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ORIGINAL RESEARCH

Subclinical atherosclerotic predictive value of inflammatory markers in thalassemia intermedia patients

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ABSTRACT

Background: A high incidence of thromboembolic events is observed in thalassemia patients. This study investigated the relationship between carotid intima-media thickness (CIMT) and lipid profile, iron metabolic indices (IMI), and inflammatory markers in β-thalassemia intermedia (β- TI) patients.

Patients and methods: Forty-five β-TI patients at Assiut University Hospital and 34 healthy individuals were enrolled in the study. We measured Lipid profile, IMI, high sensitive CRP (Hs-CRP), and interleukin-6 (IL-6) and compared the results between both groups. We used CIMT measurement as a marker for subclinical atherosclerosis. We used both univariate and multivariate analyses to test relations and independent predictors of CIMT.

Results: β-TI patients had higher CIMT *(P = 0.000)*. CIMT was positively correlated with absolute neutrophil count (ANC) *(r = 0.320, p = 0.032)*, ferritin *(r = 0.544, p = 0.000)*, Hs-CRP *(r = 0.603, p = 0.000)*, and IL-6 *(r = 0.520, p = 0.000)*. Hs-CRP was an independent predictor of CIMT *(p = 0.000)*. Hs-CRP cut off value of 60.4 ug/dl has sensitivity of 63.3% and specificity of 93.3% in predicting premature atherosclerosis.

Conclusion: β-TI patients had higher CIMT despite the protective lipid profile. Hs-CRP was an independent predictor of CIMT.

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KEYWORDS
Carotid intima-media thickness; subclinical atherosclerosis; thalassemia; thalassemia intermedia

1. Background

Thalassemia is an inherited autosomal recessive blood disorder in which abnormal hemoglobin is produced [1]. In Egypt, thalassemia cases represent about 1000/1.5 million live births per year [2]; β-thalassemia is the most common type [3].

Endothelial dysfunction that occurs in β-thalassemia patients is attributed to peroxidative tissue injury because of continuous blood transfusions [4]. Transfusion-related iron overload in β-thalassemia major (β-TM) has been associated with the onset of cardiovascular complications [5]. Carotid atherosclerosis has been positively associated with serum ferritin independent of other traditional cardiovascular risk factors [6]. In patients with thalassemia, high incidence of thromboembolic events has been found [7].

Early detection of cardiovascular risk and subclinical atherosclerosis should represent a priority, along with the detection of new cardiovascular active substances [8]. Carotid intima-media thickness (CIMT) was used in assessing the presence and the progression of the atherosclerotic process for its value as an approved marker of early atherosclerosis [9,10]. It has been used in clinical trials frequently for its quantitative value and its trustable results in detecting subclinical atherosclerosis in the clinical setting [4,11].

There is a growing evidence about the contribution of inflammation to the pathophysiological features of atherosclerosis [12,13], as well as the role of high sensitive C reactive protein (Hs-CRP) and interleukin-6 (IL-6) as inflammatory biomarkers in the development of vascular events [14,15]. Hs-CRP and IL-6 levels have showed correlations with endothelial dysfunction, arterial stiffness, and extent of subclinical atherosclerosis [16–19]. Moreover, there was an appreciable importance of subtype leukocyte and platelet count in the occurrence of cardiovascular events in healthy individuals [20,21].

It has been a matter of concern in research to assess atherosclerosis and its predictors in patients with TI [22]. Due to the improving survival of thalassemia patients, more studies are needed to evaluate precisely the prevalence of atherosclerotic disease in adult patients with TI and TM [23].

In our study, we aimed at comparing CIMT between patients with TI and healthy control, besides investigating the relationship between CIMT (as a subclinical atherosclerotic biomarker) and lipid profile, iron metabolic indices, and inflammatory markers in TI patients.

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'Clinical trial registration: www.clinicaltrials.gov identifier is NCT03170245 (Unique Protocol ID: ADICIBT)'
All authors were responsible for all aspects of the work.

