Carotid Doppler ultrasonography as a screening tool of early atherosclerotic changes in children and young adults with β -thalassemia major

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Abstract

Purpose β -thalassemia major (β -TM) patients had an increased incidence of cardiovascular complications secondary to iron overload. They showed early carotid atherosclerosis as showed by increased carotid intima media thickness (CIMT) that may occur early even when significant iron overload is absent. We aimed to test the diagnostic performance of CIMT measurement by Doppler ultrasonography as a structural indicator for premature atherosclerosis in β -TM patients.

Methods Case-control study included 42 β -TM patients (24 males and 18 females) aged from 3 to 30 years and 36 age- and sex-matched healthy controls. Carotid Duplex was used for measurement of CIMT in all subjects.

Results The frequency of abnormal CIMT among patients was 19%. Mean CIMT of right anterior wall was 0.8 ± 0.16 (range 0.5–1.2) mm, of right posterior wall was 0.80 ± 0.17 (range 0.5–1.2), of right lateral wall was 0.8 ± 0.17 (range 0.5–1.1) mm. CIMT of left anterior wall ranged from 0.5 to 1.2 with mean 0.81 ± 0.17 , CIMT of left posterior wall ranged from 0.5 to 1.1 with mean 0.80 ± 0.17 mm. Mean CIMT of left lateral wall was 0.81 ± 0.18 mm (range 0.5–1.2). CIMT of right anterior, right posterior and left anterior walls were thicker in patients compared to controls ($P = 0.003, 0.015, < 0.001$, respectively). There was no observable difference in CIMT between males and females, splenectomised and non-

splenectomised, or well and poorly chelated subgroups ($P > 0.05$). CIMT of right lateral wall correlated with the disease duration ($r = 0.3, P = 0.04$).

Conclusions Carotid ultrasound was a useful tool to detect subclinical atherosclerosis thorough CIMT evaluation in β -thalassemia major patients. β -thalassemia major children proved to have an increased CIMT regardless the state of iron overload.

Keywords Carotid intima · Media thickness · Beta-thalassemia · Iron overload · Blood transfusion

Sommario

Background I pazienti con β -Talassemia major (β -TM) mostrano una aumentata incidenza di complicanze cardiovascolari dovute al sovraccarico marziale. Si verifica una precoce aterosclerosi carotidea dimostrata da un aumento dello spessore medio-intimale, che si verifica anche in assenza di significativo sovraccarico di ferro. Ci siamo proposti di valutare la performance diagnostica dell'ultrasonografia Doppler nella misurazione dello spessore medio-intimale della carotide, quale indicatore di precoce aterosclerosi nei pazienti con β -TM.

Metodi e risultati Studio caso-controllo che ha compreso 42 pazienti con β -TM (24 maschi e 18 femmine) di età tra i 3 e i 30 anni e 36 controlli sani “matched” per età e sesso. Tutti i soggetti sono stati sottoposti ad ultrasonografia Doppler per la misurazione dello spessore medio-intimale della carotide. La prevalenza di spessore medio-intimale patologico tra i pazienti è stata del 19%. A destra lo spessore medio della parete anteriore è risultato di mm. $0,8 \pm 0,16$ (range 0,5-1,2), della parete posteriore di mm. $0,8 \pm 0,17$ (range 0,5-1,2), della parete laterale mm. di $0,8 \pm 0,17$ (range 0,5-1,1). A sinistra lo spessore della parete anteriore ha dimostrato un range di mm. 0,5-1,2 con

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una media di mm. $0,81 \pm 0,17$, della parete posteriore di mm. $0,5\text{--}1,1$ con una media di mm. $0,80 \pm 0,17$ e della parete laterale di mm. $0,5\text{--}1,2$ con una media di mm. $0,81 \pm 0,18$. Lo spessore medio-intimale delle pareti anteriore e posteriore destra e anteriore sinistra è risultato maggiore nei pazienti che nei controlli ($p.003$, $.015$ e $<.001$ rispettivamente). Non si sono riscontrate differenze riguardo al sesso, funzione splenica o chelazione ($p > .05$). Lo spessore medio-intimale della parete laterale destra ha correlato positivamente con la durata di malattia ($r = .03$; $p = .04$).

Conclusioni L'ultrasonografia è un metodo utile nell'identificare aterosclerosi subclinica grazie alla valutazione dello spessore medio-intimale carotideo nei pazienti con β -TM, che hanno dimostrato avere un aumento di tale spessore indipendentemente dallo stato di sovraccarico marziale.

