Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 01, 2025

A STUDY ON FACTORS AFFECTING PREMATURE ATHEROSCLEROSIS IN CHILDREN WITH BETA THALASSEMIA MAJOR

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ABSTRACT

Background: Thalassemia refers to a group of genetic disorders of globin chain production in which there is an imbalance between alpha globin and beta globin chain. Iron overload in patients with beta-thalassemia major (BTM) leads to alterations in the arterial structures and the thickness of the carotid arteries. Carotid artery doppler is a non-invasive screening tool to identify children at increased risk of atherosclerosis. This study aims to evaluate beta thalassemia major patients for premature atherosclerosis and correlate it with iron overload.

Methods: A cross-sectional analytical study was conducted involving 50 thalassemia patients and 50 age- and sex-matched controls. Data was analysed for demographic characteristics, clinical parameters, serum ferritin andcarotid intima media thickness (CIMT) measurements by carotid artery doppler. Statistical comparisons were made between cases and controls using appropriate tools.

Results: Thalassemia patients had a mean age of 11.84 years and received an average of 150 blood transfusions over 9.8 years. Significant differences were noted in hematologic parameters, with thalassemia patients showing lower haemoglobin levels (7.51 g/dl vs. 10.84 g/dl), higher serum ferritin (1697.64 ng/ml vs. 99.11 ng/ml), and elevated LDH and bilirubin levels. CIMT measurements were significantly higher in thalassemia patients compared to controls ($57.28*10^{-2}$ mm vs. $41.25 *10^{-2}$ mm for mean CIMT, p=<0.0001). Age, Number of transfusions, serum ferritin levels were found to be positively associated factors whereas anemia was negatively correlated to atherosclerosis.

Conclusion: Children with beta thalassemia major are at increased risk of premature atherosclerosis with iron overload as the most important predisposing factor.

Keywords: *Beta thalassemia major, Carotid intima media thickness, premature atherosclerosis, serum ferritin.*

INTRODUCTION

Thalassemia refers to a group of genetic disorders of globin chain production with an imbalance between alpha globin and beta globin chain. Thalassemia is inherited chronic extravascular nonimmune haemolytic anemia.[1]

Complications of thalassemia major due to chronic iron overload include Hypothyroidism, hypogonadotropic hypogonadism, growth hormone deficiency, hypoparathyroidism, diabetes mellitus. Iron deposition in the heart causes heart failure and arrythmias.[2]

Atherogenic vascular complications described in these patients have been largely attributed to an increase in lipid peroxidation caused by toxic hydroxyl radicals generated by the Haber-Weissreaction. According to the "iron hypothesis" chronic transfusions leading to iron overload in patients with beta thalassemia major leads to alteration in arterial structures and thickness of carotid artery, as a result coronary artery disease is a quite common complication. Structural changes in the arterial wall and thickening of the carotid arteries might

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 01, 2025

predispose them to premature atherosclerosis. Moreover, hypercoagulability, overexpression of adhesion molecules, increased platelet aggregation leads to early atherosclerosis and vascular damage.[3]

Increased CIMT is a structural indicator of early atherosclerosis, and correlates with the vascular risk factors. Increased arterial stiffness and endothelial dysfunction have been reported in adult patients with beta -TM but no reports are available on this risk inchildren.[4]

The conventional diagnostic tests to confirm atherosclerosis have been angiography and stress testing, these tests have been replaced by a more convenient and accurate test – measurement of carotid artery intima media thickness (CIMT).[5]

As the life span of thalassemia patients has increased over time, the quality of life to be improved too. In this study we aimed to assess the factors affecting premature atherosclerosis in beta thalassemia major and role of doppler ultrasonography in the evaluation of CIMT as a predictor of atherosclerotic changes.

MATERIALS AND METHODS

This was a hospital based Cross Sectional analytical study conducted at Thalassemia ward, department of pediatrics, RNT medical college Udaipur. The study was conducted for a period of 1 year after getting clearance from departmental review committee and institutional ethical committee. Individuals with confounding risk factors for atherosclerosis like Hyperglycaemia, hypertension, hyperlipidaemia, smoking and obesity, critically sick children admitted in paediatric ICU were excluded from the study.

