Serum Copeptin as a Cardiomyopathy Predictor in Thalassemic Children: Relation to Tei Index

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ABSTRACT

Background: Among the most dangerous side effects for persons with beta thalassemia major (B-TM) are cardiac problems. Early recognition of cardiomyopathy guides early management and therefore minimize mortality and morbidity.

Objective: This study aimed to assess copeptin levels as an early predictor of myocardial dysfunction in children with B-TM and to determine its relationship to tissue doppler derived Tei index in those patients.

Subjects and methods: The study was conducted on 42 children with B-TM without known heart disease and 40 age -and sex-matched healthy controls. Serum copeptin was assayed by a commercial ELISA kit and left ventricular Tei index was measured using tissue Doppler imaging.

Results: Copeptin levels were significantly higher in thalassemic patients compared with controls **(***P*=0.014). Copeptin was correlated positively with age, disease duration, transfusion index, lactate dehydrogenase, indirect bilirubin, serum ferritin, end systolic and diastolic diameters and tricuspid regurgitant jet velocity. Copeptin, on the other hand, showed a negative correlation with fractional shortening and ejection percent. Copeptin and Tei index showed a substantial link in the investigation of correlation (p=0.001).

Conclusion: Copeptin in B-TM may be regarded as an early indicator of cardiomyopathy. It ought to be included in thalassemic follow-up in order to detect and treat subclinical problems correctly, lowering the risk of detrimental heart failure (HF).

Keywords: Copeptin, Tei index, B-TM.

INTRODUCTION

The future of thalassemic patients is bright, since scientific research in the past several years has concentrated on cardiac biology and imaging indicators in TM patients. These indicators are helping to clarify how early cardiac involvement occurs and how patients who are at risk might receive intense chelation quickly, which increases the likelihood of preventing HF, which is the primary cause of mortality in this population [1].

Copeptin is a relatively novel and promising marker of many cardiovascular events, including HF, since it indicates the arginine vasopressin (AVP) system. The AVP complex is critical for maintaining cardiovascular homeostasis [2]. Copeptin is part of the uncleaved pro-AVP, which is co-secreted with AVP, mirroring its levels[3]**.** Copeptin is therefore employed as a stand-in marker for AVP release [4].

Copeptin's significance has mostly been demonstrated in acute life-threatening illnesses mediated by the stress response system and hemodynamic instability, as copeptin is released in response to several stressors [5,6]. Nonetheless, the cardiovascular system is highlighted as the primary location of copeptin formation and the significance of copeptin measurement [7]. Besides the established biomarkers for cardiac injury, copeptin has demonstrated the ability to offer corrective diagnostic data for early discriminating and risk stratification in cardiac diseases [8]. In the context of B-thalassemia cardiomyopathy, copeptin has not yet been studied.

From an imaging standpoint, it is challenging to identify early cardiac abnormalities, and traditional echocardiographic abnormalities typically appear later in the course of the illness [9]. The use of tissue-derived doppler myocardial performance (Tei index), which has distinct benefits over more traditional established indices, has shown to be a dependable technique for assessing global cardiac performance and emerges as a marker utilised for early identification of myocardial functions [10].Ideally , for a precise cardiovascular evaluation, the application of cardiovascular biomarkers is important to assist in precise functional evaluation and risk stratification in addition to echocardiography cardiac imaging methods and to guide therapeutic decisions that may prevent cardiac remodeling and progressive dysfunction [11].

Therefore, this study was performed to assess copeptin levels as an early predictor of myocardial dysfunction in children with B-TM and to determine its relationship to Tei index in those patients.

SUBJECTS AND METHODS

Study population:

The study comprised 42 Egyptian children and adolescents with B-TM who were frequent attendees at the Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University. As a control group, forty healthy individuals of similar age and gender were enlisted.

Hemoglobin analysis information was obtained from patient records. All patients had their medical history taken, with particular attention paid to their demographics, the length of their illness, the frequency of their transfusions, and their iron chelation treatment. An extensive clinical examination was performed on the patients, which included anthropometric measurements

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such as body weight and height. The formula for calculating body mass index was weight in kilogrammes (kg) divided by height in metres squared (m^2) [12]. Blood samples were collected on the same day for hematologic and chemical measurements. The control group also underwent a full echocardiographic study and blood measurements.

