	0.533	0.152	0.472	0.943
IVSD	P.C	-.307	0.108	-0.01	0.598	0.485	0.22	Right S'	-0.368	0.087	0.086	0.506	0.398	0.145
	P-value	0.01	0.373	0.933	<0.001	<0.001	0.067		0.002	0.473	0.477	<0.001	0.001	0.230
Age	P.C	-0.458	0.022	0.081	0.699	0.618	.379	Right Peak E'	0.174	0.041	0.017	-0.073	-0.02	0.011
	P-value	<0.001	0.86	0.504	<0.001	<0.001	0.001		0.15	0.738	0.886	0.549	0.87	0.926
LVDD	P.C	-0.231	0.026	-0.024	0.419	0.39	.270	Right Peak A'	0.223	-0.058	-0.125	-0.216	-0.187	-0.094
	P-value	0.055	0.832	0.846	<0.001	0.001	0.024		0.063	0.636	0.302	0.073	0.121	0.438
PWD	P.C	-0.143	0.072	0.256	0.257	0.158	0.005	VS'	-0.028	0.075	-0.108	0.115	0.055	-0.054
	P-value	0.239	0.551	0.032	0.032	0.193	0.969		0.816	0.535	0.372	0.343	0.653	0.655
IVSS	P.C	-0.036	-0.047	-0.021	0.217	0.085	-0.115	VE'	0.044	-0.047	-0.194	-0.246	-0.234	-0.154
	P-value	0.768	0.702	0.866	0.071	0.485	0.345		0.715	0.697	0.108	0.04	0.051	0.202

LVDS	P.C	-0.306	0.203	0.138	0.331	0.309	.249	VA'	-0.126	-0.02	-0.366	0.217	0.228	0.182
	P-value	0.01	0.092	0.254	0.005	0.009	0.038		0.297	0.872	0.002	0.071	0.058	0.132
PWS	P.C	-0.148	0.049	0.234	0.241	0.148	0.007	Left MPI'	-0.307	0.096	0.057	0.354	0.277	0.149
	P-value	0.22	0.686	0.051	0.045	0.223	0.953		0.01	0.43	0.64	0.003	0.02	0.217
EF	P.C	-0.603	-0.061	-0.132	0.885	0.839	.553	Right MPI'	-0.362	0.001	-0.106	0.402	0.355	.236
	P-value	<0.001	0.613	0.277	<0.001	<0.001	<0.001		0.002	0.994	0.384	0.001	0.003	0.05
FS	P.C	-0.588	-0.049	-0.154	0.875	0.83	.540	LVMI	-0.334	0.119	0.101	0.579	0.494	.268
	P-value	<0.001	0.687	0.203	<0.001	<0.001	<0.001		0.005	0.325	0.405	<0.001	<0.001	0.025
LVM	P.C	-0.295	0.133	0.074	0.518	0.45	.255	Left A/A'	-0.171	-0.024	-0.043	0.051	-0.023	-0.127
	P-value	0.013	0.272	0.545	<0.001	<0.001	0.033		0.157	0.846	0.726	0.678	0.848	0.295
Left ET'	P.C	0.277	-0.004	-0.096	-0.234	-0.27	-.288	Left E/E'	-0.049	0.017	-0.035	0.000	-0.16	-.309
	P-value	0.02	0.973	0.429	0.051	0.024	0.016		0.686	0.888	0.774	0.998	0.185	0.009
Left ICT'	P.C	-0.375	0.043	0.051	0.528	0.41	0.152	Right E/E'	-0.229	-0.085	-0.125	0.079	-0.015	-0.11
	P-value	0.001	0.723	0.675	<0.001	<0.001	0.208		0.057	0.482	0.302	0.516	0.904	0.365
Left IRT'	P.C	0.071	0.15	0.042	-0.104	-0.143	-0.123	Right A/A'	-0.185	-0.015	-0.029	0.075	0.018	-0.07
	P-value	0.56	0.216	0.727	0.394	0.237	0.312		0.126	0.9	0.81	0.536	0.881	0.564

P .C: Pearson Correlation, Peak E: Early mitral and tricuspid valve flow velocity, Peak A: Late mitral and tricuspid valve flow velocity, ET: Ejection time (for Aorta and Pulmonary), IVSD: Interventricular septal dimension in diastole, LVDD: Left ventricular end-diastolic dimension, PWD: Posterior wall dimension in diastole, IVSS: Interventricular septal dimension in systole, LVDS: Left ventricular diameter at systole, PWS: Posterior wall dimension in systole, EF: Ejection fraction, FS: Fractional shortening, LVM: Left ventricular mass, ICT: Isovolumetric contraction time, ICT: Isovolumetric contraction time, S': Systolic myocardial velocity above the baseline in pulmonic vein, mitral and tricuspid, E': early diastolic myocardial relaxation velocity below the baseline in pulmonic vein, mitral and tricuspid. A': myocardial velocity associated with atrial contraction in pulmonic vein, mitral and tricuspid, MPI: Myocardial Performance Index (Tei Index), BMI: Body Mass Index, LVMI: Left Ventricular Mass Index.