2. Patients and methods

2.1. Patients

This study included 45 β- TI patients who were attending the Clinical Hematology Clinic at Assiut University Hospitals for health maintenance, and 34 healthy individuals served as a control group. Both groups were comparable for age, sex, and body mass index (BMI). Diagnosis of thalassemia was based on the presence of microcytic hypochromic anemia in complete blood count (CBC), reticulocytosis, and markers of hemolysis, as well as high-performance liquid chromatography, was done for hemoglobin (Hb) analysis [24]. The sample size was calculated using the G power program. To detect a significant difference between the two independent groups, with a power of 95%, an effect size of 0.9, at least 26 subjects for each group were needed. The study groups included patients aged 18 years old and older. We excluded patients with an acute medical condition, having an infection, a high CRP (>6), malignancy, known immunosuppressed, or taking non-steroidal anti-inflammatory drugs (NSAIDs) or steroids. We also excluded diabetic, hypertensive, and obese individuals (BMI > 30 kg/m2). All participants signed written informed consent.

2.2. Methods

From TI patients, a detailed history and a thorough clinical examination were done. Laboratory investigations included CBC, total bilirubin (TB), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and Low-density lipoprotein cholesterol (LDL-C), serum ferritin, IL-6, and Hs-CRP. Blood samples from both groups were withdrawn through venipuncture under an aseptic technique and were collected in EDTA, 3.2% trisodium citrate, and plain tubes. Samples in the plain tube were centrifuged for 20 min at 2000–3000 rpm at 4°C then separated into aliquots, and immediately stored at −70°C until the time of testing.

Estimation of Hb, white blood cell count (WBC), and platelet count were performed on CELL-DYN 3700 hematology analyzer. The WBC differential used VCS technology which uses three simultaneous measurements of individual cell volume (V), high-frequency conductivity (C) and laser light scatter (S). The scattergram plotted the cells based upon the measurements of these three parameters. Reticulocyte count was carried out on brilliant cresyl blue-stained smear. An indirect enzyme-linked immunosorbent assay (ELISA) kit (Diametra SRL, Italy) was used to perform the ferritin level. Total cholesterol and triglyceride concentrations were estimated using enzymatic methods (Roche Diagnostics, Mannheim, Germany). HDL cholesterol was determined after precipitation with phosphotungstic acid/magnesium chloride. LDL cholesterol was measured by direct LDL-C assay (Roche Diagnostics). IL-6 was performed using an indirect enzyme-linked immunosorbent assay (ELISA) kit (SinoGeneclon Biotech Co., Ltd, China). Hs-CRP was done using an enzyme immunoassay test kit (Perfect Ease Biotech (Beijing) Co., Ltd, USA).

All patients had echocardiography done with an HDI 5000 instrument (Philips Medical Systems, Bothell, Washington, USA) equipped with a broadband harmonic transducer. Left ventricular (LV) dimensions were calculated using 2D guided M-mode calculations. Left ventricular systolic ejection fraction (LVSEF) was measured by 2D guided M-mode calculations. The CIMT was measured as the distance between two lines; the first line represented luminal-intimal transition and the second one medial-adventitial transition. Three readings of CIMT on both sides were recorded then a mean was calculated. Results of both sides were then pooled in a single CIMT reading.

The study was approved by the Faculty of Medicine's Research Ethics Medical Review Board at Assiut University under the number of IRB17200010. It is also registered at Clinical Trials.Gov: NCT03170245 (Unique Protocol ID: ADICIBT).

2.3. Statistical analysis

We used SPSS software Chicago, IL, USA, version 21 to perform the statistical analyses. Variables were evaluated for normal distribution by using the Kolmogorov–Smirnov test and all were normally distributed. The Chi-square test was used to compare categorical variables in both groups. Student T-test was used to compare the continuous variables presented as mean ± SD. Pearson correlation was used in measuring the degree or extent of the correlation between CIMT and other variables. To test the relation between CIMT and other relevant variables, we used the univariate regression analysis. Hs-CRP was used to test the independent predictors of CIMT in the multivariate analysis. ROC analysis was used to estimate the sensitivity and specificity of Hs-CRP in detecting CIMT > 0.05 cm in TI patients (which was above the mean CIMT in the control group). We consider P-value ≤ 0.05 as a significant value.