Introduction

B-thalassemia is a monogenic hemolytic anemia which results from defective hemoglobin synthesis [1]. B-thalassemia major patients (B-TM) are at increased risk for iron overload as a consequence of frequent blood transfusions and increased gastrointestinal iron absorption [2]. Several studies have suggested a link between iron load and risk of atherosclerosis [3]. According to “iron hypothesis”, iron is supposed to be injurious to the cardiovascular system, thus inducing atherosclerosis development and progression [4]. The pro-atherogenic properties of iron resulted from the generation of active free radicals, the oxidation of lipoproteins, and the activation of platelets [5]. Iron overload in β -TM patients may lead to structural changes in the arterial wall and thickening of the carotid arteries which might predispose them to premature atherosclerosis [6, 7]. Increased carotid artery intima media thickness (CIMT) is a structural indicator of early atherosclerosis, and correlates with the vascular risk factors, besides to the severity and extent of coronary artery disease [8]. Doppler ultrasound scanning of extra-cranial carotid arteries is well established as a non-invasive tool that may identify children at increased risk of stroke that would otherwise be missed. Vascular abnormalities with increased arterial stiffness and endothelial dysfunction have been reported in adult patients with β -TM [9]. But no reports are available on this risk in children, adolescents and young adults. In this study, we aimed to assess the role of Doppler ultrasonography in the evaluation of carotid artery intima media thickness (CIMT) as a predictor to atherosclerotic change in transfusion-dependent β -TM children and young adults with iron overload.

Patients and methods

This case–control study was carried out on 42 patients with β -TM (24 males and 18 females with a sex ratio 1.3). Their age ranged from 3 to 30 years with a mean age of 14.10 ± 7.67 years. Patients were followed up at the Paediatric Haematology Clinic, New Children's Hospital, Cairo University (NCHCU) in the period from April 2015 to March 2016. Further 36 healthy subjects (12 males and 24 females) were included and served as a control group. Their age ranged from 4 to 30 years with a mean age of 15.9 ± 9.0 years. Patients with associated diseases that speed up atherosclerotic process, those with carotid artery obstruction or echocardiographic evidence of heart failure and those with familial hypercholesterolemia were excluded from the study. An informed consent was obtained from the patients or their legal guardians before the enrollment. The protocol was approved by the Investigational Review Board (IRB), according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland.

Data were retrieved by reviewing medical records and by interviewing all the patients and their legal guardians. Clinical data and the most recent laboratory data were recorded. Carotid duplex study: patients and controls were subjected to B-mode and color-coded duplex sonography of their extra-cranial carotid. Studies were performed using a high resolution B-mode system (Sonoline Elegra Siemens Medical Systems), (INC, Issaquah WG 7L), with a large broadband linear array transducer of 7.0–12.0 MHz. The ultrasound system was connected to a computer system that calibrated the ultrasound images and converted them to numerical data. Examination started by locating the common carotid artery (CCA) in the lower neck in the transverse plane. Then, CCA was followed until the transducer was blocked by the clavicle. We tried the caudal angulations to evaluate the common carotid origin if possible. At CCA bifurcation, the transducer was rotated 90° to be parallel to the CCA to have longitudinal scanning of the CCA, the bifurcation, the internal carotid artery (ICA) and external carotid artery (ECA). ICA was then followed distally as far as possible and until it was lost behind the mandible. Vessels were tested for the exclusion of obstructive lesions and for detection of the presence of sub-intimal lucency, and atherosclerotic plaques that bulge into the lumen, followed by measuring CIMT. CIMT was measured in 1-cm segment proximal to the dilation of the carotid bulb, referred to as CCA, and in plaque-free segments. CIMT defined as the distance between the junction of the lumen and intima and that of the media and adventitia [10, 11] (Fig. 1). For each subject, three

Fig. 1 Long axis view in B-mode showing the measurements of the CIMT in the posterior wall (far wall) of the CCA proximal to the carotid bulb delineated by the arrows and cursors at the interfaces of measurements in one healthy control

measurements on both sides were got on the anterior, lateral, and posterior projections of the far wall [8] (Figs. 2, 3). Abnormal CIMT was considered as any reading exceeding the calculated cut off value of normal IMT which equals “mean + 2 standard deviation” of the control group for each variable.

Statistical methods

Data were analyzed by SPSS program version 22. Nominal data were expressed as frequencies. Numerical data were expressed as mean $\pm$ standard deviation and compared using the *t* test for parametric data or Mann–Whitney test for non-parametric data. Pearson’s correlations were used to explore the associations between the

numerical variables. *P* values < 0.05 were considered significant [12].