For both pulmonary hypertension and cardiovascular abnormalities, all of the participants in the study had clinically no symptoms. Also, all patients received transfusions. The transfusion index—which is the mean value over the previous three years—was used to determine the volume of transfused packed red blood cells per kilogramme of body weight annually. Iron chelation treatment was given to each patient.

We excluded patients with cardiovascular diseases such as congenital or rheumatic heart disease to rule out structural heart disease that might dilate the ventricles, such as regurgitant valvular lesions. We also excluded from this study patients with any of the following: infection, sepsis, pneumonia, endocrine diseases, neurological diseases, renal insufficiency, nocturnal enuresis, chronic inflammatory condition, or autoimmune diseases. We also excluded patients with a history of taking non-steroidal anti-inflammatory drugs or glucagon hormones or antibiotics within half a month prior this study.

Blood sampling:

On potassium-ethylene diamine tetra-acetic acid (K_2-EDTA) (1.2 mg/mL), peripheral blood samples were taken for CBC. Clotted samples were acquired for chemical analysis and ELISA. Serum was separated by centrifugation for 15 minutes at 2000–3000 *g, and it was then refrigerated at -20℃ until it was needed for the ELISA.

Laboratory analysis:

The Sysmex XT-1800i (Sysmex, Kobe, Japan) was used for CBC tests in the laboratory. Other tests included the Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany) for measuring serum ferritin and indirect bilirubin, as well as a differential WBC count on Leishman-stained smears. Serum ferritin levels were determined, and a cut-off value of 2500 ug/L was employed as a predictor of a bad prognosis, which included a higher risk of cardiac problems and a shorter survival time [13].

Using an ELISA kit for Human Copeptin (Shanghai Korian Biotech Co. Ltd., Shanghai, China), the serum level of copeptin was measured.

Echocardiography:

The research groups had conventional transthoracic echocardiography, which involved 2D, M mode, and Doppler analysis utilising phased array transducers at frequencies appropriate for their age and body weight (Vivid E9, Vingmed, GE, Horten, Norway) for estimation of:

Cardiac dimensions [the IVSd and in systole (IVSs), LVIDd and systole (LVIDs) as well as LVPWd and in systole (LVPWs)] in addition to cardiac systolic functions [EF and FS] [14]. LV global systolic function was considered normal with FS (26-45%) and EF (≥ 55%). While mild systolic dysfunction was considered with (FS 20-25%) or (EF 41- 55%) [15].

Using the Bernoulli equation, which states that the systolic pressure of the pulmonary arteries = 4xTRV2 + RAP, non-invasive Doppler measurement of the TRV was used to screen for pulmonary hypertension. Patients who were at risk for PH could be identified by a TRV of less than ≥2.5 m/s [16]. An experienced operator who was blind to the patients' test results completed this.

Assessment of TDI derived LV Tei index:

The mitral annulus's tissue Doppler imaging waveforms were captured and examined. Waves in the diastolic phases were: early (E/), late (A/), and systolic (S/). From the TDI recordings, the duration between the end of diastole (A) and the beginning of the mitral annular velocity pattern was calculated. From the beginning to the end of the S// wave, the length of wave (B) was measured. (A-B)/B was used to compute the LV-TDI Tei index (Figure 1).

From the conclusion of the A/wave until the start of the S/wave, the ICT was calculated. From the conclusion of the S/ wave until the start of the E// wave, the IRT was determined. For recorded waves and intervals, the average of the lateral and septal mitral annular values was calculated. Five successive beats were averaged to get the mean values of the waves [17].

