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Biochemistry

A Study of Biochemical Abnormalities in Thalassemia and Hemoglobinopathies Prevalent in North-East India

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Abstract **Original Research Article**

Introduction: Thalassemia and Hemoglobinopathies are the two most common monogenic disorders involving synthesis of hemoglobin molecule. In India the prevalence of β thalassemia carriers is 3-4%. Several ethnic groups have a much higher prevalence of 4-17%. HbE is the most common hemoglobin variant found in Asian population, In India it is prevalent in NE India and Eastern region where the frequencies of HbE carriers range from 3 to over 50%. Coinheritance of HemoglobinE and βthal variants is not uncommon in regions where both are prevalent. The clinical manifestations in thalassemia is due to resultant anemia due to ineffective erythropoiesis, and is mainly treated with blood transfusion which results in deposition of iron and hemosiderin in the renal tubules , heart, liver, spleen and endocrine organs. ***Aim:*** 1. To evaluate the kidney function and liver function of βthalassemia major and heterozygous βthal-HbE. 2. Correlate the hematological and biochemical status of βthal major and βthal-HbE patients with iron overload. ***Materials and methods:*** This study designed as a case-control study, was conducted in Biochemistry Section of Central Clinical Laboratory, Gauhati Medical College & Hospital during the time period from October 2019 to July 2020. Patients suffering from βthalassemia major, HbE-βthalassemia were selected as cases and age matched controls were selected from healthy relatives of patients, and patients attending ENT, Ortho and Surgery OPD. For Liver function assessment AST, ALT, ALP, Tbil, TP was done and for Kidney function assessment urea, creatinine, uric acid, calcium was done. To assess the iron overload Iron, TIBC, Ferritin was done. ***Results & Observations***: Hb levels in the case group were decreased to 60% of levels reported in the control group (7.08±1.66 vs 11.72±1.17). Iron level in the case group, all of whom were receiving regular blood transfusions, was found to be significantly high with a p-value of <0.0001. The level of Ferritin, showed 29 fold higher values in the case group as compared to controls. Mean value of AST was 95.89± 65.75U/L in βthal major while 91.56± 48.53U/L in HbE-βthal patients. ALT in thalassemia major was 73.64± 58.36U/L and in HbE-β thal patients 63.61± 44.10U/L.A positive correlation has been found between serum ALT(r=.008) and AST(r=.103) concentration and serum ferritin levels in the case group. The creatinine level in the case group was lower than the control group, (0.5±0.11 vs 0.46±0.14). But a p-value of 0.0078 suggesting very significant difference in creatinine levels between thalassemia major and HbE-βthal patients was found with a higher level in the former group. ***Conclusion:*** Regular monitoring of the biochemical profile is an effective way of detecting any organ dysfunction at an early stage in βthal major and HbE-β thal patients.

Keywords: βthalassemia, hemoglobinopathy, erythropoiesis, blood transfusion.

INTRODUCTION

Thalassemia and Hemoglobinopathies are the two most common monogenic disorders involving synthesis of hemoglobin molecule. Hemoglobinopathies refer to the structural variants of the normal hemoglobin molecule which are formed mostly due to mutations resulting in amino acid substitution. However, in thalassemia, the synthesis of either of the globin chains of hemoglobin is compromised, either reduced or total absence of synthesis. β thalassemia characterized by a defect in the synthesis of β globin chains of hemoglobin is more widely distributed

Another group is the compound heterozygous of HbE-βthal, a group predominant in South-East Asia. Severe anemia is the most common manifestation in the β thal homozygous and HbE-βthal compound heterozygous states [1-3]. HbE-βthal group shows varied presentation with moderate and severe forms clinically presenting similar to thalassemia intermedia and the more severe forms with manifestations same as thalassemia major.

With an Autosomal Recessive inheritance an estimated 300,000 to 400,000 babies are born each year with a severe hemoglobin disorder [4]. Worldwide 56000 conceptions would have a major thalassemia disorder and among them around 30,000 would have β thal major. In India the prevalence of β thalassemia carriers is 3-4%. Several ethnic groups have a much higher prevalence of 4-17% [5, 6]. ICMR multicenter study in 6 cities of 6 states found an overall incidence of β thal trait of 2.78% [7].

HbE is the most common hemoglobin variant found in Asian population, affecting 30 million inhabitants of South East Asia [8]. In India it is prevalent in NE India and Eastern region where the frequencies of HbE carriers range from 3 to over 50% [9-11]. Coinheritance of HemoglobinE and βthal variants is not uncommon in regions where both are prevalent.