Results

Clinical and laboratory data for patients are shown in Table 1. The frequency of abnormal CIMT among our patients was 19%. Table 2 shows comparison of CIMT between patients and controls. Mean CIMT of the right anterior wall, right posterior wall and left anterior wall were thicker in patients compared to control group ($P = 0.003$, 0.015 , < 0.001 , respectively), while other CIMT measurements were comparable between patients and control group ($P > 0.05$). Twenty-seven patients

Fig. 2 Long axis view in B-mode showing the measurements of the CIMT in the lateral, posterior and anterior walls of the left CCA that measures (1.2, 0.9 and 1 mm respectively) in one of the patients

Fig. 3 Long axis view in B-mode showing the measurements of the CIMT in the lateral, posterior and anterior walls of the right CCA that measures (1.0, 0.8 and 0.7 mm respectively) in another patient

Table 1 Baseline clinical and laboratory data of the studied cases ($n = 42$)

Variable	Mean $\pm$ SD	Range	Median
Heart rate (beats/min)	102.0 $\pm$ 11.5	85–110	100
Systolic blood pressure (mmHg)	101.7 $\pm$ 7.5	80–120	100
Diastolic blood pressure (mmHg)	64.5 $\pm$ 6.7	50–80	62.5
Hemoglobin (g/dL)	7.46 $\pm$ 1.57	4.30–13.30	7.45
Total leukocyte count (thousands/cmm)	37.57 $\pm$ 49.58	5.40–193.00	15.10
Platelet count (thousands/cmm)	597.65 $\pm$ 370.90	79.00–1521.00	515.00
Reticulocytic count (%)	5.26 $\pm$ 8.80	0.10–32.90	1.50
Serum ferritin (ng/mL)	2063.42 $\pm$ 1664.77	166.00–7139.70	1448.00
Random blood sugar (mg/dL)	86.00 $\pm$ 19.75	62.00–117.00	80.00
Serum creatinine (mg/dL)	0.51 $\pm$ 0.52	0.10–3.40	0.40
ALT (U/I)	56.59 $\pm$ 53.34	7.00–260.00	36.00
AST (U/I)	67.29 $\pm$ 60.39	19.00–301.00	54.00
Bilirubin total (mg/dL)	1.96 $\pm$ 1.18	0.30–7.00	1.70
Bilirubin direct (mg/dL)	0.46 $\pm$ 0.41	0.01–2.40	0.40

ALT alanine aminotransferase, AST aspartate aminotransferase

(65.9%) had cardiovascular symptoms as dyspnea or palpitation on exertion; their mean CIMT of the right anterior and left anterior walls were increased when compared to patients with no symptoms ($P = 0.015$, 0.019 , respectively) (Table 3). Six patients (14.3%) were on blood transfusions every 2 weeks, and 36 (85.7%) on blood transfusions every month. The frequently transfused patients had decreased mean CIMT of the right lateral wall compared to non-frequently transfused patients ($P < 0.05$)

(Table 4). Mean CIMT was comparable between males and females ($P > 0.05$). Twenty-eight patients (66.7%) had been splenectomised and no significant difference in CIMT was found between splenectomised and non splenectomised patients ($P > 0.05$). Thirty-five (83.3%) were on chelating agent and there were no significant difference between patients receiving chelating agent and those receiving no chelation of CIMT measurements ($P > 0.05$) (data not shown). As shown in Table 5, significant direct

Table 2 Comparison of Doppler data (CIMT in mm) between patients and control

CIMT (mm)	Patients (<i>N</i> = 42)		Control (<i>N</i> = 36)		<i>P</i> value
	Mean ± SD	Range	Mean ± SD	Range	
Right anterior wall	0.80 ± 0.16	0.5–1.2	0.69 ± 0.16	0.4–1.0	0.003*
Right posterior wall	0.80 ± 0.17	0.5–1.2	0.71 ± 0.15	0.5–1.1	0.015*
Right lateral wall	0.80 ± 0.17	0.5–1.1	0.75 ± 0.17	0.5–1.2	0.248
Left anterior wall	0.81 ± 0.17	0.5–1.2	0.68 ± 0.09	0.5–0.9	< 0.001*
Left posterior wall	0.80 ± 0.17	0.5–1.1	0.77 ± 0.12	0.6–1.1	0.375
Left lateral wall	0.81 ± 0.18	0.5–1.2	0.77 ± 0.19	0.5–1.2	0.258

* Statistically significant

Table 3 Comparison of the measured Doppler data (CIMT in mm) in relation to cardiovascular symptoms