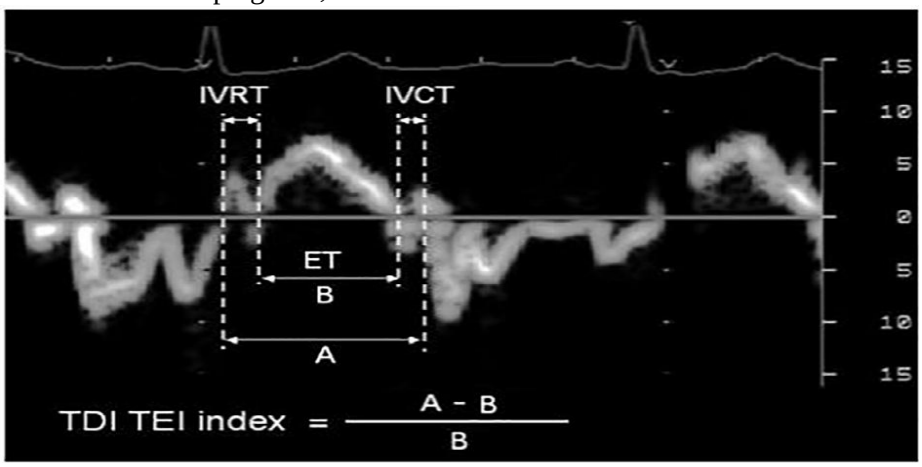

Fig. (1): TDI derived Tei index (A-B/B). IVRT: isovolumetric relaxation time, IVCT: isovolumetric contraction time. ET: ejection time.

Ethical approval:

Ain Shams Medical Ethics Committee of the Ain Shams Faculty of Medicine gave its approval to this study. The caregivers of all the participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis:

IBM SPSS V. 20.0 was updated, modified, programmed, and filled with data. The Kolmogorov-Smirnov test of normality was used to analyse quantitative data. Data with parametric distribution were shown as means± standard deviation (SD) and ranges, whereas data without parametric distribution were shown as medians with interquartile ranges (IQR). Numbers and percentages were used to represent the qualitative data. Parametric quantitative variables were compared between the 2 groups using the Student t-test. To compare non-parametric quantitative variables between two groups, the Mann-Whitney test was created. A p-value of less than 0.05 was considered significant in all analyses.

RESULTS

Patients' characteristics:

We included 42 patients with B-TM with non-overt cardiac disease (Table 1) and 40 age and sex matched controls.

Table (1): Baseline characteristics of studied patients

	Patients group (No. = 42)
Demographic characteristics, Age (Years)	
Sex, **n(%)**	15.67 ± 1.72
Female	21 (50.0%)
Male	21 (50.0%)
Clinical characteristics	
Disease Duration (Years)	14.85 ± 1.71
BMI (kg/m^2)	20.87 ± 1.96
Splenectomy, n(%)	28 (66.7%)
PH risk, n(%)	20(47.6%)
Family History, n(%)	
Negative	23 (54.8%)
Positive	19 (45.2%)
Tanner score, **n(%)**	
Delayed	42 (100.0%)
SBP mmhg	113.57 ± 4.17
DBP mmhg	73.10 ± 4.27
HR (bpm)	89.12 ± 6.23
Mild LV systolic dysfunction by Echo, n(%)	9 (21.4%)
Chelation, n(%)	42 (100.0%)
Name of chelator	
DFX	32 (76.2%)
DFP	17 (40.5%)
DFO	6 (14.3%)
Transfusion index (mL/kg/year)	317.14 ± 102.04
Hematological parameters	
WBC (10^9/L)	14.84 ± 3.34
Hemoglobin (g/dL)	8.07 ± 0.83
HbF%	36.92 ± 9.14
Biochemical measurement	
LDH (mg/dL)	477.79 ± 117.74
Total bilirubin **(mg/dL)**	1.38 ± 0.29
Indirect blirubin (mg/dL)	1.18 ± 0.26
Ferritin (µg/L)	1972.45 (896.9 - 2997)
Ferritin (µg/L) cutoff	
<2500	30 (71.4%)
>2500	12 (28.6%)

Data are presented as mean ± SD, frequency (%), or median (IQR), BMI: body mass index, PH: pulmonary hypertension, SBP: systolic blood pressure, DBP: diastolic blood pressure, LV: left ventricle, DFO: Desferrioxamine, DFP: deferiprone DFX: deferasirox

Serum copeptin levels among thalassemic patients and healthy controls:

Median copeptin levels were higher in the patients compared with the controls [P=0.014] (Figure 2).