The clinical manifestations in thalassemia are due to resultant anemia due to ineffective erythropoiesis caused by α chain aggregates in erythroblasts and resultant destruction. It is mainly treated with blood transfusion which results in deposition of iron and hemosiderin in the renal tubules, heart, liver, spleen and endocrine organs. Various complications caused by this disease including growth retardation, endocrine dysfunction, hypothyroidism, progressive liver failure and abnormal kidney function have been reported.

Aim

1. To evaluate the kidney function and liver function of βthalassemia major and heterozygous βthal-HbE .
2. Correlate the hematological and biochemical status of βthal major and βthal-HbE patients with iron overload.

MATERIALS AND METHODS

This study designed as a case-control study, was conducted in Biochemistry Section of Central Clinical Laboratory, Gauhati Medical College & Hospital during the time period from October 2019 to July 2020. Patients suffering from βthalassemia major, HbE-βthalassemia who reported to Biochemistry laboratory for blood tests were chosen for the study after taking informed consent. Age matched controls were selected from healthy relatives of patients, and patients attending ENT, Ortho and Surgery OPD. This study was approved by Institutional Ethical Committee. After taking informed consent from either of the parents of the child, detail personal history, history of the disease and of blood transfusion was noted down in a prestructured proforma. Random venous blood sample was collected in clot tubes and EDTA tubes and the estimations of all the biochemical parameters as advised by the physician and all additional tests for the purpose of this study was done in Vitros 5600 Integrated System. For Liver function assessment AST, ALT, ALP, Tbil, TP was done and for Kidney function assessment urea, creatinine, uric acid, calcium was done. To assess the iron overload Iron, TIBC, Ferritin was done. Complete blood count was done in Pathology department according to attending physician's advice and reports collected for the study. The results in the groups were expressed as mean ±SD, and t-test used to compare the means using Graph Pad software. The p-value <0.05 was considered statistically significant and < .01 as highly significant.

Cases

Inclusion criteria:

1. Diagnosed cases of either βthalassemia major or heterozygous HbE-βthal visiting OPD for regular checkup and blood transfusion.
2. Age 1year to 17 years.

Exclusion criteria:

1. Acutely ill patients.
2. Hospitalized patients.
3. Patients suffering from renal and liver complications.

Controls

Inclusion criteria:

1. Healthy relatives of patients.
2. Age 1 year to 17 years
3. Patients attending ENT, Surgery, Ortho OPD without liver or renal complaints.

Exclusion criteria:

1. Hospitalized patients
2. Patients suffering from liver and renal diseases.

This study included 28 βthal major cases and 41 HbE-βthal cases and 30 controls.

RESULTS & OBSERVATIONS

This study was done in Central Clinical laboratory-Biochemistry section of Gauhati Medical College & Hospital. A total of 69 cases, of which 28 were β thalassemia major, and 41 HbE-βthal and 30 controls were recruited for the purpose of this study after obtaining informed consent. The mean age of the case group was 9.58±4.45years and that of controls 12.5± 3.63years.All the cases were receiving blood transfusion 1-2units per month. Table 1 shows the biochemical profile of the cases as compared to the controls. In table 2 the case group has been divided into thalassemia major and HbE-βthal and along with the control group the difference in the biochemical profile has been studied. Table 3 projects the biochemical evaluation of the cases who had received blood transfusion >5years versus those who received for ≤5years.

Table-1: Biochemical Profile of cases and controls

Parameters	Cases N=69	Controls N=30	Independent Sample Test	
			t	P value
Age(yrs)	9.58±4.45	12.5±3.63	3.163	.0021
Hb(g/dl)	7.08±1.66	11.72±1.17	13.867	<.0001
Glucose(mg/dl)	88.51±17.27	81.6±11.61	2.001	0.0482
Iron Profile				
Fe(µg/dl)	187.30±69.97	69.43±21.48	9.021	<.0001
TIBC(µg/dl)	275.55±83.71	307.47±90.19	1.703	0.0917
Ferritin(ng/ml)	2909.67±2170.6	98.77±94.93	7.069	<.0001
Liver Function Tests				
AST(U/L)	93.32±55.74	38.17±11.32	5.357	<.0001
ALT(U/L)	67.68±50.21	26.43±12	4.433	<.0001
ALP(U/L)	185.14±66.5	143.5±75.02	2.753	0.007
TBIL(mg/dl)	2.43±1.61	0.6±0.27	6.171	<.0001
TP(gm/dl)	8.10±1.2	7.94±0.94	0.648	0.5183
Kidney Function Tests				
Urea(mg/dl)	23.84±6.24	20.96±5.61	2.174	0.0322
Creat(mg/dl)	0.35±0.11	0.46±0.14	4.2	<.0001
Uric acid(mg/dl)	4.81±1.25	4.16±0.89	2.575	0.0115
Calcium(mg/dl)	9.35±0.64	9.46±0.84	0.7127	0.4777

