ORIGINAL RESEARCH PAPER

Pathology

STUDY OF VARIOUS BIOCHEMICAL PARAMETERS IN ß-THALASSEMIA MAJOR PATIENTS AND ITS CORRELATION WITH SERUM FERRITIN LEVEL AND TRANSFUSION INDEX

KEY WORDS: Thalessemia, Lipid Profile, Serum Calcium

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ABSTRACT

BACKGROUND: Thalassemia is a heterogeneous group of single gene disorder characterized by decreased or absent beta globin chain synthesis, leading to transfusion dependent severe anemia, therapy related iron overload, resulting in many biochemical and metabolic disturbances.

OBJECTIVE: The aim of the study was to focus primarily on the frequency of abnormal lipid profile, serum calcium, phosphorus and alkaline phosphatase and their correlation with serum ferritin level among the thalassemic major patients on regular blood transfusion in central part of India.

METHOD: The study was hospital based crosssectional study in which demographic data as well as history of blood transfusion was taken. Serum lipid profile, calcium and phosphorus, alkaline phosphatase, and serum ferritin level were measured .

RESULT: Out of 60 thalassemia patients, 37 were male and 23 were female. Total as well HDL and LDL cholesterol were significantly low as compared to reference control group of similar age group, whereas, serum triglyceride levels were significantly high in present study group as compared to reference control group. Hypocalcemia was found in 25% patients. Hyperphosphatemia was found in 22 (36.67%) patients and 2 (3.33%) patients had hyper alkaline phosphatasia. 66% patients of hypocalcemia had hyperphosphatemia simultaneously.

CONCLUSION: Hypocalcemia, hypocholesterolemia and hypertriglyceridemia are more common in second decade of life and should be routinely monitored in thalassemic patients to improve the quality of life and life expectancy of these patients.

INTRODUCTION:

ß thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis, resulting in reduced Hb in red blood cells (RBC), decreased RBC production and anemia. Every year approximately 60,000 children are born worldwide with thalassemia major out of which 10,000 are born in India.[1] Children with ß thalassemia major are generally diagnosed between 6 months to 2 years of life. If remain undiagnosed or untreated, more than 90% do not survive beyond 3 to 4 years of age. The regular blood transfusion remains the cornerstone of treatment of these patients. These patients are transfused 10ml of packed red cells/ kg body weight every 3-4 weeks. Each ml of red cells contains 1mg of iron. This leads to iron overload, which causes damage to parenchymal cells of various organs particularly liver, endocrine glands (thyroid, parathyroid and pancreas) and heart.

Patients maintained on a regular transfusion regimen progressively develop hypogonadism (35-55% of the patients), hypothyroidism (9-11%), hypoparathyroidism (4%), diabetes mellitus (6-10%), liver fibrosis, and heart dysfunction (33%)[2, 3]. The aim of our study is to see the severity and frequency of some of the metabolic and biochemical abnormalities in thalassemic patients of Bhopal.

MATERIALS AND METHOD:

The study was a descriptive hospital based, cross sectional study and carried out in the Department of Pathology and associated Blood Bank Gandhi Medical College, Bhopal from 1st Oct 2012 to 30th Sep 2013. Ethical permission for the study was obtained. Participation was fully voluntary and an informed consent was taken from the parents after explaining the purpose of study.

Patients with established diagnosis of β thalassemia major and who is on blood transfusion were included in the study whereas, patients with any cardiac or congenital anomaly or with any metabolic syndrome were excluded from the study.

The detailed clinical history as well as transfusion history was taken. Fasting blood sample was withdrawn of which Serum calcium, serum phosphorous and serum alkaline phosphatase, Serum total cholesterol, serum LDL, serum HDL and serum triglycerides by Biosystems commercial kit ; and Serum Ferritin by Sandwich ELISA method by Accu-bind kit were estimated.

RESULTS:

A total of 65 β thalassemic patients were screened from the 1st October 2012 to 30th September 2013. Out of which, two patient's parent did not give informed consent. Three patients had mixed haemoglobinopathies (two with sickle-thalassemia, one with thalassemia plus HbE).Hence total 5 cases were excluded from the study. So a total of 60 confirmed cases of β thalassemia were included in the analysis.