CIMT (mm)	Cardiac or vascular symptoms				<i>P</i> value
	Yes (<i>n</i> = 27)		No (<i>n</i> = 19)		
	Mean ± SD	Range	Mean	Range	
Right anterior wall	0.85 ± 0.15	0.60–1.2	0.72 ± 0.15	0.5–1.0	0.015*
Right posterior wall	0.81 ± 0.17	0.50–1.2	0.80 ± 0.18	0.50–1.1	0.955
Right lateral wall	0.83 ± 0.16	0.50–1.1	0.74 ± 0.17	0.50–1.1	0.174
Left anterior wall	0.86 ± 0.16	0.60–1.2	0.73 ± 0.15	0.5–1.0	0.019*
Left posterior wall	0.77 ± 0.17	0.50–1.1	0.87 ± 0.15	0.60–1.10	0.078
Left lateral wall	0.82 ± 0.18	0.50–1.2	0.82 ± 0.17	0.60–1.20	0.989

* Statistically significant

Table 4 Comparison of the measured Doppler data (CIMT in mm) in relation to transfusion frequency

CIMT (mm)	Frequency of transfusion				<i>P</i> value
	Frequent (every 2 weeks) (<i>n</i> = 6)		Not frequent (<i>n</i> = 36)		
	Mean ± SD	Range	Mean	Range	
Right anterior wall	0.87 ± 0.22	0.60–1.20	0.79 ± 0.15	0.5–1.1	0.432
Right posterior wall	0.88 ± .04	0.80–0.90	0.79 ± 0.18	0.5–1.2	0.097
Right lateral wall	0.65 ± .12	0.50–0.80	0.82 ± 0.17	0.5–1.1	0.018*
Left anterior wall	0.77 ± 0.19	0.60–1.10	0.82 ± 0.17	0.5–1.2	0.411
Left posterior wall	0.85 ± 0.05	0.80–0.90	0.79 ± 0.18	0.5–1.1	0.351
Left lateral wall	0.75 ± 0.10	0.60–0.90	0.82 ± 0.19	0.5–1.2	0.392

* Statistically significant

correlation was found between CIMT of left anterior wall and age ($r = 0.3$, $P = 0.035$), while left posterior wall correlated with total and direct bilirubin ($r = 0.4$, $P = 0.018$; $r = 0.4$, $P = 0.014$, respectively). CIMT of right lateral wall correlated with the disease duration ($r = 0.3$, $P = 0.04$) and showed marginally insignificant direct correlation with AST ($r = 0.286$, $P = 0.07$).

Discussion

Carotid ultrasound is the diagnostic tool used worldwide for grading of the carotid artery stenosis. However, the lack of worldwide uniformity in the interpretation of the

ultrasound criteria and in the different laboratory reports makes this work difficult [13]. Progressive increase in carotid artery intima media thickness (CIMT) is considered a structural alarm of early atherosclerosis and was showed to correlate with the vascular risk factors [10, 14]. A significant incidence of vascular complications, including premature arterial stiffness and early atherosclerosis, has been observed in adult patients with β -TM [15]. Studies performed in adolescents and adults with β -TM reported premature atherosclerosis with increased CIMT [9, 16], and suggested a causal link between iron load and premature atherosclerosis as reflected by increased CIMT [17, 18]. The present study was undertaken to test the role of Doppler ultrasonography in the evaluation of carotid artery

Table 5 Correlations between measured Doppler data (CIMT in mm) and other numerical variables

	Right anterior wall (mm)	Right posterior wall (mm)	Right lateral wall (mm)	Left anterior wall (mm)	Left posterior wall (mm)	Left lateral wall (mm)
Age						
<i>R</i>	0.140	0.120	0.293	0.326	0.136	0.023
<i>P</i>	0.377	0.450	0.060	0.035*	0.389	0.885
Hb (g/dL)						
<i>R</i>	0.229	0.068	− 0.161	0.150	− 0.058	− 0.102
<i>P</i>	0.154	0.677	0.320	0.354	0.721	0.531
Serum ferritin (ng/mL)						
<i>R</i>	− 0.148	− 0.096	0.140	0.097	0.045	− 0.100
<i>P</i>	0.376	0.566	0.400	0.563	0.788	0.552
ALT (U/I)						
<i>R</i>	− 0.129	0.098	0.289	0.103	− 0.011	− 0.006
<i>P</i>	0.420	0.541	0.067	0.523	0.945	0.972
AST (U/I)						
<i>R</i>	− 0.180	0.112	0.286	0.158	0.205	0.087
<i>P</i>	0.261	0.484	0.070	0.323	0.199	0.588
Bilirubin total (mg/dL)						
<i>R</i>	− 0.065	0.026	0.159	− 0.103	0.393	0.125
<i>P</i>	0.708	0.882	0.353	0.549	0.018*	0.469
Bilirubin direct (mg/dL)						
<i>R</i>	− 0.186	0.096	0.166	0.003	0.424	0.105
<i>P</i>	0.301	0.595	0.356	0.986	0.014*	0.561
Disease duration						
<i>R</i>	0.130	0.148	0.318	0.288	0.101	0.024
<i>P</i>	0.411	0.351	0.040*	0.064	0.525	0.879