3. Results

3.1. Demographic, laboratory, and CIMT data

The ages of TI patients ranged from 18 to 49 years old, where males were 22 (48.9%) and females were 23 (51.1%), while the ages of the control ranged from 18 to 53 years old, where males were 16 (47.1%) and females were 18 (52.9%). Out of the studied patients, 11 patients (24.4%) were splenectomized. Age of diagnosis of the studied patients ranged from 12 − 38 years old. As for the transfusion history, 26 patients (57.8%) had transfusion history while 19 patients (42.2%) had no previous transfusion history. There were only 15 patients (33.3%) on iron chelation therapy. Out of the 15 patients on iron chelation, 11 patients (73.3%) were on deferasirox while 4 patients (26.7%) were on deferiprone. Patients started iron chelation at variable durations from the time of diagnosis, four (26.6%) patients started it on diagnosis, nine (60%) patients within the first 5 years of diagnosis while the last 2 patients (13.4%) started iron chelation later on. No history of previous cardiovascular or thromboembolic events in the studied group. No smoking history in all TI patients and only 4 of the healthy control group were mild smokers. Laboratory, echocardiography, and CIMT values of both groups are shown in (Table 1). Based on a cutoff point of ferritin

Table 1. Comparison of laboratory parameters and CIMT between thalassemia intermedia patients' group and control group.

Variables	β- thalassemia intermedia patients	Control	P = Value
Age (years)	**29.87 ± 7.65 Range 18–49**	**26.38 ± 7.97 Range 18–53**	**0.054**
Male Sex	**22 (48.9%)**	**16 (47.1%)**	**0.815**
Female sex	**23 (51.1%)**	**18 (52.9%)**	
BMI(kg/m^2)	**23.41 ± 2.29**	**22.99 ± 2.6**	**0.452**
Age at diagnosis (years)	**19.8 ± 6.16 Range 12–38**	-	
History of transfusion No. (%)	**26 (57.8%)**	-	
Splenectomy No. (%)	**11 (24.4%)**	-	
Iron chelation No. (%)	**15 (33.3%)**	-	
WBC (×10^9/L)	**8.78 ± 2.87**	**5.44 ± 1.33**	**0.000***
ANC (×10^9/L)	**4.75 ± 1.7**	**2.74 ± 1.14**	**0.000***
Hb (g/dl)	**7.39 ± 1.03**	**13.54 ± 2.14**	**0.000***
PLTs (×10^9/L)	**410.24 ± 115.8**	**282.41 ± 66.74**	**0.000***
Reticulocytes (%)	**4.23 ± 2.59**	**1.18 ± 0.57**	**0.000***
TB (umol/L)	**43.9 ± 16.82**	**10.89 ± 5.04**	**0.000***
IL-6 (ng/L)	**8.45 ± 3.69**	**5.18 ± 1.26**	**0.000***
Hs-CRP (ng/ml)	**54.85 ± 32.44**	**39.50 ± 30.49**	**0.036***
Serum ferritin (ng/ml)	**670.81 ± 335.67**	**110.66 ± 29.08**	**0.000***
Total cholesterol (mg/dl)	**91.83 ± 22.25**	**158.47 ± 25.55**	**0.000***
Triglycerides (mg/dl)	**103.07 ± 31.89**	**79.12 ± 31.16**	**0.001***
HDL-C (mg/dl)	**37.37 ± 11.31**	**44.44 ± 11.26**	**0.007***
LDL-C (mg/dl)	**36.42 ± 13.71**	**99.03 ± 20.83**	**0.000***
CIMT(cm)	**0.06 ± 0.01**	**0.05 ± 0.01**	**0.000***
LVSEF (%)	**59.87 ± 5.74**	**64.65 ± 4.70**	**0.000***
PASP(mmHg)	**29.49 ± 6.59**	**24.68 ± 5.28**	**0.001***

BMI: Body mass index, ***WBC:*** White blood cell, ***ANC:*** Absolute neutrophil count, ***Hb:*** Hemoglobin, ***PLTs:*** Platelets,
TB: Total bilirubin, ***IL-6:*** Interleukin-6, ***Hs-CRP***: High sensitive C- reactive protein, ***HDL-C:*** High-density lipoprotein cholesterol, ***LDL-C:*** Low-density lipoprotein cholesterol, ***CIMT:*** Carotid intima-media thickness,
LVSEF: Left ventricular systolic ejection fraction, ***PASP:*** Pulmonary artery systolic pressure
*Statistically significant