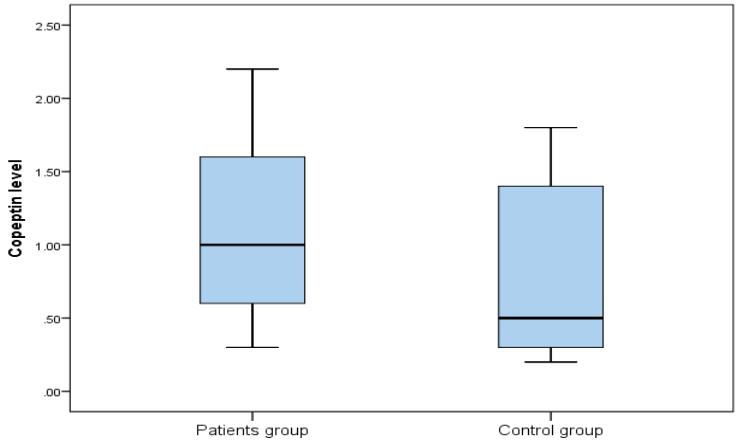

Figure (2): Serum copeptin levels among thalassemic patients and controls

Serum Copeptin levels in relation to qualitative characteristics of B-TM patients:

As shown in table 2, the median levels of copeptin were significantly higher in thalassemic patients with PH risk and in those with mild systolic dysfunction. No association was found between copeptin and other clinical variables including sex and history of splenectomy.

Table (2): Copeptin levels in relation to qualitative characteristics of B-TM patients.

		Copeptin level		Test value•	P-value
		Median (IQR)	**Range**		
Sex	Female Male	2 (1.5 2.5) 1.8 (1.4 2.7)	1 – 6 0.9 – 4	0.973	0.330
Splenectomy	Negative Positive	1.5 (1.4 2.5) 2.2 (1.5 3)	0.9 – 5 0.9 – 6	1.059	0.289
PH risk	Negative Positive	1.5 (1 1.5) 2.5 (1.9 3.5)	0.9 – 2.7 1.2 – 6	4.407	<0.001
Mild LV systolic dysfunction by Echo	Negative Positive	1.5 (1.2 – 2.4) 3.5 (2.7 – 4)	0.9 – 5 2.5 – 6	-3.913	<0.001

Correlation of copeptin and quantitative data of studied patients:

Copeptin was correlated positively with age, disease duration, transfusion index, levels of hemolytic markers, as well as serum ferritin, which is a measure of iron overload. Copeptin levels were negatively correlated to the degree of anemia (Table 3).

Table (3): Correlation of copeptin and quantitative data of studied patients

	Copeptin level	
	r	**P-value**
Age (Years)	**0.308***	**0.047**
Disease Duration (Years)	**0.318***	**0.040**
BMI (kg/m^2)	-0.118	0.458
Transfusion index(mL/kg/year)	**0.839****	**<0.001**
WBC	-0.164	0.300
Hemoglobin	**-0.423****	**0.005**
HbF%	-0.090	0.572
LDH (mg/dL)	**0.791****	**<0.001**
Total bilirubin	**0.760****	**<0.001**
Indirect bilirubin (mg/dL)	**0.732****	**<0.001**
Ferritin (µg/L)	**0.963****	**<0.001**
TRV (m/sec)	**0.882****	**<0.001**
Ejection fraction (%)	**-0.714****	**<0.001**
Fractional shortening	**-0.701****	**<0.001**
LVESD(cm)	**0.597****	**<0.001**
LVEDD (cm)	**0.492****	**0.001**
Tei index	0.0487	0.001

Echocardiographic evaluation:

Echocardiographic characteristic of studied patients are shown in table 4.

Table (4): Echocardiographic characteristic of studied patients.

Echocardiographic characteristic	Mean ± SD	Range
TRV (m/sec)	2.55 ± 0.52	2 – 4.2
Tei index	0.43 ± 0.02	0.4 – 0.46
Ejection Fraction (%)	61.48 ± 6.72	52 – 75
Fractional shortening	31.29 ± 4.71	23 – 38
LVESD (cm)	3.40 ± 0.77	2.6 – 4.8
LVEDD (cm)	4.92 ± 0.78	4.2 – 6.3

In this study we evaluated thalassemic patients with no signs/symptoms of heart failure. Conventional echocardiographic assessment was performed to evaluate features of cardiomyopathy such as systolic dysfunction and dilatation of the left ventricle, as well as poor global contractility. We found increased left ventricular dimensions (LVESD, LVEDD) in thalassemic individuals. On echocardiography, all of the included patients exhibited normal systolic LV function with the exception of nine. However, we observed a considerably reduced FS in thalassemia patients compared to healthy controls (31.29% ± 4.7 versus 34.20%± 1.62, P value=0.042).