Table-2: Biochemical Profile of βThalassemia major, HbE-βthal and controls

Parameters	βThal major N=28	HbE-βthal N=41	Control N=30	P-value
Age(yrs)	11.04±5.03	8.59±3.75	12.5±3.63	0.0005
Hb(gm/dl)	7.04±1.17	7.11±1.94	11.72±1.17	<0.0001
Glucose(mg/dl)	89.11±12.96	88.10±19.83	81.6±11.61	0.1350
Iron Profile				
Iron(µg/dl)	198.36±85.71	179.76±56.74	69.43±21.48	<.0001
TIBC(µg/dl)	282.29±82.59	270.95±85.18	307.47±90.19	0.2104
Ferritin(ng/ml)	3019.11±2800.42	2834.93±1643.74	98.77±94.93	<.0001
Liver Function Test				
AST(U/L)	95.89±65.75	91.56±48.53	38.17±11.32	<.0001
ALT(U/L)	73.64±58.36	63.61±44.1	26.43±12	<.0001
ALP(U/L)	200.61±78.17	174.59±55.78	143.5±75.02	0.0083
TBIL(mg/dl)	2.88±2.14	2.13±1.04	0.6±0.27	<.0001
TP(gm/dl)	8.1±1.6	8.1±0.87	7.94±0.94	0.8146
Kidney Function Test				
Urea(mg/dl)	23.68±6.04	23.96±6.44	20.96±5.61	0.0991
Creatinine(mg/dl)	0.39±0.11	0.32±0.1	0.46±0.14	<.0001
Uric acid(mg/dl)	5.01±1.46	4.67±1.08	4.16±0.89	0.0207
Calcium(mg/dl)	9.48±0.59	9.27±0.66	9.46±0.84	0.3798

Table-3: Biochemical Profile of cases according to period of blood transfusion and controls

Parameter	Cases receiving blood transfusion		Control	F	Anova P-value	Post Hoc test P-value		
	≤ 5years	>5 years				Transfusion≤ 5yrs vs >5yrs	Transfusion ≤ 5yrs vs Control	Transfusion> 5 yrs vs Control
Age(yrs)	6.4±2.7	12.5±3.63	11.75±4.1	26.997	<.0001	<.0001	<.0001	0.4184
Hb(gm/dl)	7.19±1.91	7.01±1.49	11.72±1.77	77.674	<.0001	0.662	<.0001	<.0001
Glucose(mg/dl)	87.14±20.61	89.44±14.76	81.6±11.61	2.163	0.120	0.5906	0.2085	0.0185
Iron Profile								
Iron(µg/dl)	171.79±61.14	197.9±74.27	69.43±21.48	43.258	<.0001	0.1289	<.0001	<.0001
TIBC(µg/dl)	267.75±91.36	280.88±78.79	307.47±90.19	1.635	0.2003	0.5263	0.1014	0.1908
Ferritin(ng/ml)	2432.43±1205.06	3235.59±2600.2	98.77±94.93	27.25	<.0001	0.1322	<.0001	<.0001
Liver Function Test								
AST(U/L)	87.6±46.12	97.21±61.7	38.17±11.32	14.644	<.0001	0.4859	<.0001	<.0001
ALT(U/L)	70.04±46.06	66.07±53.36	26.43±12	9.811	0.0001	0.7497	<.0001	0.0002
ALP(U/L)	195.82±64.11	177.85±67.89	143.5±75.02	4.358	0.0154	0.2735	0.0062	0.0479
TBIL(mg/dl)	2.3±1.83	2.52±1.45	0.6±0.27	19.262	<.0001	0.5801	<.0001	<.0001
TP(gm/dl)	7.74±1.41	8.34±0.98	7.94±0.94	2.651	0.0757	0.0407	0.5250	0.0884
Kidney Function Test								
Urea(mg/dl)	24.34±6.63	23.5±6.01	20.96±5.61	2.509	0.0867	0.5864	0.0402	0.0749
Creatinine(mg/dl)	0.31±0.08	0.37±0.12	0.46±0.14	12.145	<.001	0.0237	<.0001	0.0049
Uric acid(mg/dl)	4.79±0.81	4.82±1.49	4.16±0.89	3.258	0.0427	0.9230	0.0068	0.0344
Calcium(mg/dl)	9.34±0.56	9.36±0.69	9.46±0.84	0.2512	0.7784	0.8991	0.5278	0.5841