800 ng/ml, the results revealed that CIMT, Hs-CRP, and IL-6 were significantly higher in patients with serum ferritin 800 ng/ml and more than those in patients with serum ferritin less than 800 ng/ml (Figure 1)

3.2. Correlation data

Positive correlations were found between CIMT and absolute neutrophil count (ANC), WBC, serum ferritin, IL-6, and Hs-CRP as shown in (Table 2). While CIMT was not correlated with age, BMI, Hb, Platelets (PLTs), cholesterol, triglycerides, HDL-C, LDL-C, LVSEF, or PSAP. Strong positive correlations were found between Ferritin and both Hs-CRP and IL-6, and between Hs-CRP and IL-6 (Figure 2).

3.3. Univariate and multivariate analysis

In univariate analysis, there were significant relations between CIMT, BMI, ANC, and Hs-CRP (Table 3). The multivariate analysis was conducted after the exclusion of the co-linear variables. The best multivariate model included Hs-CRP. In the multivariate regression, Hs-CRP was an independent predictor of CIMT (Table 4).

3.4. ROC analysis

To test the diagnostic characteristics of Hs-CRP as a diagnostic test in detecting abnormal CIMT (more than the mean of normal control), a receiver operating characteristic had been conducted. A cut off value of 60.4 ng/ml had a sensitivity of 63.3% and a specificity of 93.3% (Figure 3).

4. Discussion

We concluded that the main findings of this study were: (1) CIMT, serum ferritin, IL-6, and Hs-CRP were significantly higher in TI patients than control and were higher in patients with serum ferritin ≥ 800 ng/ml, (2) LVSEF was significantly lower and PSAP was significantly higher in TI patients than control (3) Both serum ferritin and inflammatory markers had significant positive correlations with CIMT, (4) CIMT was not correlated with the lipid profile in thalassemia intermedia patients, (5) Hs-CRP was an independent predictor of abnormal CIMT.

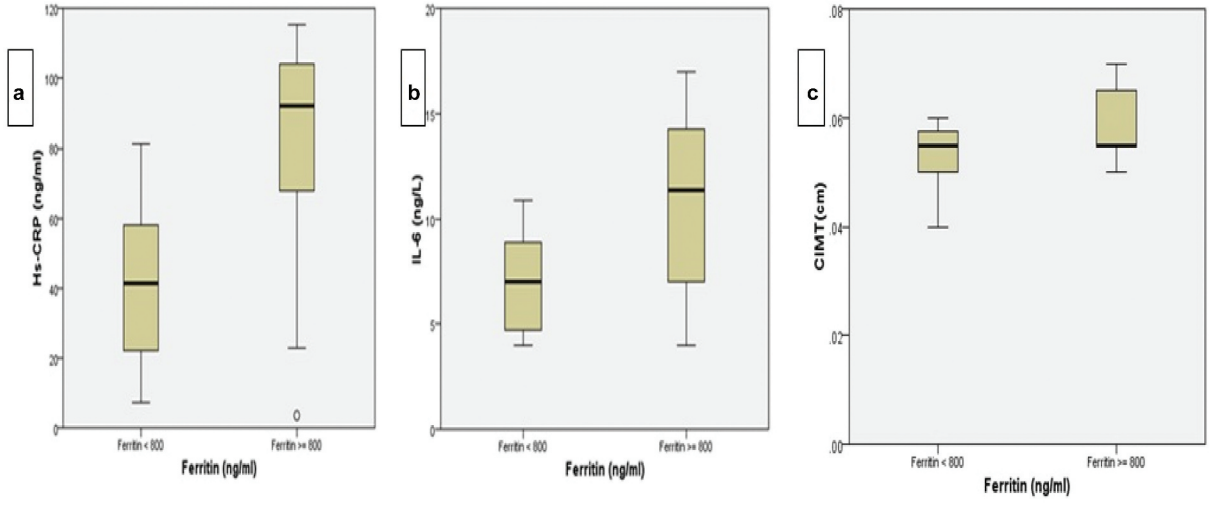


Figure 1. Comparison of IL-6 (A), Hs-CRP (B), and CIMT(C) results in B-thalassemia intermedia patients based on a cutoff point of ferritin 800 ng/ml.