According to the aforementioned criterion of PH risk, 47.6% of the patients (n = 20) had it, and 21.4% had mild LV systolic dysfunction as detected by echocardiography. Furthermore, the Tei index was considerably higher in investigated patients compared to normal control participants (0.43 ± 0.02 vs. 0.37 ± 0.02, P value<0.0001), indicating subclinical systolic dysfunction.

In terms of the relationship between copeptin and echocardiographic data among patients with B-TM, we discovered a positive correlation between copeptin and TRV, LVEDD, and LVESD. Whereas the correlations between EF and FS and copeptin were negative correlations. Interestingly, our correlation analysis between copeptin and Tei index was significant (p=0.001) (Table 5).

Table (5): Correlation of copeptin and echocardiographic data of studied patients

	Copeptin level	
	r	P-value
TRV (m/sec)	**0.882****	**0.000**
Ejection. Fraction (%)	**-0.714****	**0.000**
fractional shortening	**-0.701****	**0.000**
LVESD(cm)	**0.597****	**0.000**
LVEDD (cm)	**0.492****	**0.001**
Tei index	0.0487	0.001

DISCUSSION

Even in the absence of overt HF, patients with thalassemia exhibit early regional systolic and/or diastolic dysfunction. Many thalassemic individuals don't show any symptoms until they start to decompensate. But only 50% of patients survive after overt HF is evident [18].

Thus, the objective is to start therapy as soon as possible while the cardiomyopathy is still curable. However, because global left ventricular function and exercise capacity in patients receiving continuous transfusions may stay normal until late in the course of the disease, it has been challenging to identify patients at risk of HF at an early stage [19].

Copeptin has gained recognition in recent years as a significant marker for identifying individuals who are at high risk for severe cardiovascular events, such as HF, as it is a unique signal of AVP activation [20].

The existing data on the relationship of copeptin with cardiomyopathy in B-TM are inadequate. As a result, the purpose of this investigation was to identify copeptin levels as an early predictor of myocardial dysfunction in children with B-TM, and to the best of our knowledge, this is the first study to investigate the association between copeptin and Tei index in pediatric thalassemics.

We examined copeptin levels in the study individuals and found that B-TM patients had considerably higher copeptin levels than healthy controls. These changes were more pronounced in thalassemic individuals with mild LV systolic insufficiency and PH risk.

Studies on neurohumoral activation in patients with cardiovascular illnesses have frequently demonstrated the involvement of AVP/copeptin in HF patients [3, 20]. Baroreceptor activation, which is involved in the control of blood pressure and volume, initiates the release of AVP/copeptin. AVP causes ventricular hypertrophy, reduced contractility, and the development of myocardial fibrosis by stimulating the creation of proteins and cardiac fibroblasts through its actions on V1 and V2 receptors. As a result, AVP exacerbates both diastolic and systolic wall stress, which leads to LV dysfunction [21].

Patients with PH are known to have higher sympathetic nervous system activity, as described in patients with left ventricular HF, and it is probable that this activation also affects the AVP/copeptin system. Thus, the neurohumoral axis is also activated in pulmonary hypertension, right ventricular strain and right-sided cardiac dysfunction [3].

When considered collectively, these findings may indicate that copeptin levels, which are a measure of the activation of the AVP system, may serve as an early marker of neurohumoral stimulation, even in the absence of obvious clinical signs of cardiac failure. This concept underlines the importance of copeptin as an early predictor of cardiomyopathy.

Similarly, copeptin was investigated as a HF predictor in a population- based prospective cohort by **Schill *et al.*** [22]**.** Copeptin was analysed in 5297 adults without prevalent HF. It was determined that copeptin was a risk factor for VP-driven HF vulnerability and a potential candidate to direct HF preventive initiatives that target the VP system.

On the contrary of our results, **Saadatifar *et al.*** [23] concluded that it was not possible to use the blood levels of inflammatory factors (NT-proBNP, copeptin, and hs-CRP) to identify cardiac siderosis in B-TM patients early. But since our study only included children, it's possible that this strong correlation was shown because children's illnesses usually progress more slowly than adults'.