Based on study done by Abdel Hakeem GL et al[4] sample size was calculated using p value a statistical application. Sample size was around 39. By adding 10% error sample size was rounded to 50 (50 controls and 50 cases).

Sampling technique-cases were taken by convenient sampling method. Age and sex matched children from OPD with no prior history of transfusion were taken as controls.

After taking informed consent, detailed history including the duration of illness since the first blood transfusion, whether the transfusion was frequent or not (frequent $\geq$ 2 times/month) and the intake of iron chelating agents were collected. Clinical examination including Vital parameters, General physical Examination, Anthropometry measurements, systemic examination including abdomen and chest was recorded. Relevant laboratory investigations including complete blood count, renal function tests, liver function tests, serum ferritin levels were done. All patients and controls were subjected to B-mode and color-coded Doppler sonography of their extra-cranial carotid arteries. All studies were performed using a Philips Affinity 70 G ultrasound system (GE medical systems, Milwaukee, WI) with a 3-10-MHz linear array transducer any time during their hospital stay.

All ultrasound examinations were performed by a radiologist who was unaware of the clinical and laboratory details of the examined children. For each subject, both right and left side IMT measured was then averaged. The average of the two sides was considered the patient's overall mean CIMT.

Data was entered in Microsoft Excel and later imported in SPSS V.26 for statistical analysis. CIMT as an indicator of premature atherosclerosis was correlated with age, duration of transfusions, number of transfusions, Total RBC count, pre transfusion hemoglobin values and serum ferritin. Numerical variables were measured as mean and standard deviations while categorical variables were expressed as frequencies and percentages. Chi-Square Test was performed in comparison of proportions. p-value less than 0.05 was considered as significant.

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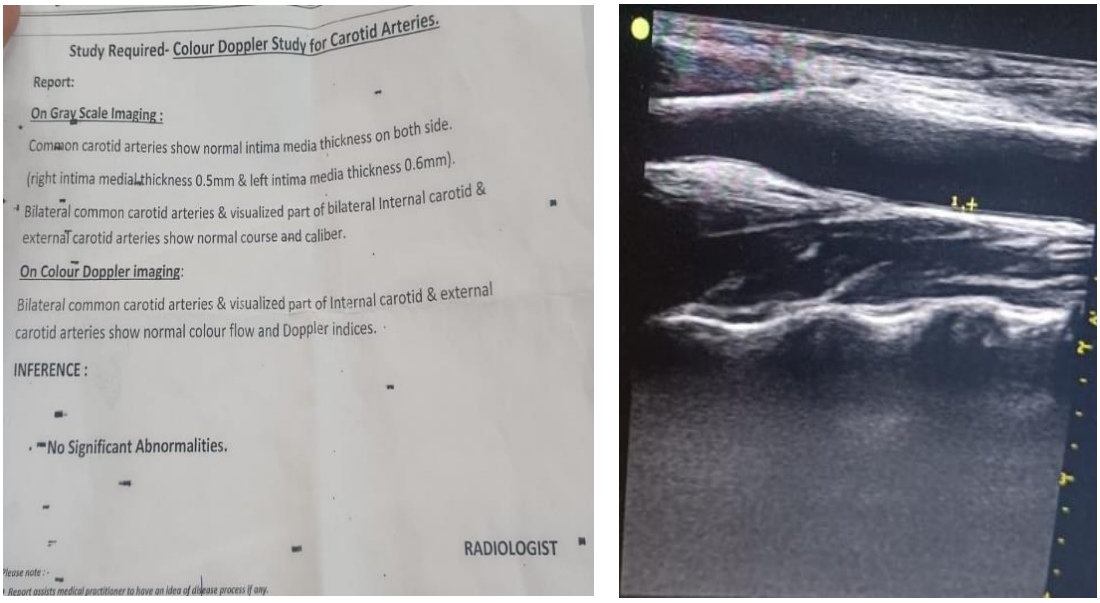

Figure 1- Carotid artery doppler for measuring CIMT.