The data was entered in Epidata software, and analyzed using statistical software STATA version 12 (College Station, TX). We presented descriptive data as mean values with standard deviation for continuous variables and numbers with percentages for categorical variables.

TABLE 1: Baseline characteristics of 60 thalassemic patients

Variable	Observation	Mean	Std. Dev.	Min	Max
Age (yrs)	60	8.75	4.01	2	18
Sex	60				
Male	37 (61.7%)				
Female	23 (38.3%)				
Address					
Urban	37 (61.7%)				
Rural	23 (38.3%)				
Consanguinity	4 (6.7%)				
Calcium (mg/dl)	60	9.67	0.94	7.67	10.8
Phosphorus (mg/dl)	60	5.28	1.00	3.7	8.34
AlkPo4(U/L)	60	235.26	79.46	81	477
Cholesterol (mg/dl)	60	113.5	30.81	71	184
TG(mg/dl)	60	228.95	96.25	68	568
HDL(mg/dl)	60	24.33	5.39	10	38

LDL(mg/dl)	60	44.48	24.66	3	95
TC/HDL ratio	60	4.87	1.60	2.15	12.4
S.Ferritin(ng/dl)	60	557.25	198.66	125.3	818.4
Transfusion Index	60	165.36	44.95	100	240
Chelation therapy	60				
Regular	48 (80%)				
Irregular	12 (20%)				
T.Frequency	60				
Regular	48 (80%)				
Irregular	12 (20%)				

The mean age of the study population was 8.75 years (ranging from 2 years to 18 years). Out of the 60 patients included in the study, 37 patients (61.7%) were male patients. 48 patients (80%) were on regular blood transfusion and chelating therapy was mostly oral.

STUDY OF LIPID PROFILE

Study of various lipid parameters were done in all 60 thalassemic patients. Table 1 shows the mean of various lipid parameters. Two control groups of same age were taken from two different studies conducted to find out normal lipid level in Indian children . Table 2 shows the comparison of various lipid parameters between present study group and reference control group formed by 410 normal healthy children of age group between 3 to 12 years. Present study showed significantly low level of total as well as HDL and LDL cholesterol as compare to reference control group of similar age group (p value < 0.001). Serum triglyceride levels were significantly high in present study group as compare to control group (p value < 0.001). No patient in present study has total or LDL cholesterol above the suggested cut off limit i.e. 190 mg/dl for total cholesterol and 130 mg/dl for LDL cholesterol.

TABLE 2: Comparison of various lipid parameters between study and control group

Lipid Parameter	Study Group (60)	Control Group[4] (410)	P value
Total Cholesterol (mg/dl)	113.5±30.81	134.5±27.1	< 0.001
Triglyceride (mg/dl)	228.95±96.25	91.1±29.85	< 0.001
HDL(mg/dl)	24.33±5.39	34.15±13.05	< 0.001
LDL(mg/dl)	44.48±24.66	80.1±21.65	< 0.001
TC/HDL ratio	4.87±1.60	4.3±1.4	0.004

TABLE 3: Comparison of various lipid parameters between study and control group

Lipid Parameter	Study Group (60)(Male 37,Female 23)		Control Group (71) (Male 16,Female 55)		P value
Total Cholesterol (mg/dl)	Male	115.83±30.65	Male	150.31 ± 25.68	<0.001
	Female	109.73±31.38	Female	143.61± 25.08	<0.001
	Total	113.5±30.81	Total	145.11±25.21	<0.001
Triglyceride (mg/dl)	Male	226.81±11.95	Male	74.81 ± 19.78	<0.001
	Female	232.39±88.40	Female	94.69 ± 33.95	<0.001
	Total	228.95±96.25	Total	90.21±30.75	<0.001
HDL (mg/dl)	Male	25±5.24	Male	45.00 ± 25.68	0.007
	Female	23.26±5.56	Female	36.71 ± 25.08	0.013
	Total	24.33±5.39	Total	38.57±25.21	<0.001
LDL(mg/dl)	Male	47.29±24.74	Male	90.69 ± 25.68	<0.001
	Female	39.95±24.38	Female	82.51 ± 25.08	<0.001
	Total	44.48±24.66	Total	84.35±25.21	<0.001

Table 3 shows the comparison of various lipid parameters between present study group and reference control group of 71 children between the age group of 0 to 15 years taken from a study conducted to establish normal reference value of lipid profile in Indian children. Present study showed statistically significant lower level of serum total, HDL and LDL cholesterol in both male and female as compare to control groups. Serum triglyceride levels were significantly high in both male and female in study group as compare to reference control group (p value < 0.001).

