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CORRELATION OF SERUM LIPID PROFILE WITH SERUM IRON, TIBC & FERRITIN LEVELS IN BETA THALASSEMIA MAJOR PATIENTS

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ABSTRACT: *Correlation of serum lipid profile with serum iron, TIBC & ferritin levels in beta thalassemia major patients. Background- Beta thalassemia major, a common disorder, causes severe anemia. This is treated by regular blood transfusion, leading to iron overload and increased serum ferritin. There is considerable variation between studies as to the serum lipid levels in these patients. Aims- The study was carried out to observe the alteration of serum lipid levels and also whether the alteration could be correlated with ferritin and other hematological indices. Methods and materials-The study was carried out on 50 beta thalassemia major patients and 47 controls. Estimation of serum lipid profile, iron, ferritin, TIBC (Total iron binding capacity), hemoglobin and hematocrit was done. Statistical analysis-Data analysis was performed using standard methods. Results-Compared to controls, the cases had significantly higher ferritin and significantly lower HDL-C (high density lipoprotein- cholesterol) levels. There was inverse correlation between high ferritin and low HDL-C levels. Conclusion-Taken together, high ferritin and low HDL-C levels might be important predictors for mortality in thalassemia.*

KEYWORDS: lipid profile, iron, TIBC, ferritin, thalassemia

INTRODUCTION

Beta-thalassemia major is a fairly common and serious hematological problem that causes life threatening anemia. Regular blood transfusions and chelation therapy have prolonged survival considerably in thalassemic patients [1]. Recurrent transfusions lead to iron overload manifested by increased serum ferritin levels, for which chelation therapy is required. The accumulation of iron results in progressive dysfunction of the heart, liver and endocrine glands [2-5]. There is considerable variation between various studies as to the serum lipid levels in thalassemia patients [6-8]. Awareness of physicians of these alterations in lipid profile is helpful to avoid unnecessary work up in these patients. The present study had been undertaken to observe the alteration of blood lipid levels in thalassemic patients receiving repeated transfusion and also whether the alteration would be correlated with the haematologic indices like serum ferritin, iron and TIBC.

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MATERIALS AND METHODS

The present study was a hospital based, non-interventional, cross-sectional case control study. The work was undertaken in the Department of Biochemistry of a medical college and hospital of West Bengal, India in collaboration with the Thalassemia Control Unit of the same Hospital.50 cases [The Mean (SD) age was 8.2(2.768) years] including 19 women with thalassemia syndrome were randomly selected from the patients attending the thalassemia clinic of the same Hospital. Clinical history and relevant data were collected from patient's files with prior permission of the attending physician. The time from last transfusion to the time of sampling was at least thirty days. The sampling was done just before the next transfusion. The cases had beta thalassemia major as diagnosed by HPLC (High performance liquid chromatography) and subsequent molecular characterization. Cases had a mean (SD) pretransfusion haemoglobin of 8.5(2.5) gm/dl and a mean (SD) pretransfusion ferritin level of 1548(752) µg/l. All patients had significant hepatosplenomegaly, 16%(8/50) of which had undergone splenectomy and had received repeated blood or erythrocyte transfusion (at least ten units of blood at 3 to 4-week intervals) with the aim of maintaining pretransfusion haemoglobin levels above 9 g/dL. All the patients belonged to the same socioeconomic group and hence it may be assumed that their dietary habits were more or less similar. All of them were on calcium and folic acid supplementation in equivalent dosage. None of the patients or control subjects enrolled in this study received antioxidant supplementations or vitamin E which could affect the results. None of the cases were on iron chelation therapy as they could not afford it.

The age and sex matched 47 controls [The Mean (SD) age was 7.978(2.557) years; 3 controls dropped out] including 21 women were randomly selected from apparently healthy individuals who were neither thalassemic trait nor carrier. None had any history of blood transfusion, anaemia, infection and any acute or chronic disease state. A total of 97 children were enrolled in this study and examined. Patients were free of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

Blood samples were obtained from the study subjects after 12 hours of overnight fasting. Serum triglyceride and cholesterol were measured in Semi-automated Clinical Chemistry Analyzer (Coralab 300, Crest) using standard commercial reagents by Glycerol-3-phosphate oxidase-peroxidase (GPO-PAP)[9] and Cholesterol oxidase-peroxidase (CHOD-PAP) method[9] respectively. Measurement of serum HDL-C was performed by phosphotungstic acid precipitation method in the presence of magnesium ions. [9] The levels of LDL-C and VLDL-C were estimated by calculation using the formula of Friedewald and Levy.[10] Both serum iron and TIBC were estimated by Ferrozine method[11] in the same instrument using standard commercial reagents. Hemoglobin of whole blood was assayed in a blood analyzer (KX-21 Sysmex Auto Hematology Analyzer, Sysmex International Co. LTD. Japan, Marketed in India by Transasia Biomedical Pvt. Ltd.). For ferritin serum was separated and stored at -20^{0}C. Ferritin levels were performed by ELISA [12].