OBSERVATIONS

A total of 50 thalassemia patients and 50 age and sex matched controls were recruited during the study period. After analyzing the data with appropriate software both cases and controls were found to be comparable with respect to demographic details. There was no significant difference in age, gender, socioeconomic status, place of residence, BMI of the cases and controls. Mean age of the cases and controls was 11.84(3.472), 11.32(3.782) years respectively. Among cases 56% were males whereas among controls 46% were male patients.

Mean BMI of cases was 17.90(2.5) kg/m^2 and of controls was 18.39(4.40) kg/m^2. There was a significant difference among cases and controls with respect to total RBC count. Mean RBC count of cases and controls was 2.91(0.7) $*10^6$/microlitre , 4.04(1.2) $*10^6$/microlitre respectively. Mean Hb of cases was 7.51(1.1) g/dl and that of controls was 10.84(2.1) g/dl with a significant $p<0.05$. There was a significant difference in bilirubin profile and serum LDH among cases and controls. There was no significant difference among other liver function tests, renal function tests, serum electrolytes and lipid profile of the cases and controls. Serum ferritin levels were compared, among cases mean ferritin levels was1697.6(533.01) ng/ml whereas that of controls was 99.11(94.6) ng/ml with a significant p value of <0.0001(Table 1) (Fig).

Mean duration of the disease among thalassemia patients was 9.85±3.570 years. Majority (80%) of the patients were receiving blood transfusions frequently. Mean number of transfusions received till date was 150.46±74.776, whereas mean transfusion rate was 153.77±44.189ml/kg/year. Majority of the patients were inadequately chelated (88%) and non-splenectomised (94%) (Table 2)

Carotid intima media thickness was calculated using carotid artery doppler and compared with controls. Mean right CIMT among cases and controls was $57.62±6.074*10^{-2}$mmand $41.38±5.660*10^{-2}$mmrespectively. Mean left CIMT among cases and controls was $56.94±6.662*10^{-2}$mmand $41.12±6.255*10^{-2}$mmrespectively. Mean CIMT of cases and controls was $57.28±5.908*10^{-2}$mm and $41.25±5.755*10^{-2}$mm respectively. All the parameters were significantly higher among cases than controls ($p<0.0001$). These findings demonstrate increased risk of atherosclerosis among thalassemia patients when compared to controls (Table 3) (Fig 2).
Univariate analysis was done to detect correlation of CIMT with Age, Gender, Duration of the disease, number of transfusions till date, BMI, lipid profile, pre transfusion Hb and Total RBC count and serum ferritin

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levels. There was a significant positive correlation between Age(r=0.730, p<0.0001), duration of transfusion (r=0.598, p<0.0001), number of transfusion (r=0.616, p<0.0001), serum ferritin levels (r=0.767, p<0.0001). There was a negative and significant correlation between pre transfusion Total RBC count and CIMT values (r=-0.287, p<0.0001). No significant correlation was found with gender, BMI, pre transfusion Hb and lipid profile. (Table 4)(Fig 3)

Multivariate analysis of the significant factors found to contribute to premature atherosclerosis showed positive correlation with age (b=0.296 p=0.016), Number of blood transfusions (b=0.27 p=0.033), Ferritin (b= 0.45 p<0.0001) and negative correlation with Total RBC count (b= -0.199 p=0.012)(Table 5)(fig 4).

Table 1- Comparison of Demographic and Lab parameters among cases and controls

	Cases	**Controls**
Age (years)	11.84(3.472)	11.32(3.782)
Male	28(56.0%)	23(46.0%)
BMI(Kg/m^2)	17.90(2.5)	18.39(4.40)
RBC (10^{6}cells/microliter) *	2.91(0.7)	4.04(1.2)
Hb(g/dl) *	7.51(1.1)	10.84(2.1)
RBS (mg/dl)	97.26(17.4)	98.50(22.7)
Total Bilirubin(mg/dl) *	1.44(0.59)	0.61(0.49)
Indirect bilirubin(mg/dl) *	0.79(0.45)	0.32(0.33)
SGPT(U/dl)	52.52(23.23)	50.46(59.72)
LDH(U/L) *	282.02(122.3)	109.48(51.7)
Total cholesterol(mg/dl)	101.41(28.6)	109.00(17.4)
Triglycerides(mg/dl)	149.54(24.7)	140.16(30)
Ferritin(ng/ml) *	1697.6(533.01)	99.11(94.6)

Values are expressed in mean (SD), * p<0.005.BMI- Body mass index, RBC-Red blood cell count, Hb-Hemoglobin, RBS- Random blood sugar, SGPT- serum glutamic pyruvic transaminase, LDH- Lactate dehydrogenase.