SERUM CALCIUM, PHOSPHOROUS AND ALKALINE PHOSPHATASE STUDY

Mean serum calcium level was 9.67±0.94 mg/dl. Hypocalcemia was found in 15 (25%) patients.10 patients (66%) of hypocalcemia has hyperphosphatemia simultaneously. Mean serum ferritin levels was 557.56±195.17 ng/dl in hypocalcemic group and 557.15±201.99 ng/dl in normocalcemic group which did not differ significantly (p value 0.99). Hypocalcemic group had significantly higher transfusion index (204±41.19) as compare to normocalcemic group (152.48±38.61) with a p value of less than 0.001.

TABLE 4: Comparison of age, serum ferritin and transfusion index between two groups of calcium

Variables	Calcium Study		
	Hypocalcemia (15)	Normocalcemia (45)	P value
Age(yrs)	10.0±4.2	8.33±3.88	0.16
S.Ferritin (ng/dl)	557.56±195.17	557.15±201.99	0.99
Transfusion Index	204±41.19	152.48±38.61	<0.001

Mean serum phosphorus level was 5.28±1.0 mg/dl. Hyperphosphatemia was found in 22 (36.67%) patients and 2 (3.33%) patients had hyper alkaline phosphatasia.45% of patients (10 patients) of hyperphosphatemia has hypocalcemia simultaneously. Patients with hyperphosphatemia had significantly more mean age as compare to normophosphatemic group. However, there was no significant difference in mean serum ferritin level or mean transfusion index between two groups (p value 0.21 and 0.18 respectively) (Table 5).

TABLE 5: Comparison of age, serum ferritin and transfusion index between two groups of phosphorus

Variables	Phosphorus study		
	Hyperphosphatemia (22)	Normophosphatemia (38)	P value
Age(yrs)	11.09±3.90	7.39±3.45	<0.001
S.Ferritin (ng/dl)	599±156.55	533.09±217.68	0.21
Transfusion Index	175.45±49.15	159.52±41.89	0.18

CORRERATION OF VARIOUS PARAMETERS WITH AGE SERUM FERRITIN, AND TRANSFUSION INDEX

Since most of the β thalassemic patients become symptomatic by the age of 6 months to2 years, hence age of patients in thalassemia nearly corresponds to duration of the disease. Similarly serum ferritin levels and transfusion index are the indicators of iron overload in these patients. Hence, we studied the correlation of these parameters with lipid profile, serum calcium, phosphorus and alkaline phosphatase. (Table 6)

TABLE 6: Pearson's correlation coefficient of serum ferritin, Age, Transfusion index with various parameters

Variables	Serum Ferritin	Age	Transfusion Index
Calcium	-0.1055	-0.2883*	-0.3736*
Phosphorus	0.0840	0.3440*	0.2783*
Alkaline Phosphatase	-0.1066	-0.3320*	0.0121

Total Cholesterol	-0.0615	0.1145	0.1745
HDL	-0.1431	0.0227	-0.0457
LDL	0.0125	0.1231	0.1732
Triglyceride	-0.0202	0.0100	0.0946
Total cholesterol/HDL ratio	0.1577	0.0747	0.1246

* p value < 0.05

TABLE: Age wise comparison of various Hypocalcemia and Hyperphosphatemia

Vari ables	<10 years (37 pts.)	≥ 10 years (23 pts.)	Chi Square test		Odds test	
			Chi Square value	P value	Odds ratio	95% Confidence Interval
Hypocalcaemia	6	9	3.97	0.046	2.71	0.81- 9.02
Hyperphosphatemia	9	13	6.33	0.011	3.12	1.05 – 9.31