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Data analysis was performed using SPSS statistical analysis software (SPSS version 17.0, Chicago IL, USA). Statistically significant difference was determined by the Student's T test. All *P* values are 2-sided, with values less than 0.05 considered significant. Correlation coefficients were calculated according to the Brave-Pearson function.

The study was in compliance with the ethical principles contained in the Helsinki Declaration. All the cases and controls were informed about the purpose of the study and written consent for inclusion in the study and for the publication of the study report was obtained. The study was approved by the Institutional Ethics Committee.

RESULTS

Out of the total 50 cases of thalassemia studied in this study, 31 were males and 19 females. The age of patients at the time of diagnosis ranged from 6 months to 2 ½ years with a mean of 1 year 6 months. The age at the time of this study ranged between 4 years and 15 years. The interval between successive transfusions varied between 7 days to 5 weeks in different patients. It is evident that there is no significant difference in means of age between the case and control groups. (Table 1)

In Table 2, Hb, Hct, ferritin, Iron and TIBC values of the test and control groups are displayed. Hb and Hct values of the group with thalassemia were significantly lower than those of the control group ($P<0.001$). Ferritin values in the cases were found to be significantly higher than those of the control group ($P<0.001$). It is seen that serum iron was significantly higher in diagnosed cases with multiple blood transfusions than control groups ($P<0.001$). Serum TIBC was significantly lower in cases than control ($P<0.001$).

Table 3 displays the results of unpaired t-test for equality of means of the control population and diagnosed cases of thalassemia who received chronic blood transfusion. As seen in Table 3, cholesterol, HDL-C, and LDL-C levels in patients with thalassemia syndrome were found to be significantly lower than those of the control group ($p<0.001$), while the triglyceride levels were found to be higher ($p<0.001$).

Table-1: Mean age distribution of cases and controls

Variable	Cases (n=50) Mean (+/-SD)	Controls (n=47) Mean (+/-SD)	t test (Level of significance)
Age(Years)	8.2 (+/-2.768)	7.978 (+/-2.557)	0.4096 (NS)

**P value reached from independent sample studen*t's "t" test. NS= not significant (p>0.05)

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Table-2: Hematological parameters of cases and controls

Variable	Cases (n=50) Mean (+/-SD)	Controls (n=47) Mean (+/-SD)	t test (Level of significance)
Haemoglobin(gm/dl)	8.5 (+/-2.5)	11.9 (+/-1.1)	8.5741 (<0.001)
Hematocrit(%)	27.04 (+/-2.6)	36.61 (+/-3.8)	14.5514 (<0.001)
Serum Ferritin(µg/dl)	1548.06 (+/-751.67)	77.72 (+/-11.09)	13.4047 (<0.001)
Serum Iron(µg/dl)	268.60 (+/-58.60)	86.14(+/-20.876)	20.1716(<0.001)
Serum TIBC (µg/dl)	205.16 (+/-48.274)	327.97(+/-33.359)	14.4879(<0.001)

**P value reached from independent sample student's "t" test. NS= not significant (*P*>0.05)*

Table 3: Plasma lipid levels of cases and controls

Parameters	Cases (n=50) Mean (+/-SD)	Controls (n=47) Mean (+/-SD)	t test (Level of significance)
Serum Triglyceride (mg/dl)	157.24 (+/-33.04)	86.46 (+/-12.263)	13.8157 (<0.001)
Serum Total Cholesterol(mg/dl)	97.9 (+/-16.865)	152.1 (+/-16.575)	15.9565(< 0.001)
Serum HDL-C (mg/dl)	24.44 (+/-5.268)	45.65(+/-4.621)	21.0255(<0.001)
Serum VLDL-C (mg/dl)	31.34 (+/-7.63)	17.25(+/-2.48)	12.0716(<0.001)
Serum LDL-C (mg/dl)	41.86 (+/-11.87)	86.65(+/-12.16)	18.3544(<0.001)

**P value reached from independent sample student's "t" test. NS= not significant (*P*>0.05)*

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Table 4

Correlation between HDL-C and ferritin levels

		FERRITIN	HDL
FERRITIN	Pearson Correlation	1	-0.536*
	Sig. (2-tailed)		0.000
	N	50	50
HDL	Pearson Correlation	-0.536**	1
	Sig. (2-tailed)	0.000	
	N	50	50

*. Correlation is significant at the 0.01 level (2-tailed).

