

Comparative assessment of serum liver enzymes (AST and ALT) in thalassemia patients of Murshidabad and matched controls

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Abstract:

Patients with thalassemia syndrome, a group of inherited autosomal recessive haematologic disorder, need multiple blood transfusions, a complication of which is iron overload. Though life saving, but chronic blood transfusion can lead to lethal iron overload injury of liver, often characterized by the development of fibrosis and eventually, cirrhosis and increased serum ferritin. Liver enzymes are raised and indicative of liver injury in transfusion dependant thalassemia patients. To our knowledge no data are available on values of liver enzymes in patients with thalassemia in Murshidabad district. The study was carried out on 50 thalassemia cases and 50 age, sex matched controls to observe any alteration of serum AST and ALT and also whether the alteration could be correlated with common haematological indices. Compared to controls, the cases had significantly higher liver enzymes and there was positive correlation between high ferritin and high liver enzymes level. Taken together, high ferritin and liver enzymes levels might be important predictors for morbidity and mortality in thalassemia.

Keywords: AST, ALT, ferritin, iron, thalassemia

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I. Introduction

Thalassemia syndrome (from Greek $\theta\alpha\lambda\alpha\sigma\sigma\alpha$,thalassa-sea, $\alpha\dot{\iota}\mu\alpha$,haima-blood) are a group of inherited autosomal recessive haematologic disorder in which genetic defect results in reduced rate of synthesis of one of the globin chains leading to formation of abnormal hemoglobin molecule.¹ Typical treatment of thalassemia is multiple and chronic blood transfusions, a complication of which is iron overload. It starts another pathological mechanism leading to oxidative damage of RBC membrane, the so called "second disease".^{1,2} A clear improvement is seen in about 90 % of cases after a bone marrow transplant in thalassemia major. But as it is very costly, frequent blood transfusion is still the mainstay of therapy which can damage the heart, liver³ and other organs. "Iron chelators" can prevent or delay problems related to iron overload.⁴ Iron overload is both due to increased absorption of iron from gut secondary to hyperactive bone marrow and also from frequent blood transfusions. It results in inappropriate suppression of the iron regulating peptide hepcidin.⁵ Though it affects almost all systems of body, liver is the earliest site of iron toxicity.⁶ Iron overload occurring both in hepatocytes and reticuloendothelial cells can be evaluated easily and verified by measuring serum ferritin levels and other haematological parameters.^{7,8} Liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are raised in transfusion dependent β -thalassemia major patients. Relatively simpler way of knowing the liver damage is by estimation of liver enzymes which are raised due to oxidative injury and direct toxic effect of iron on liver cells.⁹ This study was planned to reveal the liver enzymes values in thalassemia patients compared with controls and whether any correlation exists with serum ferritin levels and other haematological parameters in children with transfusion dependent thalassemia.

II. Materials And Methods

A case control, observational, non-interventional, tertiary care hospital based research was conducted using a cross sectional design in the Department of Biochemistry of Murshidabad Medical College and Hospitals of West Bengal, India in collaboration with the Thalassemia Control Unit of the same Hospital.

50 cases [The Mean (SD) age was 8.22(2.743) years] including 19 women with thalassemia syndrome were randomly selected from the patients attending the thalassemia clinic of the same Hospital. 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 thalassemia syndrome (thalassemia major/E beta thalassemia) 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(751.67) 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 50 controls [The Mean (SD) age was 8.08(2.522) years;] including 21 women were randomly selected from apparently healthy individuals who were neither thalassaemic trait nor carrier. None had any history of blood transfusion, anaemia, infection and any acute or chronic disease state. A total of 100 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).

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.

Blood samples were obtained from the study subjects after 8-10 hours of overnight fasting. Serum AST and ALT were measured in Fully Automated Clinical Chemistry Analyzer (EM-360, Transasia) using standard commercial reagents by IFCC kinetic assay, without Pyridoxal Phosphate.¹⁰ Both serum iron and TIBC were estimated by Ferrozine method¹¹ 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 C. Ferritin levels were performed by sandwich ELISA.¹²

Data found from clinical history and laboratory investigations, were compiled in MS excel sheet and analyzed by applicable statistical methods. Data display was done by charts and tables. Data were described by mean, SD, range, median etc. Statistical tests like Student's T test, correlation coefficient (Brave-Pearson function) were done by suitable statistical software program. All P values are 2-sided, with values less than 0.05 considered significant to discard the null hypothesis at 5% precision and 95% confidence interval.