Table 7 shows the comparison of Hypocalcemia and Hyperphosphatemia between first and second decade of life using chi square test and odds ratio. Hypocalcemia was found in 6 patients during first decade of life as compare to 9 patients during second decade of life (p value 0.046) indicating hypocalcemia is significantly more common in second decade. A odds ratio of 2.7 suggest that hypocalcemia is 2.7 times more common in second decade life as compare to first decade. Similarly hyperphosphatemia is more common in second decade as compare to first decade with odds ratio of 3.12

DISCUSSION

LIPID PROFILE STUDY

Thalassemic patients are subjected to peroxidative tissue injury. It has been documented that circulating LDL in thalassemic patients show marked oxidative modification. Free-radical production is increased in patients with iron overload. Iron-loaded patients have elevated plasma levels of thiobarbituric acid reactants and increased hepatic levels of aldehyde-protein adducts, indicating lipid peroxidation. All these factors may adversely affect the levels of various lipid parameters in β thalassemic patients.

Present study showed significant lower level of total, HDL and LDL cholesterol in study population as compared to reference healthy control of same age group. Serum triglyceride levels were significantly more in study group as compared to control. No patient in present study has total or LDL cholesterol above the suggested cut off limit i.e. 190 mg/dl for total cholesterol and 130 mg/dl for LDL cholesterol. These findings suggest that patients with thalassemia are at lower risk of coronary artery disease with regards to their total and LDL cholesterol levels. However on diverting our focus on HDL cholesterol levels which is considered as good cholesterol, we found that study patients also had significant lower level of HDL cholesterol as compared to control. Inviduals with normal levels of total cholesterol, risk for myocardial infarction is high when HDL cholesterol is low[6,7]. This highlights the importance of total-to-HDL cholesterol ratio for the evaluation of blood lipids and the prevention of atherosclerotic disease. It has also been reported that the rise in total cholesterol-to-HDL cholesterol ratio is better predictor of coronary heart disease risk as compare to absolute LDL and HDL cholesterol[7]. It has been reported that total, HDL and LDL-cholesterol were significantly decreased, while triglycerides were significantly increased in the thalassemic patients compared to the control subjects[8,9,10]. Scientists found a positive correlation between age and triglycerides levels.

Adolescents with beta-thalassemia intermedia have significantly lower cholesterol levels than patients with beta-thalassemia major[11]. The author suggested that low lipid levels in thalassemia is related to their disorder and not influenced by age, sex, hemoglobin, or ferritin levels and hence needless investigations for hypolipidemia should be avoided.

Various studies from India have showed significant higher level of triglyceride in thalassemic patients[12-14]. However some researchers have found results contrary to these and they did not find any significant difference in triglycerides level between thalassemia and control[15,16]. However small number of patients included in these studies as compared to present study may be the likely reason for difference.

Various other mechanisms has been proposed which include increased erythropoietic activity resulting in increased cholesterol requirements, iron overload leading to free radical generation and liver injury, macrophage system activation with cytokine release, plasma dilution due to anemia and hormonal disturbances. There is significant hypocholesterolemia in anemia patients associated with high-erythropoietic activity [17]. It has been postulated that increased cholesterol requirements by the proliferating erythroid cells because of high erythropoietic activity could be responsible for hypocholesterolemia in thalassemic patients. Another very well known fact is that patient with thalassemia intermedia have more erythropoietic activity as compared to thalassemia major patients. Various previous studies have shown that thalassemia intermedia patients have significantly more hypocholesterolemia as compare to thalassemia major patients [11,18,19].

Iron overload is another important factor which may be responsible for hypocholesterolemia in thalassemic patients. Two important factors which causes iron overload in these patients is repeated blood transfusion and increase in absorption from the guts due to ineffective erythropoiesis. Excessive iron overload leads to free radical generation. These free radical generation leads to lipid peroxidation and may be responsible for low levels of cholesterol and lipid in thalassemia. Another effect of iron overload and repeated blood transfusion is induction of an acute phase response in thalassemic patients which can lead to shift of LDL cholesterol towards protein-rich, denser particles leading to low LDL cholesterol level[20]. Decrease synthesis of cholesterol from the liver due to severe anemia is also important factor for low cholesterol level leading to hypocholesterolemia. Increased uptake of low-density lipoprotein (LDL) by macrophages and histiocytes of the reticuloendothelial system has been mentioned as important determinants of low plasma cholesterol in thalassemia [9, 15]. The accelerated erythropoiesis and increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system are the main determinants of low plasma cholesterol levels in beta thalassemia major[15]. In addition, Giardini et al [21] observed that total serum phospholipids, their fractions and cholesterol were significantly lower among patients with thalassemia major. These changes were referred to hepatic damage and to severe anemia.

