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Coronary atherosclerosis burden is not advanced in patients with β-thalassemia despite premature extracardiac atherosclerosis: a coronary artery calcium score and carotid intima-media thickness study

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Abstract

Background Thalassemic patients demonstrate an increased rate of extracardiac vascular complications and increased carotid wall intima-media thickness (cIMT), but very low prevalence of coronary artery disease (CAD). We investigated the atheroma burden by assessing the coronary artery calcium (CAC) and cIMT in these patients. **Methods** We examined 37 patients with β-thalassemia and 150 healthy control volunteers with multi-detector computer tomography (CT) and ultrasonography to determine CAC score and cIMT, respectively. **Results** Propensity score matching (C-statistic: 0.88; 95% CI: 0.83–0.93) resulted in 27 pairs of patients; severe CAC was observed in 2 (7.4%) and 0 of β-thalassemia patients and healthy volunteers respectively (P = 0.5). Median calcium score was 0 (0–0) in β-thalassemia patients and 0 (0–4) in healthy volunteers (P = 0.8). Median intima-media thickness was higher in β-thalassemia patients compared to control group [0.45 (0.06–0.65) *vs*. 0.062 (0.054–0.086); P = 0.04]. **Conclusions** Patients with β–thalassemia in comparison with healthy control subjects exhibit similar CAC score and increased cIMT. Our findings indicate a disparate rate of progression of atherosclerosis between coronary and extracardiac arteries in these patients lending support to the epidemiological evidence.

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1 Introduction

The thalassemias are anemias of variable severity, which result from mutations of the genes encoding the synthesis of α- and β-globin chains of hemoglobin.[1] Serious thalassemias are associated with iron overload, tissue damage and increased risk of cardiovascular (CV) complications.[2–5] In contrast to β-thalassemia major (β-TM) which is a transfusion-dependent disease, patients with the β-thalassemia intermedia phenotype (β-TI) exhibit only sporadic, if any, blood transfusion needs for maintenance of life, more severe anemia, less iron overload, and profound red cell disturbances.[3] The remarkably high incidence of venous thromboembolic events and strokes in these patients, while the coronary artery bed appears to be spared, suggests a distinct interplay of an atheroprotective lipid pattern with a proatherogenic environment.[3–10] In fact, multiple clot-promoting and proinflammatory abnormalities have been shown in patients with β-TM and β-TI and linked with endothelial perturbation, procoagulant erythrocytes, hemolysis, iron overload, arterial elastic tissue injury, oxidative stress, hyperplastic bone marrow and splenectomy.[2–4,11–13]

Both venous thromboembolism and carotid atherosclerosis predict subsequent atherothrombotic episodes.[14,15] For example, increased carotid intima-media thickness (cIMT) increases the risk for future myocardial infarction (MI).[16] Moreover, microalbuminuria is associated with higher rates of venous thromboembolism, whereas rosuvastatin over placebo therapy reduces the risk of both coronary arterial and venous thromboembolic events.[17–19] Subclinical vascular disease seems therefore to develop following a unifying pathophysiological pathway, in that a latent disease in one vascular bed may indicate a more generalized impairment of vascular function. We have previously demonstrat-

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ed that both patients with β-TM and β-TI exhibit a global impairment of arterial vasorelaxation and increased cIMT as compared with control healthy subjects.[9,10] The abovementioned findings strongly support the notion that the severe arterial dysfunction in thalassemia may indicate an additional clinical vulnerability for venous thromboembolism.

Epidemiologically, vascular events appear at a relatively young age with a four times higher incidence in β-TI as compared with β-TM patients.[20] Specifically in β-TM, the about 1% rate of vascular episodes accounts for 3.3%–3.5% of total mortality,[21,22] contrasts the zero incidence of coronary artery disease (CAD) in these patients,[2,6,21] and is in accord with the authors' experience at our institution.[9] The reasons for such differential protection of the coronary artery tree are speculative,[9,10,23] and no studies heretofore have addressed the burden of coronary atheroma in thalassemic patients.

Coronary artery calcification (CAC) is highly specific for atherosclerosis, has a close association with CV risk factors and enables a refined risk prediction beyond that provided by global risk assessment tools.[24] Absence of coronary artery calcification on multidetector computed tomography in adults identifies individuals at low risk for CV disease and CV events, thus precluding the need for further downstream management.[24,25]

Therefore, the aim of the present study was to compare thalassemic patients free from CV disease and healthy control subjects with respect to both extent of CAC and cIMT.

2 Methods

In the current study, we recruited patients from our Institution with either β-TM or β-TI who were free from CV disease to undergo both a CT assessment of CAC scoring and ultrasonography to determine cIMT (patient group).[9,10] A group of healthy control subjects was enrolled for comparison (healthy control group).