In thalassemics, cardiomyopathy is a continuous, chronic, and progressive disease. Due to regular blood transfusions, iron starts getting deposited in the myocardium and is catalyzed to produce reactive oxygen species that damages the myocardium and alters cardiac function eventually leading to cardiomyopathy later in their life [24]. In line with these observations, we have reported positive correlations between copeptin -as a cardiomyopathy marker- and patients' age as well as disease duration. As we suggested copeptin as a cardiomyopathy predictor in thalassemic patients, we analysed its relation to the established risk factors of iron overload cardiomyopathy. Interestingly, serum ferritin, a sign of iron overload, and the transfusion index as well as hemolysis indicators all showed positive correlations with serum copeptin.

The gold standard imaging modality for the diagnosis of HF is echocardiography, which provides standardised measurable parameters related to HF. For this reason, echocardiographic examination is used for routine cardiac evaluation in TM [25].

Regretfully, even with its widespread availability, it isn't always enough to accurately track the disease's course. Finding a relevant and accessible laboratory marker is therefore necessary. The link between systolic and diastolic function and echocardiography markers, and copeptin serum levels, is not well-established [7].

Therefore, evaluating the correlation between copeptin serum concentrations and echocardiography measures was crucial. In our investigation, left ventricular sizes were correlated with higher copeptin concentrations. The increased cardiac output of a chronic anemia condition is the cause of these anatomical alterations in the heart. This suggests a relationship between copeptin and the ventricular volumes and myocyte stretch.

Additionally, copeptin and FS showed a negative correlation, which might be an indication of early cardiac dysfunction. Additionally, we reported that copeptin and EF had an inverse relationship. Numerous investigations demonstrated the important effect EF plays in TM patients' prognosis [26, 27]. A recent research by **Maggio *et al.*** [27] indicated that EF decrease was a substantial predictor of cardiac mortality, highlighting the significance of EF reduction during follow-up of thalassemic patients.

Based on the previous knowledge and being negatively correlated with EF, we calimed that copeptin may be a valuable marker in monitoring disease progression and could be assessed on a regular basis to identify risk stratification and implement preventative measures in time.

There is no data on the relationship between echocardiography parameters and copeptin levels in thalassemia. However, **Kelly *et al.*** [28] reported that copeptin concentrations were correlated positively with ventricular volumes at follow-up and inversely with EF at discharge and follow -up.

The assessment of systolic and diastolic left ventricular function using standard echocardiographic measures is subjected to several limitations [10]. Prior research has shown intriguing findings about the usefulness of TDI in the echocardiographic assessment of B-TM patients using derived indexes, such as the Tei index [29, 30].

The Tei index is an effective way to represent overall cardiac function that may be used to assess both the systolic and diastolic functions simultaneously. It is a sensitive HF indication that may be helpful in the early identification of myocardial dysfunction in a particular area prior to the occurrence of aberrant indices [31].

In our study, patients with β thalassemic syndrome had higher Tei index values than controls, and this difference was shown to be statistically significant. **Shahmohammadi *et al.*** [32] reported the same result in a prior research. When iron overload has not yet resulted in systolic dysfunction, measurement of the Tei index in B-TM has been shown to be helpful in the early diagnosis of LV dysfunction, particularly in young, asymptomatic individuals in an early reversible stage of the illness [32].

Finally, we analysed the correlation between copeptin and Tei index in our thalassemic patients. A novel finding by our work was the positive significant association between the two parameters. This observation could help to enforce the value of copeptin as an early predictor of cardiac dysfunction.

CONCLUSION

Copeptin, as a biomarker of neurohormonal disarray, could be considered as an early predictor of cardiomyopathy in B-TM. In clinical practice, copeptin, along with other cardiac biology and imaging indicators, should be included in the follow-up system of thalassemic patients in order to identify and treat correctly subclinical problems, hence minimising the burden of detrimental HF.

LIMITATION

There is a primary limitation in this study that could addressed in future research. In HF pathways, cardiac biomarkers are generated that reflect different molecular interactions and anatomical alterations in the heart. Investigating serum cardiac biomarkers such as hs-CRP, NT-pro-BNP in concert with copeptin and analysis of their correlations would provide additional value to our study and validate the potential clinical use of this molecule as a cardiomyopathy predictor in thalassemics.

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