Another important factor which might be responsible for hypocholesterolemia in thalassemic patients is an increased secretion of some cytokines (interleukin-1, interleukin-6, and tumor necrosis factor-alpha) due to chronic activation of monocyte-macrophage system. This affects the hepatic secretion and the receptor-mediated removal of apolipoprotein-B containing lipoproteins[22].

Role of hepatic and extra hepatic lipase enzyme has been suggested by various authors in the pathogenesis of hypocholesterolemia and high triglyceride levels in thalassemic patients. The significantly low level of triglyceride lipase in thalassemic patients, and hence decreased enzymatic activities could play a role in determining the decrease of HDL-cholesterol observed in thalassemic patients[16]. Similarly reduced extra hepatic lipolytic activity could account for the rise in circulating triglyceride levels in thalassemia [23]. Studies have shown significant correlation of high triglycerides level and serum ferritin level [24].

SERUM CALCIUM AND PHOSPHORUS

Among the various endocrinal complications in thalassemia patients, hypoparathyroidism is well known to occur in thalassemia major patients[29]. However it is thought to be uncommon as compared to other complications[25]. The cause of hypoparathyroidism in thalassemia is assumed to be iron deposition in the parathyroid glands. A number of possible mechanisms have been described to be responsible for glandular

damage through iron overload. These include free radical formation and lipid peroxidation resulting in mitochondrial, liposomal and sarcolemmal membrane damage and a number of surface transferrin receptors in the cells and the ability of the cell to protect itself against inorganic iron. Hypoparathyroidism, thought to be a more rare complication is also known to cause hypocalcemia[26, 28] and hyperphosphatemia[27]. In our study, prevalence of hypocalcaemia and hyperphosphatemia was 25% and 36.6% respectively. Overall 16.66% of patients (i.e. 66% of hypocalcemic) had both hypocalcemia and hyperphosphatemia.

There were no significant differences in serum ferritin levels between those with hypocalcemia and those without hypocalcemia. Similarly no significant difference in serum ferritin level or transfusion index was observed between hyperphosphatemia and normophosphatemia group. Several explanations can be put forth for this finding. The serum ferritin level can be subjected to changes with intercurrent infection and hence it is not a reliable indicator of the development of hypocalcemia. Another explanation is the individual susceptibility to iron toxic effect or the development of organ damage by severe iron overload in the many years preceding the initiation of chelation therapy. Present study showed significant difference in transfusion index between hypocalcemic group and normocalcemic. Hence it can be concluded that duration and amount of blood transfusion which is indicated by transfusion index is more important factor in predicting hypocalcemia rather than absolute or present value of serum ferritin.

Contrary to expectations of a negative correlation between serum ferritin levels and calcemia, present study did not show any significant negative correlation between serum ferritin and calcium. However there was significant negative correlation of age with serum calcium and transfusion index with serum calcium. Similar finding were observed with regards to serum phosphorus level showing no significant positive correlation with serum ferritin but significant positive association with age and transfusion index.9 patients (out of 23) above the age of 10 years had hypocalcemia, while only 6 patients (out of 33) under the age of 10 years (Odds ratio 2.7) developed hypocalcemia. Similarly 13 patients had hyperphosphatemia in second decade of life as compared to 9 patients in first decade (odds ratio 3.12).So hypocalcemia and hyperphosphatemia in thalassemia mainly occurs in the second decade of the life and two to three times more common after the age of 10 years.

CONCLUSION

In conclusion, lipid profile, serum calcium and phosphorus are altered in -thalassemia major patients, which is the complications occurring due to repeated blood transfusion and should be monitored regularly mostly in the second decade of life for early diagnosis of these complications and to improve the life expectancy as well as quality of life of these patients

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