Table 2. Correlation between CIMT and other variables.

	CIMT (cm)	
	r-value	P-value
Age (years)	**0.155**	**0.308**
BMI (kg/m^2)	**0.208**	**0.171**
WBC (×109/L)	**0.333**	**0.025***
ANC (×109/L)	**0.320**	**0.032***
Hb (g/dl)	**−0.036**	**0.812**
PLTs (×109/L)	**0.168**	**0.269**
Serum Ferritin (ng/ml)	**0.544**	**0.000***
IL-6 (ng/L)	**0.520**	**0.000***
Hs-CRP (ng/ml)	**0.603**	**0.000***
Total cholesterol (mg/dl)	**0.155**	**0.310**
Triglycerides (mg/dl)	**0.010**	**0.949**
HDL-C (mg/dl)	**0.212**	**0.162**
LDL-C (mg/dl)	**−0.012**	**0.937**
LVSEF (%)	**−0.064**	**0.678**
PASP (mmHg)	**0.291**	**0.052**

CIMT: Carotid intima-media thickness, ***BMI:*** Body mass index, ***WBC:*** White blood cell, ***ANC:*** Absolute neutrophil count,
Hb: Hemoglobin, ***PLTs:*** Platelets, ***IL-6:*** Interleukin-6, ***Hs-CRP***: High sensitive C-reactive protein, ***HDL-C:*** High-density lipoprotein cholesterol, ***LDL-C:*** Low-density lipoprotein cholesterol, ***LVSEF:*** Left ventricular systolic ejection fraction,
PASP: Pulmonary artery systolic pressure
*Statistically significant

This study showed that TI patients had significantly higher CIMT than control. CIMT is a good determinant of subclinical atherosclerosis [25–28]. Increased CIMT was considered as a mirror of atherosclerotic burden, and could predict subsequent events including myocardial infarction and stroke [4,25]. Studies conducted to investigate CIMT in thalassemia patients had controversial results. *Hahalis et al*. and *Adly et al*. found higher CIMT in TI patients [29,30], while *Nassef et al*. did not find a difference between cases and control despite the finding that concluded the occurrence of early premature atherosclerosis and arterial resistance in TI confirmed by the mean flow velocity (MFV) and peak systolic velocity (PSV) of middle cerebral and basilar arteries [30].

In the current work, lower levels of total cholesterol, HDL-c, and LDL-c with higher triglycerides levels were found in TI patients compared to the control. Most studies demonstrated hypocholesterolemia and hypertriglyceridemia in TI compared to healthy participants [30–35]. The major mechanisms that had been claimed for hypocholesterolemia in thalassemia were: (1) The accelerated erythropoiesis that enhances the bone marrow to consume cholesterol to be provided for the erythroid progenitor cells [31,36], (2) The reduced hepatic secretion and increased catabolism of LDL-C due to increased production of inflammatory cytokines [37,38], and (3) The increased clearance of cholesterol by the macrophages of the spleen and the liver [37]. The increased triglycerides levels could be contributed to the reduced extrahepatic lipolytic activity [39].

The absolute level of LDL-C was not considered as more important in atherogenesis than the modification of LDL in the arterial wall. In particular by oxidation, it appears to be more important so that the decreased LDL-C level in thalassemia patients would not appear to have a protective effect against atherosclerosis [35]. It was established that coronary heart disease can occur at any level of serum cholesterol [40], and there was a crucial role for the oxidation in atherosclerotic plaque formation [41]. The increased oxidative stress in patients with thalassemia was attributed to abnormal iron homeostasis [42,43].