2.1 CAC measurement

All subjects were examined in a 16-MDCT scanner (LightSpeed 16 ×; General Electric, Milwaukee, Wisconsin) with prospective ECG triggering during a single breath hold (typically 8 s). Data were obtained from the entire heart using sequential acquisition. Scans were prospectively initiated at 70% of the RR interval.[26] Forty-eight contiguous 2.5-mm thick slices (120 kVp, 250 mA, gantry rotation time 0.5 s and exposure time/rotation 0.3 s) were acquired. All scans were read by the same experienced readers (C. K. and A.D) for the presence and amount of CAC using a dedicated workstation (Advantage Workstation 4.2). Quantification of CAC was provided by a computerized program (SmartScore 3.5, GE Medical Systems) that calculated the CAC score by the Agatston method.[27] A calcified lesion was defined as an area ≥ 3 connected pixels with an attenuation > 130 Hounsfield Units applying 3-dimensional connectivity criteria (6 points). CAC score was calculated by multiplying the area of each lesion with a weighted attenuation score dependent on the maximal attenuation within the lesion. Presence of CAC was defined as calcium score > 10 Agatston units (AU), and severe CAC as calcium score > 400 AU.

2.2 IMT measurement

Examinations of the left and right common carotid were examined by experienced sonographers (I.T. and A.R.) in multiple directions as previously described.[9,10] In brief, the sonographer recorded the distance between the boundaries of lumen-intima and media-adventitia interfaces at the far wall of the common carotid arteries. The mean IMT of the six measurements in each patient was then calculated. An intraobserver variability analysis has shown previously a mean percentage error of < 5% for cIMT.

All healthy volunteers were mainly hospital workers who responded to a campaign for CAC screening and were considered to be at usual CV risk.[25] All study subjects denied any history or symptoms of heart disease and had normal physical examinations and resting electrocardiograms. Systolic and diastolic blood pressure was measured and phlebotomy was done for analysis of total cholesterol. Smoking history was determined and considered positive if the subject had been smoking in the past year. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg, or if subjects were receiving antihypertensive treatment. Hypercholesterolemia was considered if total cholesterol concentration was > 200 mg/dL or subjects were on treatment with statins. Diabetes was defined according to World Health Organization criteria. A family history of CAD was thought to be present if a parent or sibling aged ≤ 55 years had had documented CAD.

2.3 Statistical analysis

Categorical data are presented as frequencies and group percentages. Continuous data with normal and skewed distribution are presented as means ± SD and medians (first to third quartile) respectively. The Kolmogorov-Smirnov test was used to examine data distribution normality.

Intima-media thickness and calcium score were compared after propensity matching in patients with β-thalassaemia and in healthy volunteers. Propensity scores were calculated for each patient using multivariable logistic regression modeling fitted with all the variables listed in Table 1.

Table 1. Demographic characteristics.

	Overall (n = 187)	Healthy volunteers (n = 150)	β-thalasaemia (n = 37)	*P*-value
Age, yrs	51.7 ± 7.8	53.2 ± 3.3	45.7 ± 6.9	< 0.001
Male gender	119 (63.6)	101 (67.3)	18 (48.6)	0.04
Smoking	123 (65.8)	106 (70.7)	17 (45.9)	0.007
Hyperlipidemia	99 (52.9)	93 (62.0)	6 (16.2)	< 0.001
Hypertension	64 (34.2)	59 (39.3)	5 (13.5)	0.003
Diabetes mellitus	26 (13.9)	20 (13.3)	6 (16.2)	0.6
Family history of CAD	52 (27.8)	44 (29.3)	8 (21.6)	0.4

Variables are expressed as mean ± SD or *n* (%). *P*-value refers to comparisons between healthy individuals and β-thalassaemia patients. CAD: coronary artery disease.

The logistic regression model was tested for discriminative power by the C statistic [area under the receiver operating characteristic (ROC) curve]. A greedy, nearest neighbor 1: 1 matching was used to match subjects with a caliper width of 0.2 of the standard deviation of the logit of the propensity score. Each matched pair was unique and patients without a suitable match were excluded from further analysis. Standardized differences of the mean < 10% were taken to indicate good balance in the matched sample. Using the matched pairs, we conducted Wilcoxon signed rank test to compare intima-media thickness and the calcium score between the two groups. Rates of CAC and severe CAC between the matched pairs were compared with Mc Nemar's test.

All tests were two-sided and statistical significance was considered for *P*-values < 0.05. The SPSS for windows (version 16.0; SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis.

3 Results

We studied 37 patients with β-thalassemia and used 150 healthy volunteers as a control group. Individuals' demographic characteristics are presented in Table 1**.** Patients with β-thalassemia were younger, less frequently smokers and had less frequently hyperlipidemia and hypertension as well as lower rate of male gender compared to control group. Propensity score matching resulted in 27 pairs of patients (C-statistic: 0.88, 95% CI: 0.83–0.93). The standardized differences between the two groups were less than 10% for all variables in the matched sample (Figure 1). In the propensity matched sample, CAC was observed in 5 (18.5%) and 6 (22.2%) of β-thalasaemia patients and healthy volunteers, respectively ($P > 0.99$), whereas severe CAC was observed in 2 (7.4%) and 0 (0%) of β-thalasaemia patients and healthy volunteers, respectively ($P = 0.5$). Median calcium score was 0 (0–0) in β-thalasaemia patients and 0(0–4) in healthy volunteers ($P = 0.8$). Median cIMT was higher in β-thalasaemia patients compared to control group [0.45 (0.06–0.65) *vs*. 0.062 (0.054–0.086); $P = 0.04$].