Table 2- Blood transfusion details of Thalassemia patients

Variable	**Frequency/Mean±SD**	**Percentage (%)**
Mean Duration(years)	9.85±3.570	-
Frequency		
Frequent	40	80.0
Infrequent	10	20.0
Mean Number of transfusions tilldate	150.46±74.776	
Mean Transfusion Rate(ml/kg/year)	153.77±44.189	
Adequacy of chelation		
Adequate	6	12.0
Inadequate	44	88.0
Splenectomised		
Yes	3	6.0
No	47	94.0

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Table 3- Comparison of Carotid intima media thickness (CIMT) between cases and controls

	Cases	Controls	P value
Right CIMT (10^{-2}mm)*	57.62±6.074	41.38±5.660	**<0.0001**
Left CIMT (10^{-2}mm)*	56.94±6.662	41.12±6.255	**<0.0001**
Mean CIMT (10^{-2}mm)*	57.28±5.908	41.25±5.755	**<0.0001**

Values are expressed in mean (SD), * p value significant

Table 4 – Univariate analysis of factors affecting CIMT values

	Correlation coefficient(r)	p value
Age(years)	0.730	<0.0001*
Gender		0.752
Duration of transfusion(years)	0.598	<0.0001*
Number oftransfusions	0.616	<0.0001*
BMI ($kg/m^{2)}$	0.036	0.803
Haemoglobin(g/dl)	-0.261	0.067
RBC count(*10^6/microlitre)	-0.287	0.043*
Serum ferritin (ng/ml)	0.767	<0.0001*
LDL (mg/dl)	-0.078	0.591

*p value significant, BMI- Body Mass Index, RBC- Red Blood Cell, LDL- Low Density Lipoprotein

Table 5- Multivariate analysis of risk factors affecting premature atherosclerosis

Variables	Regression Coefficient (b)	p-value	Significance
Constant	44.781	**<0.0001***	Significant
Age(years)	0.296	**0.016***	Significant
Duration of Transfusion(years)	-0.063	0.656	Not Significant
No. of Blood Transfusion	0.279	**0.033***	Significant
RBC count (*10^6/microlitre)	-0.199	**0.012***	Significant
Ferritin(ng/ml)	0.451	**<0.0001***	Significant

