RESEARCH PAPER

Premature Atherosclerosis in Children With Transfusion-Dependent Thalassemia: A Twin-Center Cross-Sectional Study

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Objective To analyze the risk of premature atherosclerosis in children with transfusion-dependent thalassemia (TDT) compared to controls by measuring carotid intima-media thickness (CIMT) and correlating it with clinical and biochemical parameters. **Methods:** Case-control study among children aged 2 to 15 years. **Results:** Significantly higher CIMT values were observed across all age groups. Mean (SD) CIMT in controls were 0.27(0.07) mm, 0.39 (0.03) mm, and 0.46 (0.05) mm in 2 to 5 years, 6 to 10 years, and 11 to 15 years age groups respectively, as against 0.43 (0.08) mm, 0.55 (0.07) mm and 0.63 (0.08) mm in cases in similar age groups (P<0.001). Mean triglycerides and liver enzymes were significantly elevated in cases. Logistic regression analysis demonstrated that older age group and higher serum ferritin levels, but not dyslipidemia, were significantly associated with high CIMT. **Conclusion:** Children with TDT are at increased risk for premature atherosclerosis.

Keywords: *Complications, Ferritin, Outcome, Survival*.

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The life expectancy and quality of life of children with transfusion-dependant thalassemia (TDT) have improved in recent years. However, non-hematological complications like atherosclerosis can cause severe morbidity and mortality [1]. Many studies have demonstrated an association between abnormal serum lipid levels and increased risk of premature atherosclerosis in these children [1-3]. Studies have also demonstrated an association between iron load and increased risk of premature atherosclerosis in them [4]. The conventional diagnostic tests to confirm atherosclerosis have been angiography and stress testing. Recently, these tests have been replaced by a more convenient and accurate test – the measurement of carotid intima-media thickness (CIMT) [5]. CIMT measurement has been widely used in many studies to analyze the risk of atherosclerosis in children and adults [5-8]. But there is a paucity of similar studies in children with TDT.

As the life expectancy of these children is improving, the early diagnosis of premature atherosclerosis should be a research priority. This study was planned to analyze the risk of premature atherosclerosis in children with TDT by measuring CIMT, a marker for atherosclerosis, and correlating it with clinical and biochemical parameters.

METHODS

This cross-sectional study was conducted in the Thalassemia care units of two public sector medical colleges in Tamil Nadu, between January, 2022 and March, 2022. Children with TDT aged 2 to 15 years, and receiving a regular transfusion regimen for more than 6 months were considered for inclusion. Children with nephrotic syndrome or familial hypercholesterolemia were excluded. Age and sex-matched healthy volunteers were recruited from the outpatient department and taken as controls. Informed consent and clearance from institutional human ethics committee were obtained from both the centers.

Invited Commentary: Pages 910-11.

Baseline data of participants including age, sex, height, weight, body mass index (BMI), blood pressure (BP), and iron chelation history were recorded. Based on the BP, children were classified as normotensive, pre-hypertensive, or hypertensive [9]. Complete blood counts (CBC), lipid profile, C-reactive protein (CRP), serum ferritin, liver enzymes, and fasting blood sugar were estimated at enrolment. The common carotid arteries were analyzed for intima-media thickness at 1cm segment proximal to its bifurcation and expressed in millimeters using electronic calipers. CIMT was defined as the distance from the junction of the lumen and intima to the junction of media and adventitia [5]. The CIMT measurements were done using a Mind ray duplex ultrasound system with a linear array high-frequency transducer at 7.5 MHz scanning frequency in B mode by a trained radiologist who performed measurements in both the centers.

The sample size was estimated using nMaster software. In the study by Jindal, et al. [10] the reported mean difference of CIMT value between cases and control was 0.106 (0.058-0.153). Using pooled standard deviation of 0.09, with a least possible difference 0.058 for CIMT values between groups and to achieve a power of 80% at a level of significance of 5% (two sided), the required minimum sample size was 40 in each group. However, all eligible children were included in the study.