Table 5

Paired Samples Test between HDL-C and ferritin levels

		Paired Differences					t	df	Sig(2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	HDL - FERRITIN	-1.52364E3	754.45027	106.69538	-1738.05240	-1309.22760	-14.280	49	0.000

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Table 6
Descriptive Statistics for HDL-C and TIBC levels

	Mean	Std. Deviation	N
HDL	24.4200	5.15906	50
TIBC	2.0516E2	47.85154	50

Table 7
Correlation between HDL-C and TIBC levels

		HDL	TIBC
HDL	Pearson Correlation	1	-.137
	Sig. (2-tailed)		.342
	N	50	50
TIBC	Pearson Correlation	-.137	1
	Sig. (2-tailed)	.342	
	N	50	50

Table 8
Descriptive Statistics for HDL-C and iron levels

	Mean	Std. Deviation	N
IRON	2.6860E2	58.00774	50
HDL	24.4200	5.15906	50

Table 9
Correlation between HDL-C and iron levels

		IRON	HDL
IRON	Pearson Correlation	1	.205
	Sig. (2-tailed)		.152
	N	50	50
HDL	Pearson Correlation	.205	1
	Sig. (2-tailed)	.152	
	N	50	50

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DISCUSSION

The main modality of treatment in beta thalassemia major is regular blood transfusion every 2–4 weekly coupled with iron chelation.[13] without effective iron chelation therapy, patients with transfusional iron overload are at risk of iron deposition in vital organs such as the liver and heart. Iron overload in thalassemic patients is caused by blood transfusions, excessive hemolysis and by a decrease in the life span of red blood cells.[14] In addition, thalassemic patients absorb too much iron from food because of the abnormally low levels of a small peptide, hepcidin, which regulates iron uptake from the gut. Lastly, as already stated, none of our cases were on iron chelation therapy as they could not afford any form of iron chelation.

Myocardial siderosis is the most common cause of death in patients with beta thalassemia major(TM). [15] Elevated serum ferritin is commonly used to diagnose iron overload in thalassemic patients. [16] Subclinical systolic dysfunction appears more likely in thalassemia subjects with a history of repeated blood transfusions, which further correlated with serum ferritin levels.[17] Moreover, Shivanna et al demonstrated significant negative correlation between serum ferritin and left ventricular EF. [18] Further, Shodikin et al found a positive correlation between cardiac troponin I, a marker of myocardial infarction, and ferritin in beta thalassemia major patients.[19]

The haematologic findings in thalassemia and their control groups are shown in Table 2. All patients' Hb concentrations were significantly decreased compared to healthy controls ($p < 0.05$). This is expected in thalassemic patients, where normal Hb synthesis is impaired. In our study all cases had highly significant increase in serum ferritin levels compared to the healthy controls ($p < 0.001$). Ferritinemia was about twenty times more in patients as compared to controls. This increase in serum ferritin indicates an existing iron overload in cases, due to multiple blood transfusions and probably also due to intestinal hyperabsorption of iron.[20]

In our study it was found that the majority of the cases had lower total cholesterol, HDL-C as well as LDL-C levels, and higher triglycerides levels in cases as compared to controls.The pathogenesis of these abnormalities can be caused by many mechanisms including plasma dilution because of anemia, accelerated erythropoiesis resulting in increased cholesterol uptake by macrophages and histiocytes of the reticuloendothelial system, defective liver functioning because of iron overload, macrophage system activation with cytokine release, and hormonal disturbances [21-23]

Also, the existence of a state of oxidative stress as reflected by the measurement of peroxidation products and depletion of lipid-soluble antioxidants, possibly related to the presence of unpaired hemoglobin chains and abnormal iron homeostasis, has been demonstrated in β-thalassemia major patients. This is supported by recent in vitro studies which stated that there is oxidative interactions of unpaired hemoglobin chains with LDL ApoB, and this can cause oxidative modification of LDL-C.[24,25] Such oxidation processes could conceivably alter the metabolic behaviour of both LDL-C and HDL-C and result in increased uptake of the two modified lipoproteins by the already hyperplastic macrophage/monocyte system (scavenger pathway).[26,27] Triglyceride lipase activities (both hepatic and extrahepatic) were significantly lower in thalassemic patients.

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Christina et al speculated that the decreased levels of these enzymatic activities could play a role in determining the decrease of HDL-C observed in thalassaemic patients.[7]

The major cause of mortality in thalassemia is cardiac disease, due to iron deposition and otherwise. Ferritin represents a risk marker for thalassemic patients and a predictive factor for progression to endocrine disorders.[28] In addition, it is well known that low plasma levels of HDL-C represent an important independent risk factor for cardiovascular disease.[29] In our study, we found inverse correlation between high ferritin and low HDL-C levels (tables 4 and 5) So, taken together, high ferritin and low HDL-C levels might be important predictors for mortality in thalassemia. On the other hand, there was no correlation between HDL-C levels and iron or TIBC levels (tables 6-9).

This study has limitations that must be considered. The number of patients in the study groups was not large. Thus, care must be taken in extrapolating the present findings to other populations.
In conclusion, based on the present results, low levels of HDL-C and high levels of ferritin in patients with thalassemia should be a motive for concern of better evaluation of the cardiovascular risk factors in these patients; however more future research is needed for confirmation and explanation of this relationship as well as clarification of the exact mechanism and clinical consequences of decrease in lipids and also increase in ferritin in patients with beta-thalassemia.

Conflict of interest
There was no conflict of interest

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