*p value significant. RBC- Red Blood Cell

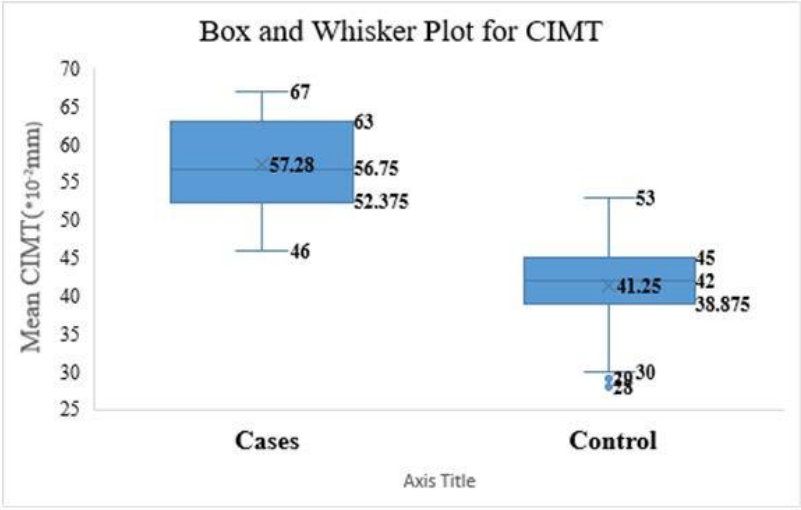

Figure 2 – Box and whisker plot for mean CIMT levels among cases and controls

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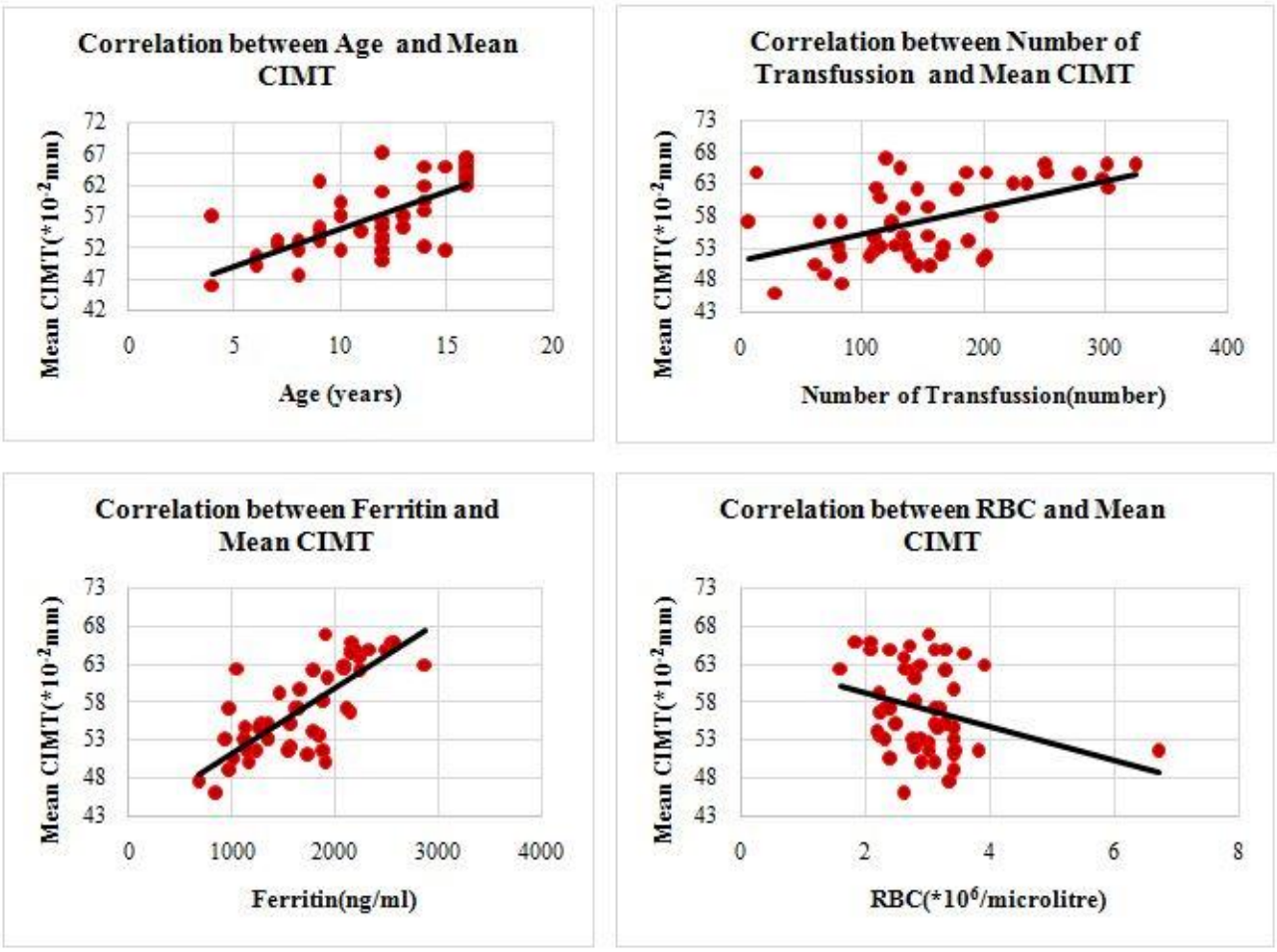