High ferritin level in TI patients was found in many clinical studies [33,44–46] in concordance to the current study. The enhanced iron absorption secondary to ineffective erythropoiesis was the contributing factor for the iron overload in TI patients [44,47]. The oxidation of LDL in the vascular subintima was enhanced by the increased iron-catalyzed free radical reactions secondary to the iron overload state [48]. This theory had been supported by clinical, and epidemiological studies that concluded that there were alterations of arterial

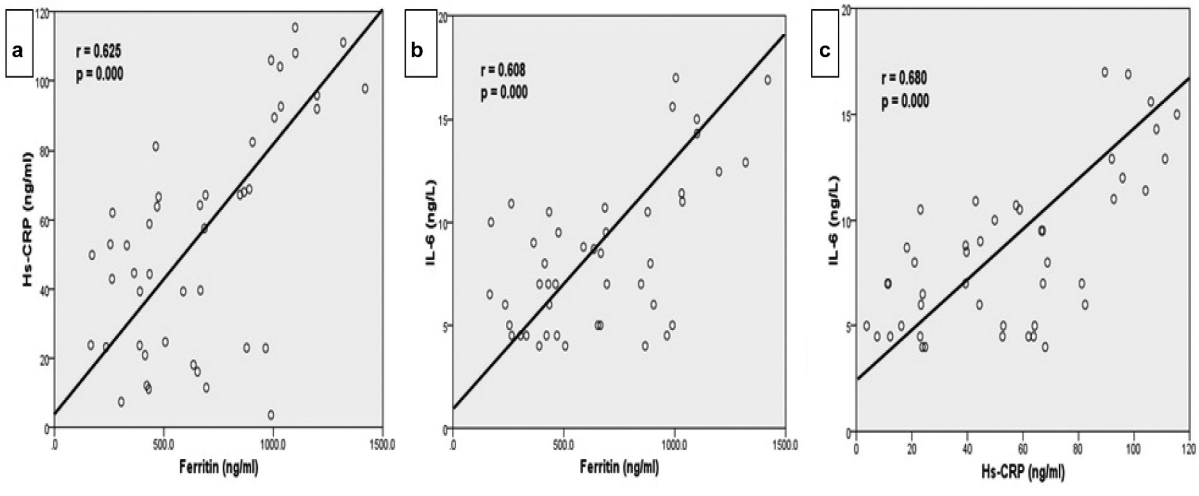
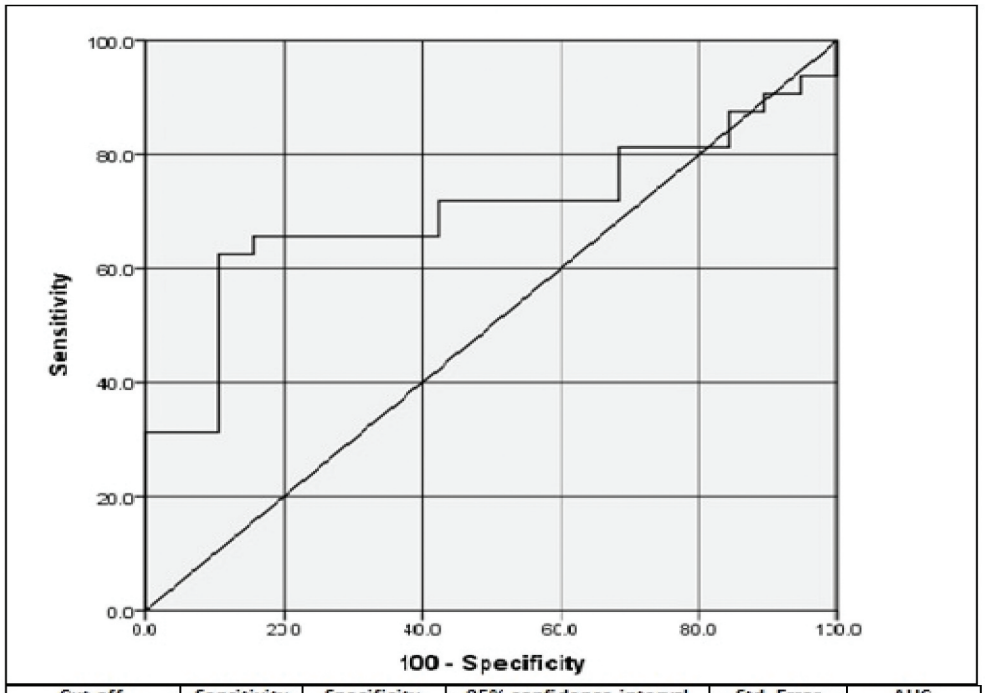


Figure 2. Scatter blot and correlations between (A) Ferritin and Hs-CRP, (B) Ferritin and IL-6, and (C) Hs-CRP and IL-6.