Hb hemoglobin, *TLC* total leucocytic count, *PLt* platelet count, *Retics* reticulocytic count, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

* Statistically significant

intima media thickness (CIMT) as a predictor of atherosclerotic changes in β -TM patients with iron overload.

Our data showed that the frequency of abnormal CIMT in our cohort was 19%. CIMT ranged from 0.5 to 1.2 mm and their mean IMT of right anterior wall, right posterior wall and left anterior wall were higher in patients compared to the control group. But CIMT of right lateral wall left posterior wall and left lateral wall were comparable between patients and control group. This was in line with previous studies which found increased carotid CIMT in patients with β -TM as compared with healthy subjects [7, 9, 15, 16, 18–22]. Gursel and colleagues showed that CIMT in patients with β -TM aged 11 years or less was increased compared with healthy controls in the same age group. This finding suggested that subclinical atherosclerosis started at an early age in β -TM children. They suggested early assessment of CIMT in patients with β -thalassemia major [23]. Stoyanova and associates compared the CIMT between β -TM patients and healthy

subjects (0.67 ± 0.20 vs. 0.66 ± 0.15 mm), and reported insignificant difference [24]. Our data showed no significant difference in mean CIMT between males and females. This come in harmony with other studies that found no significant differences in mean CIMT in relation to gender [17]. Among our patients, mean CIMT of the right anterior and left anterior walls were higher in patients having cardiac or vascular symptoms. In addition, frequently transfused patients had lower mean CIMT of the right lateral wall compared to none frequently transfused patients but no significant difference in means CIMT was found between splenectomised and non splenectomised patients, or between patients receiving chelating agent and those did not receive chelating agent. Which contradicts other studies that found significant difference in CIMT in β -TM children in relation to features suggestive of iron overload like duration since first blood transfusion, frequency of blood transfusion, poor chelation and splenectomy [25, 26]. The difference between our results and reported data might be justified by the differences in ethnicity, age group,

comorbidities and the smaller sample size in our study. Our data showed that CIMT values correlated with age and disease duration and this agreed with previous studies reported significant statistical correlation between CIMT and patient's age [16, 20]. Dogan and Citak reported positive correlation between mean common CIMT and disease duration ($r = 0.535$) [18]. A striking finding in our data was the direct correlation between CIMT values and indicators of hemolysis including serum bilirubin and aspartate transaminase (AST) which reflected the disease severity. But none of the measures correlated with serum ferritin. This disagree with previous reports of positive correlation between CIMT values and serum ferritin [16, 18, 20] and total body iron as reflected by liver iron concentration (LIC) [17]. Amongst our cohort none of the measures correlated with total hemoglobin. Our results agreed with earlier studies reported no significant difference of CIMT in relation to patient's hemoglobin level [16, 20]. Our data might highlight that β -TM patients with intense hemolysis would be more prone to premature atherosclerotic changes regardless their serum ferritin values.

In fact, ultrasound with color Doppler and spectral analysis remains the primary modality for screening of the extracranial part of carotid arteries offering assessment and grading of carotid disease. Nevertheless, this technique has some limitations, including flow-related artefacts that sometimes make complete assessment of a stenotic part of the vessel difficult. Recently, contrast-enhanced ultrasound (CEUS) was introduced as an adjunctive ultrasonographic technique offering improved flow visualization free of artefacts and detailed plaque surface delineation, thus being able to accurately grade stenosis [27]. Lack of this complementary technique in our study, as well as earlier studies might explain the heterogeneity of the available data and the discrepancies between different studies that were mentioned earlier.

In conclusion, carotid ultrasound is a useful tool to detect subclinical atherosclerosis thorough CIMT evaluation in thalassemia major patients with increased CIMT which is an early change in carotid atherosclerosis. Vascular abnormalities, although often subclinical, occur at an early stage of β -TM even in the absence of significant iron overload.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval The protocol was approved by the Investigational Review Board (IRB), according to the Institutional Committee for the protection of human subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland.

Informed consent Written informed consent was obtained from the patients for the publication of this case report and accompanying images.

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