Fig 3 – Univariate analysis of factors affecting premature Atherosclerosis

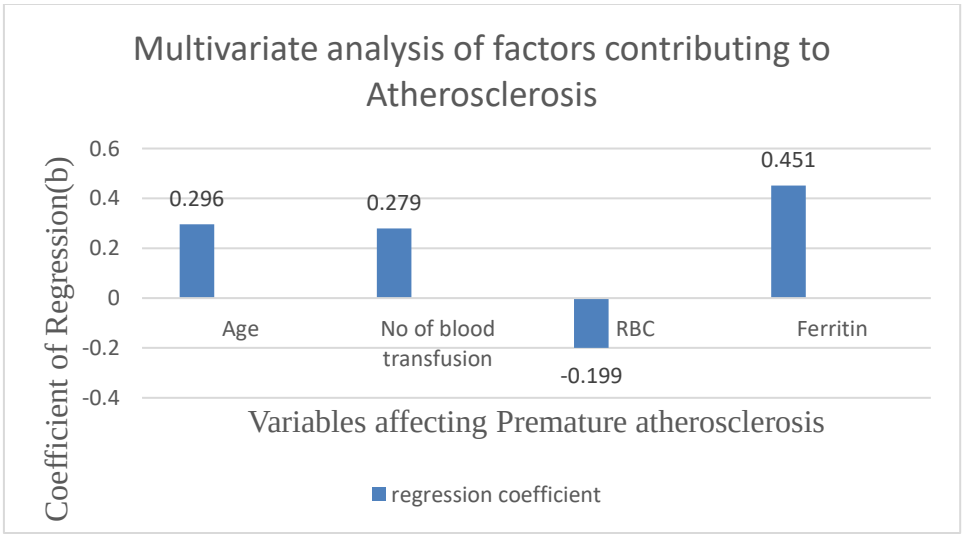

Figure 4- Bar diagram showing Multivariate analysis affecting premature atherosclerosis

DISCUSSION

The present study "Carotid Artery Doppler As A Screening Method For Evaluation Of Premature Atherosclerosis In Children With Beta Thalassemia Major" was conducted in Thalassemia ward of tertiary care center of Southern Rajasthan, for a duration of 1 year from August 2023 to July 2024.

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During the study period a total of 50 cases and 50 controls were recruited after fulfilling the inclusion criteria. Patients were screened by convenient sampling. Age and Sex matched controls with no prior blood transfusion history were selected from wards.

Out of total 50 cases and 50 controls enrolled; mean age of the children was 11 years. There was no significant difference between cases and controls with respect to Age distribution, Gender, Area of residence and Socioeconomic status. As matching was done prior to control selection, confounding factors were nullified. In a similar study conducted by Seif El-din Abaza et al[6] including 42 patients of thalassemia and 36 healthy subjects, mean age of cases was 14 years and of controls was 15 years.Most of the cases and controls in our study were underweight. Obesity being one of the confounding factors for atherosclerosis was excluded in our study. As majority of transfusion dependent patients are chronically malnourished and anemic, undernourishment among them is more likely. (Table 1)

The table also showed comparison of lab parameters. The mean Hb and RBC counts were significantly lower when compared to controls. Due to chronic extravascular haemolysis and ineffective haematopoiesis anaemia is more prevalent in thalassemia patients.

Among Cases and controlsmean Ferritin levels were 1697.64±533.016ng/ml, 99.11±94.665ng/ml respectively with a statistically significant ($p<0.0001$) difference. As thalassemia major patients are transfusion dependent, chronic iron overload leads to hyperferritinemia. Increased iron absorption due to anaemia is also a contributing factor. Poor adherence to chelating drugs, availability of only one chelating agent in our centre could be contributing factors for raised serum ferritin levels. In similar studies done by KS KUMARAVEL et al[7],Seif El-din Abaza et al[6],Abdel Hakeem GL et al[4],the serum ferritin levels were found to be significantly higher among cases compared to controls.