Statistical analysis: Data were analyzed using the R software. To study the association of clinical parameters between the groups, an independent sample *t* test or Mann-Whitney *U* test was applied for the continuous measurements, after checking the normality assumption. Chi-square test was applied for the categorical observations based on the expected frequency. Univariate and multiple linear regression analyses were carried out to examine the predictors of CIMT among cases. *P* value was considered significant at 5% level of significance.

Table I General Characteristics of the Study Groups

Parameters	*Children without thalassemia (n=49)*	*Children with thalassemia (n=49)*
Age (y)[a]	6.38 (3.65)	6.38 (3.65)
Male gender	20 (41)	20 (41)
Anthropometry		
Thinness	2 (4)	5 (10)
Normal	41 (84)	42 (86)
Overweight/obese	6 (12)	2 (4)
Pre hypertension	0	2 (4)
Laboratory values		
CRP (mg/L)[b]	0.6 (0.2,1.2)	2.1 (0.3,3.1)
WBC count ($X10^9$/L)[b,c]	6.7 (6.2,9.2)	9.2 (7.6,12)
SGOT (U/L)[a,c]	24 (5.68)	44.4 (12.39)
SGPT (U/L)[a,c]	28.8 (6.9)	45.7 (11.3)
SAP (U/L)[a,c]	118.7 (37.0)	190.6 (42.6)
Total cholesterol (mg/dL)[a,c]	128.8 (15.8)	90.5 (13.9)
HDL (mg/dL)[a,c]	41.6 (7.6)	25.4 (9.2)
LDL (mg/dL)[a,c]	84.5 (6.6)	71.2 (10.7)
Triglyceride (mg/dL)[a,c]	84.3 (20.4)	225.2 (59.9)
Serum ferritin level (ng/mL)[a]		
2-5 y	83.7 (29.36)	3740.2 (2120.96)
6-10 y	106.7 (55.87)	5180.4 (3071.43)
11-15 y	87.8 (35.95)	4222.6 (4467.93)
Overall	91.7 (41.02)	4316.8 (3072.47)
Carotid intima-media thickness (mm)[a]		
2-5 y[c]	0.27 (0.07)	0.43 (0.08)
6-10 y[c]	0.39 (0.03)	0.55 (0.07)
11-15 y[c]	0.46 (0.05)	0.63 (0.08)
Overall[c]	0.35 (0.09)	0.51 (0.11)

Values in no. (%), [a]mean (SD) or [b]median (IQR). [c]P<0.001. CRP-C-reactive protein, WBC-white blood cell, SGOT-serum glutamic oxaloacetic transaminase; SGPT-serum glutamic oxaloacetic transaminase, SAP-serum alkaline phosphatase, HDL-high density lipoprotein, LDL-low density lipoprotein.

RESULTS

We studied 49 children each with and without thalassemia. No statistically significant difference was observed between the cases and the control group in terms of age, sex, BP, and BMI (**Table I**). Significant differences were observed in the mean WBC count, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (SAP) levels between the two groups. The mean serum total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were significantly lower in cases than in the controls. On the contrary, the mean triglyceride levels were significantly elevated in cases. The mean (SD) serum ferritin was 4316.8 (3072.47) ng/mL in children with TDT against 91.7 (41.02) ng/mL in controls. All cases were receiving oral iron chelation with tablet deferasirox.

The mean (SD) CIMT in the thalassemia group was significantly higher than the control group [0.51 (0.11) vs 0.35 (0.09) mm; *P*<0.001]. Significantly higher values of CIMT were observed across all age groups. Mean (SD) CIMT in the controls were 0.27 (0.07) mm, 0.39 (0.03) mm, and 0.46 (0.05) mm in 2 to 5 years, 6 to 10 years, and 11 to 15 years age groups respectively, as against 0.43 (0.08) mm, 0.55 (0.07) mm and 0.63 (0.08) mm in cases in the similar age groups (*P*<0.001). These findings demonstrate that the risk of atherosclerosis in children with TDT is evident in all age groups.

On univariate analysis, older age group and higher serum ferritin levels were significantly associated with high CIMT. The factors like BMI, pre-hypertension, CRP, and WBC count were not significantly associated. Multivariate analysis also demonstrated that the older age group and higher serum ferritin levels were found to be significantly associated (**Table II**).