III. 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. (Table1)

Serum ferritin and iron values in the cases were found to be significantly higher than those of the control group ($P<0.001$). Hb and TIBC values of the cases were significantly lower than those of the control group ($P<0.001$). Serum AST and ALT levels in patients with thalassemia syndrome were found to be significantly higher than those of the control group ($p<0.001$) as seen in Table-1.

Table-1: Descriptive statistics of different attributes and significance of difference between thalassaemic individuals (Cases) and age, sex matched apparently healthy individuals (Controls)

Attributes	Age (years)		AST(IU/L)		ALT(IU/L)		Serum Iron(µg/dl)		Serum TIBC (µg/dl)		Serum Ferritin(µg/L)		Haemoglobin(gm/dl)	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Mean	8.22	8.08	65.60	19.94	54.52	17.11	268.60	85.86	205.16	327.62	1548.06	78.30	8.50	11.85
Median	8.00	8.00	65.85	20.30	54.30	16.70	268.00	84.50	197.00	325.00	1315.30	79.50	9.65	11.80
Mode	8.00	7.00	63.00	21.00	57.00	15.00	265.00	65.00	181.00	289.00	2112.00	65.00	9.80	11.00
SE of Mean	0.388	0.357	1.491	0.429	1.50	0.368	8.203	2.872	6.767	4.599	106.30	1.56	0.353	0.155
SD	2.743	2.522	10.547	3.03	10.607	2.603	58.00	20.31	47.85	32.52	751.67	11.03	2.50	1.10
Variance	7.522	6.361	111.24	9.211	112.528	6.781	3364.898	412.531	2289.77	1057.832	565014.5	121.765	6.259	1.213
Minim	4.00	4.00	45.0	14.80	34.0	13.00	176.	47.00	123.	247.00	702.00	53.00	5.00	9.80

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Maxim um	15.00	15.00	82.7 0	26.500	76.9 0	23.00	414. 00	140.00	335. 00	387.00	3635.0 0	97.00	12.0 0	15.00
t – test (Level of signific ance)	0.397(0.693)NS		29.713 (<0.001)		23.632 (<0.001)		20.688(<0.001)		-15.815<0.001)		13.794 (<0.001)		-8.824 (<0.001)	

P value reached from paired sample T-test. NS= not significant (p>0.05)

Table-2: Bivariate Correlation analysis among different parameters in thalassemic individuals (Cases)

Pearson's correlation in cases		AST	ALT	Iron	TIBC	Ferritin	Hb
AST	correlation coefficient	1	0.863	-0.008	0.05	0.676**	-0.312*
	Significance(2-tailed)		<0.001	0.957	0.730	<0.001	0.027
ALT	correlation coefficient	0.863	1	-0.097	0.076	0.459**	-0.258
	Significance(2-tailed)	<0.001		0.503	0.601	0.001	0.071
Iron	correlation coefficient	-0.008	-0.097	1	-0.701**	-0.041	0.124
	Significance(2-tailed)	0.957	0.503		<0.001	0.779	0.392
TIBC	correlation coefficient	0.05	0.076	-0.701**	1	0.008	-0.04
	Significance(2-tailed)	0.730	0.601	<0.001		0.956	0.783
Ferritin	correlation coefficient	0.676**	0.459**	-0.041	0.008	1	-0.234
	Significance(2-tailed)	<0.001	0.001	0.779	0.956		0.102
Hb	correlation coefficient	-0.312*	-0.258	0.124	-0.04	-0.234	1
	Significance(2-tailed)	0.027	0.071	0.392	0.783	0.102	

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

* Correlation is significant at the 0.05 level (2-tailed)

Table 3: Partial correlation of Liver Enzymes with other one eliminating the effect of remaining two haematological parameters in cases