Cut-off	Sensitivity	Specificity	95% confidence interval	Std. Error	AUC
> 60.4	63.3 %	93.3%	0.576 - 0.873 (P value= 0.015)	0.076	0.724

Figure 3. Diagnostic characteristics of Hs-CRP in B-thalassemia intermedia patients.

structures which provoke the process of atherosclerosis due to iron overloading in patients with TM [49–51]. A significant correlation was detected between CIMT and ferritin [52,53].

Moreover, this iron overload state could also overstimulate the macrophages which in turn increase IL-6 production [54]. The production of CRP and other acute-phase proteins in the liver and their release into the bloodstream is stimulated by IL-6 [55].

In agreement with the current finding, *Kanavaki et al*. had found an increased hs-CRP and both *Aggeli et al*. and *Tselepis et al*. had found an increased IL-6 level in patients with TI [33,56,57], and Hs-CRP had been associated with arterial stiffness [58–62]. Moreover, circulating IL-6 was independently correlated with CIMT [63]. In apparently healthy men and women, independent associations of IL-6 and CRP were found with cardiovascular events and mortality [64–66].

TI patients were found to have higher ANC than the control. In addition, ANC had a positive correlation with CIMT. Circulating neutrophils are recruited to the arterial walls by IL-

Table 3. Univariate linear regression of variables and CIMT **.

	Standardized coefficients Beta	T	P-value	95.0% C.I. for B Lower	Upper
Age	**− 0.100**	**− 0.742**	**0.464**	**0.000**	**0.000**
BMI	**0.403**	**2.859**	**0.008***	**0.000**	**0.002**
WBC	**− 0.035**	**− 0.217**	**0.830**	**− 0.001**	**0.001**
ANC	**0.344**	**2.073**	**0.047***	**0.000**	**0.003**
Hb	**− 0.099**	**− 0.836**	**0.409**	**− 0.002**	**0.001**
PLTs	**0.122**	**1.063**	**0.296**	**0.000**	**0.000**
Cholesterol	**0.391**	**1.120**	**0.271**	**0.000**	**0.000**
Triglycerides	**− 0.088**	**− 0.533**	**0.598**	**0.000**	**0.000**
HDL-C	**0.065**	**0.237**	**0.815**	**0.000**	**0.000**
LDL-C	**− 0.374**	**− 1.929**	**0.063**	**0.000**	**0.000**
Ferritin	**0.062**	**0.360**	**0.721**	**0.000**	**0.000**
Hs-CRP	**0.564**	**2.944**	**0.006***	**0.000**	**0.000**
IL-6	**0.017**	**0.092**	**0.927**	**− 0.001**	**0.001**

CIMT: Carotid intima-media thickness, ***BMI:*** Body mass index, ***WBC:*** White blood count, ***ANC:*** Absolute neutrophil count,
Hb: Hemoglobin, ***PLTs:*** Platelets, ***HDL-C:*** High-density lipoprotein cholesterol, ***LDL-C:*** Low-density lipoprotein cholesterol,
Hs-CRP: High sensitive C- reactive protein, ***IL-6:*** Interleukin-6
* Statistically significant
** Dependent Variable: CIMT

Table 4. Multivariate linear regression of variables and CIMT **.

	Standardized coefficients Beta	t	P-value	95.0% C.I. for B Lower	Upper
Age	**0.055**	**0.399**	**0.693**	**0.000**	**0.000**
WBC	**0.167**	**1.274**	**0.211**	**0.000**	**0.001**
Hb	**− 0.163**	**−1.276**	**0.210**	**− 0.003**	**0.001**
PLTs	**0.111**	**0.895**	**0.377**	**0.000**	**0.000**
Cholesterol	**0.235**	**0.654**	**0.517**	**0.000**	**0.000**
Triglycerides	**− 0.001**	**− 0.004**	**0.997**	**0.000**	**0.000**
HDL-C	**0.165**	**0.552**	**0.584**	**0.000**	**0.000**
LDL-C	**− 0.286**	**−1.448**	**0.156**	**0.000**	**0.000**
Hs-CRP	**0.586**	**4.617**	**0.000***	**0.000**	**0.000**