Blood transfusion details of thalassemia patients. Mean duration of transfusion was 9 years. Most of the patients 40(80%) required frequent transfusions. Mean transfusion rate was 153 ml/kg/year. Only 6(12%) patients were adequately chelated. The inadequacy of chelation is explained by need of repeated transfusions in adolescent age group, availability of only one chelating agent at our center. (Table 2)

Mean right CIMT among cases and controls was $57.62\pm6.074*10^{-2}$mm and $41.38\pm5.660*10^{-2}$mm respectively. Mean left CIMT among cases and controls was $56.94\pm6.662*10^{-2}$mm and $41.12\pm6.255*10^{-2}$mm respectively. Mean CIMT of cases and controls was $57.28\pm5.908*10^{-2}$mm and $41.25\pm5.755*10^{-2}$mm respectively. All the parameters were significantly higher among cases than controls ($p<0.0001$). These findings demonstrate increased risk of atherosclerosis among thalassemia patients when compared to controls. (Table 3, Fig 2)

The CIMT levels being studied here as a non-invasive marker for atherosclerosis, there was a very strong significant difference of CIMT values among thalassemia major patients and controls who had no history of transfusion. Increased carotid artery intima media thickness (CIMT) is a structural indicator of early atherosclerosis, and correlates with the vascular risk factors.

In similar studies conducted by KS KUMARAVEL et al[7], Seif El-din Abaza et al[6], Abdel Hakeem GL et al[4] the results were in concordance with our study. The mean CIMT levels of thalassemia patients were found to be much higher when compared to controls.

Univariate analysis done to detect risk factors for premature atherosclerosis. There was a significant positive correlation between Age, duration of transfusion, number of transfusions, serum ferritin levels. There was a negative and significant correlation between pre transfusion Total RBC count and CIMT values**.** No significant correlation was found with gender, duration of the disease, BMI, pre transfusion Hb and lipid profile. (Table 4, fig 3)

In similar studies done by Abdel Hakeem GL et al[5],Ahmed Abdul-Mohsin Alshammary et al[11] positive significant correlation between age, duration of transfusion, frequency of transfusion and serum ferritin with CIMT levels. In studies done by Azza A.G. Tantawy et al[16], KS KUMARAVEL et al[10] showed a positive significant correlation between CIMT and S ferritin levels. In contrast, study done by Laila M. Sherief et al[9] no significant correlation could be detected between CIMT and S. ferritin.

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Multivariate analysis of risk factors for atherosclerosis. Our study found that On multivariate analysis S. ferritin was the major positively associated risk factor for atherosclerosis with regression coefficient(b) =0.451. Age was the next important positively associated risk factor. Other risk factors were Number of blood transfusions(positive) and Red blood cell count(negative).(Table 5 ,fig 4)

In concordance study done by Abdel Hakeem GL et al[5] on multiple regression analysis it was found that duration since first transfusion had the highest association with CIMT levels followed by regularity of iron chelation, haematocrit value, serum ferritin and there was negative correlation with splenectomy, serum cholesterol levels.

The result showed direct indicator of iron overload (S ferritin) and indirect evidence like Number of transfusions, Age of the patient were strong risk factors for premature atherosclerosis. Anaemia was also found to be an associated factor in our study. Among the risk factors Age, number of transfusions were non modifiable and hyperferritinemia and anemia were modifiable risk factors.

Limitations of present study include small sample size, cross sectional study design, unavailability of hepatic and cardiac MRI for iron overload estimation.

CONCLUSION

In present study, it could be concluded that beta thalassemia major patients exhibited significantly increased risk for premature atherosclerosis when compared to controls. These patients had markedly elevated carotid intima-media thickness (CIMT), a key indicator of atherosclerosis. The study revealed that factors such as Age, Number of blood transfusions, and Serum ferritin levels were positively correlated with CIMT, whereas Red blood cell count showed a negative correlation. The study indicates a need for regular monitoring of cardiovascular monitoring in beta thalassemia major patients.

Conflict of interest-None
Funding-None
Ethical clearance- Approved

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