Table II Multivariate Analysis of Factors Associated With High Carotid Intima-Media Thickness in Children With Thalassemia

Parameters	*β coefficient (95% CI)*	*P value*
Age group[a]		
6-10 y	0.07 (0.01,0.13)	0.02
11-15 y	0.13 (0.06,0.2)	<0.001
Serum ferritin[b]		
5,001-10,000 ng/mL	0.07 (0.02,0.12)	0.01
>10,000 ng/mL	0.12 (0.04,0.21)	0.004

Reference for [a]age-group, 2-5y and for [b]serum ferritin, <5000 ng/mL.

WHAT THIS STUDY ADDS?

- Children with transfusion-dependent thalassemia are at an increased risk of developing premature atherosclerosis and measurement of carotid intima-media thickness is a non-invasive tool for estimating it.

DISCUSSION

Increased CIMT mirrors the risk of atherosclerosis and because of its ease of use; it has been extensively used. Many researchers consider high CIMT as a gold standard in the diagnosis of atherosclerosis in children [5-7]. In this study, we have included children from the 2 years of age and analyzed their risk for the development of atherosclerosis. There were significant differences in the lipid profile between the cases and the controls. The lower total cholesterol and HDL present in children with TDT in this study have been documented in many other studies [11,12]. Elevated triglyceride levels found in this study have also been documented in other studies [12]. Oxidative stress, iron overload, and deranged lipolytic activity have all been postulated to be the mechanisms of dyslipidemia in children with TDT which places these children at atherogenic risk [13,14]. Significantly higher mean serum ferritin levels were seen in children with TDT in the present study. Lack of local transport because of the prevailing coronavirus disease (COVID-19) pandemic during the study period has left many children with TDT without iron chelation for many months. There are contradicting studies that have documented a positive correlation and no correlation between high serum ferritin levels and risk for atherosclerosis in children with TDT [4,7].

In various other studies done in adults and children, the increase in CIMT in children with TDT has been well documented [2,7]. In a study by Dogan, et al. [14] (mean age 8 years), median CIMT in thalassemic patients was significantly higher (0.87 mm) than in controls (0.74 mm). In another study by Jindal, et al. [10] (mean age 7.33 years), the mean CIMT in thalassemic children was 0.69 (0.11) mm, and in controls, the CIMT was 0.51 (0.07) mm (P<0.001). This study has demonstrated that CIMT values are significantly higher even in younger children with TDT. Most of the studies of CIMT in patients with TDT had a higher mean age than this study [10,13,14]. To the best of our knowledge, there are no studies that have documented a significant difference in CIMT in children in the 2 to 5 years age group. Hence, it is evident that the process of atherogenesis in children with TDT starts at a very early age.

The older age group and higher serum ferritin were significantly associated with higher CIMT in regression analysis in this study. The association of serum ferritin with atherogenesis in children with TDT remains elusive, with contradicting observations as discussed earlier. This study had a few limitations. High serum ferritin levels observed in this study is a confounder in analyzing the risk of premature atherosclerosis. Prevalence of atherosclerosis could not be determined as normative values are not available for CIMT in Indian children below 10 years of age [15]. Also, due to the small sample size in this study, normative values for CIMT could not be defined. Further larger studies are needed to estimate normative values of CIMT in Indian children in all age groups, which will help in screening the children with TDT for the risk of premature atherosclerosis.

Children with TDT are at increased risk for premature atherosclerosis as evidenced by high CIMT in all age groups. Higher serum ferritin and a longer duration of disease were significantly associated with high CIMT. Measurement of CIMT in children with TDT is a convenient and non-invasive tool for estimating the risk of premature atherosclerosis.

Ethics Clearance: Institutional Human Ethics Committee clearance was obtained from both the centers (Center 1: GMKMC&H/4341/IEC/2019-561 dated December 29, 2021; and Center 2: EC No: 5/II/2021 dated November 05, 2021).
Contributors: VA, MH: involved in collection and interpretation of data; KSK: drafted and critically reviewed the manuscript; PP,BR,DS: designed the study and approved the final version; KSK: guarantor of the study and is in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All the authors read and approved the final manuscript.
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