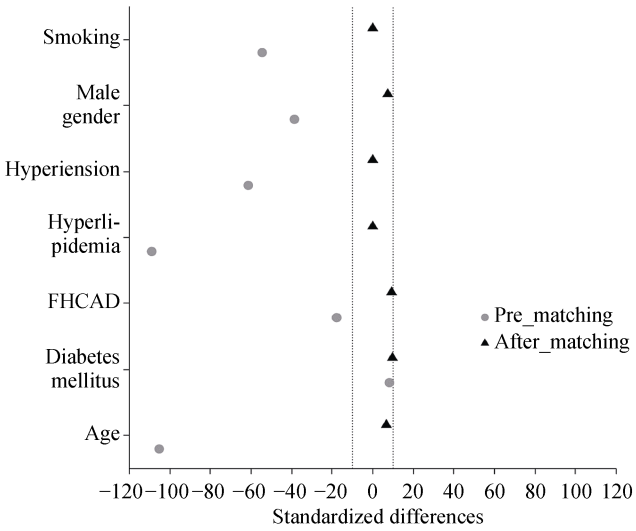

Figure 1. Percentage standardized differences in various characteristics between β-thalassaemia patients and healthy volunteers before and after propensity score matching. FHCAD: family history of coronary artery disease.

4 Discussion

The main findings of this study can be summarized as follows: CAC score was similar between thalassemic patients and healthy control subjects. Confirming our previous work, cIMT was increased and indicated premature extracardiac arterial ageing.[9,10] Coronary arterial calcification participates in atherosclerosis development and is found exclusively in atherosclerotic but not in the normal vessel wall. Although CAC occurs in small amounts in the early atherosclerotic lesions in the second and third decades of life, it is more common in advanced lesions and in older age.[24] Therefore, the current report suggest a disparate rate of atherosclerosis progression between carotid and coronary arteries among thalassemic patients.

4.1 CV complications in β-thalassemia

Initially Logothetis, *et al*, and subsequently additional investigators elucidated the susceptibility of patients with β-TM and β-TI for vascular episodes and documented herein the impact of splenectomy.[28,29] In a large survey, the prevalence of thromboembolic events was 1.65% among 8860 patients with β-TM and β-TI.[6] Venous episodes (predominantly deep vein thrombosis) were more frequent over arterial events (mostly strokes), whereas recurrent as

well as simultaneous arterial and venous thromboses were also reported.[6] Thrombosis in thalassemia was more common in females, splenectomized patients and those with profound anemia.

There is only one case-report on an ill-documented MI in a patient with β-TM.[7] Additionally, one of our patients with β-TM underwent emergency coronary angiography for suspected MI five years ago but we found patent coronary arteries and normal left ventricular contractility. As discussed elsewhere, five extracardiac thrombotic episodes and no CAD event have developed over an 20-year period at our Institution.[9]

4.2 Clinical relevance of the study findings

Carotid disease as compared with CAD exhibit both similarities and differences including plaque morphology and differential impact of serum cholesterol levels on atherogenesis.[30] Since CAC score and therefore atherosclerosis burden was found to be similar, one would expect approximate incidence of CAD between thalassemic patients and healthy volunteers. Zero frequencies of CAD however denote very strong coronary protective mechanisms of atherosclerotic plaques in thalassemia. We have recently shown in thalassemic patients a predominance of small-dense low-density lipoprotein cholesterol particles, increased oxidative stress and very high plasma levels of lipoprotein-associated phospholipase A_2 (Lp-PLA_2), which correlated with cIMT.[23] Further, we have postulated that the high plasma Lp-PLA_2 levels in the presence of a relatively anti-atherogenic cholesterol profile may contribute to premature carotid atherosclerosis but not to coronary artery disease.[23]

4.3 Limitations

This was an observational, non-randomized investigation on a small number of healthy subjects and patients with β-TM and β-TI. Propensity score matching was used, nevertheless we cannot rule-out residual bias due to either unmeasured confounding or over-adjustment. A larger study with adequate sample size may have been capable to detect meaningful CAC score differences between the study groups.

4.4 Conclusions

We showed that patients with β-TM or β-TI in comparison with healthy control subjects exhibit similar CAC score and increased cIMT. These findings demonstrate a differential rate of atherosclerosis progression between coronary and extracardiac arterial beds. Future research should focus on the possible anti-atherogenous potential of blood lipids in thalassemic patients.

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