CIMT: Carotid intima-media thickness, ***WBC:*** White blood count, ***Hb:*** Hemoglobin, ***PLTs:*** Platelets, ***HDL-C:*** High-density lipoprotein cholesterol, ***LDL-C:*** Low-density lipoprotein cholesterol, ***Hs-CRP***: High sensitive C- reactive protein
* Statistically significant
** Dependent Variable: CIMT

6 secreted from the stimulated macrophages secondary to the oxidized LDL and iron overload state in the vascular subintima which in turn promotes atherosclerosis. It was demonstrated in many studies that neutrophils infiltrate human carotid atherosclerotic plaques and contribute to the destabilization and subsequent rupture of these plaques [67,68]. Besides being an indicator of systemic inflammation, neutrophils were also considered to be a useful biomarker for predicting the risk of cardiac-cerebral vascular diseases [69–71] and associated with adverse cardiovascular events [72].

The studied group of patients was found to have lower LVSEF compared to controls which was in concordance with the findings of *Adly* study [29]. Cardiac toxicity is often insidious; less than half of the patients with β-thalassemia have detectable cardiac iron, though many of them are asymptomatic [73,74]. Electrocardiography and echocardiography signs of iron toxicity do not appear until severe cardiac iron deposition has occurred [75,76]. Cardiac iron overload is the leading cause of death in patients with TM and TI, which is associated with cardiac dysfunction and chronic cardiac failure [77,78]. Higher PSAP, which is found in our patients, was in agreement with *Moghaddam* and *Amoozgar* studies [79,80]. Endothelial activation along with chronic inflammation underlies the pathophysiology of B-thalassemia [56]. This endothelial dysfunction is characterized by reduced nitric oxide bioavailability, and pro-oxidant and pro-inflammatory stress [81]. As a consequence, blood cells adhere and migrate through the endothelium into the tissues [82,83], with the development of vascular intimal hyperplasia, platelet activation, neutrophil adhesiveness [57,84], and coagulopathy, resulting in further vasomotor instability, proliferative vasculopathy, and endothelial injury [57,84], a hallmark of the development of pulmonary hypertension (PHT) in adulthood [81].

The cross-sectional and observational nature of the study limited the ability to establish a cause and an effect relationship. We recommended conducting a prospective study to identify this relation.

Furthermore, our recommendations which based on this pro-inflammatory status and premature atherosclerosis in TI patients are to modify lifestyle, proper iron chelation, and screen for other risk factors of atherosclerosis in those patients for early management to achieve primary prophylaxis of the atherosclerotic events. Moreover, we can carry on a randomized clinical trial to verify if there is a role for anti-inflammatory drugs in lowering the studied pro-inflammatory markers and the progress of CIMT. Such a study would provide a more precise vision of the follow-up plan and treatment schedules in TI patients.

5. Conclusion

We concluded that TI patients had abnormally higher CIMT than control despite the protective lipid profile and that could be explained by the overstimulation of macrophages by the iron overload state. This iron overload state also could increase the oxidized portion of LDL and the overstimulated macrophages would oversecrete inflammatory mediators that in turn would recruit neutrophils and initiate atherosclerosis.

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Author contributions

Osama Ahmad Ibrahim: Conception and design of the work, obtaining funding, supervision, careful revision of the protocol, revision of the methodology of the study and interpretation of the results then finally, revision of the paper before submission.

Ahmad B. Ahmad: Study and revision of the study aims and methodology, responsible for doing echocardiography and doppler required in the study, interpretation of the echocardiography and doppler results in relation to the studied markers then lastly, sharing in the paper writing.

Dalia Ahmad Nigm: Substantial contribution to the conception of the work, concerned about the laboratory results, revision of the methodology, and interpretation of the results.

Asmaa Nady Hussien: The principal investigator responsible for the design of the work, collection of data, obtaining fund, analysis of study results and paper drafting then finally, approval of the version to be published.

Walaa H. Mohammad Ibrahim: Significant revision of the protocol and work design, analysis of the data and interpretation of results then final revision of the paper.

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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