Parameter	Control variables	Parameter with which AST is correlated	correlation coefficient	Significance(2-tailed)
AST(IU/L)	Serum Iron(µg/dl) & Serum TIBC(µg/dl)	Serum Ferritin(µg/L)	0.680	<0.001
	Serum TIBC(µg/dl) & Serum Ferritin(µg/L)	Serum Iron(µg/dl)	0.097	0.511
	Serum Ferritin(µg/L) & Serum Iron(µg/dl)	Serum TIBC(µg/dl)	0.111	0.451
ALT(IU/L)	Serum Iron(µg/dl) & Serum TIBC(µg/dl)	Serum Ferritin(µg/L)	0.458	0.001
	Serum TIBC(µg/dl) & Serum Ferritin(µg/L)	Serum Iron(µg/dl)	-0.044	0.767
	Serum Ferritin(µg/L) & Serum Iron(µg/dl)	Serum TIBC(µg/dl)	0.027	0.855

Serum AST as well as serum ALT levels were found to be significantly and positively correlated to serum ferritin level in cases. Haemoglobin level was found to be weakly and negatively correlated with serum AST level in thalassemic individuals. It is also evident from partial correlation (Table-3) that liver enzymes activity was significantly correlated with ferritin in cases, when iron and TIBC remain fixed.

IV. Discussion

In present study, we investigated the distribution of serum AST and ALT levels of children with thalassemia syndromes in Murshidabad district of West Bengal and age, sex matched apparently healthy controls. It was found that the majority of the cases had high AST as well as ALT level. Ferritin, iron levels were significantly higher in cases as compared to controls. Present study showed similar results in liver enzymes of thalassemic patients that were observed by other studies, where significant increase in liver enzymes were detected.^{13,14,15} Statistically significant relationship exists between iron storage and number of transfusion and it has been firmly validated in Indian scenario.¹⁶ But their survival needs recurrent blood transfusions leading inevitably to multiple organ dysfunction.¹⁷

It was shown by a study that peroxidative status was generated by iron overload in beta-thalassemia major as evident by rapid formation of marked amounts of thiobarbituric acid reactive substances (TBARS) and increase of super oxide dismutase (SOD) and glutathione peroxidase (GPX) activity.¹⁸ This work suggested that in beta-thalassemia the first organ impaired is the liver. Thalassemic individuals also have increased hepatic level of aldehyde protein adduct indicating lipid peroxidation. Collagen formation and portal fibrosis may start in liver and in absence of chelation, cirrhosis may develop in first decade of life.¹⁹ Elevated aspartate and alanine aminotransferase than controls were possibly due to hepatic necroinflammatory mechanisms as a consequenc of paroxidative injury to hepatocytes.

Nearly all thalassemia patients had abnormally very high levels of serum ferritin compared to the reference range (7-140 µg/L), indicating that these patients have iron overload, probably due to multiple blood transfusion, increased dietary iron absorption or lack of chelating therapy. ALT and AST levels were correlated significantly with serum ferritin concentrations ($r = 0.459$ and 0.676 respectively, $p < 0.001$). Suman RL, et al. showed in a study²⁰ that liver dysfunction starts when serum ferritin increases beyond 1000 ng/ml and number of blood transfusions >30 . The present findings are in agreement with those found by some other studies^{21,22} as there is significant increase in liver enzymes level.

Patients with thalassaemia syndrome are prone to metabolic complications, including different organ dysfunction. Although the actual mechanism is not definitive, the most likely explanation is related to anaemia and iron overload^{23,24} in addition to lipid peroxidation, oxidative stress and free radical release. In patients with thalassaemia, the most important cause of mortality and morbidity is organ failure due to deposits of iron.²⁵ Serum AST and ALT are raised presumably due to peroxidative injury and direct toxic effect of iron on liver cells. These changes of liver enzymes were reported to be appeared as a result of excessive iron-loading (as evidenced by high ferritin level)²⁶ and liver damage.

V. Conclusion

This study is very important in terms of the examination of the serum liver enzymes in patients with thalassemia in our country. Our results revealed that AST and ALT changed in thalassemia patients. Many factors such as iron overload, liver injury due to oxidative stress might cause these changes. Oxidative drugs and iron supplements should be avoided for the patients of thalassemia receiving blood transfusions. It is suggested that appropriate iron chelators²⁷ and antioxidant supplements improve antioxidant/oxidant balance in thalassemia patients. Liver enzymes should be measured and carefully monitored in every thalassaemic child as hepatic dysfunction is inevitable in these children who receive multiple blood transfusions as main modality of treatment.

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