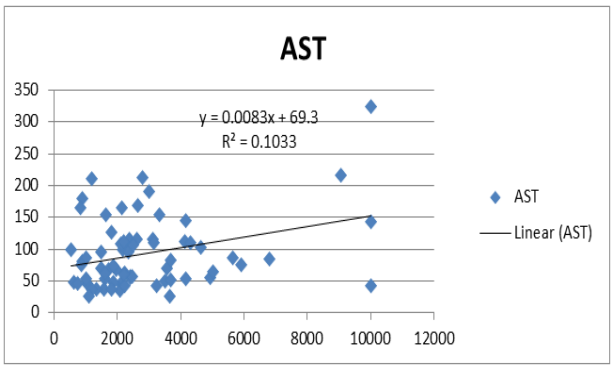

Fig-1: Correlation between AST and Ferritin in case group

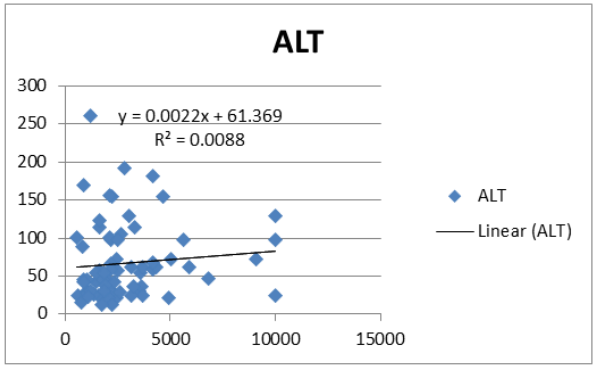

Fig-2: Correlation between ALT and Ferritin in case group

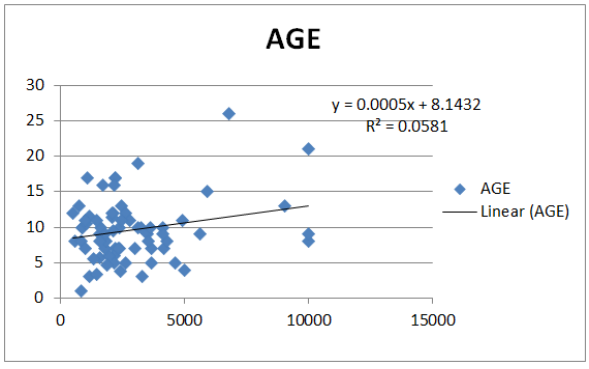

Fig-3: Correlation between age and Ferritin level in case group

DISCUSSION

Both β thalassemia and HbE are widely prevalent in this North Eastern corner of India. The Bodo Kachari have a gene frequency of 0.50 for Hb E, the highest anywhere in the world [12]. The frequencies of β Thalassemia are very high in malarial, tropical and subtropical regions such as Indian subcontinent, a fact hypothesized to be the result of a heterozygote advantage against severe forms of malaria [13].

According to published literatures, prevalence of HbE is widely variable among the different ethnic groups of NE region of India. A high frequency of 0.20-0.60 have been reported from Assam [14]. In some of the ethnic groups of the region the gene frequency for βE globin gene is as high as 0.6[15]. Both HbE and βThal prevalence being high in this region, the

prevalence of Compound heterozygotes (HbE-βThal) is also high.These single gene disorders have drawn the attention of the scientific world as they can be easily prevented with premarital testing programs.

In this study the participant cases of βthal and HbE-βthal heterozygotes have been found to be suffering from worsened clinical situation as has been revealed by their hematologic and biochemical test parameters. Anemia is the most common finding in both these conditions, resulting from the defect in producing both qualitatively and quantitatively normal globin chain of Hb. Hb levels in the case group was decreased to 60% of levels reported in the control group (7.08±1.66 vs 11.72±1.17). Low Hb levels due to ineffective erythropoiesis and hemolysis is mainly sought to be corrected by blood transfusions. Hemolysis and regular blood transfusions create a situation of iron overload in the body. Going with this explanation the iron level in the case group, all of whom were receiving regular blood transfusions, was found to be significantly high with a p-value of <0.0001(187.30±69.97 vs 69.43±21.48). Despite chelation therapy being provided, the level of Ferritin, which is the storage form of iron in the body showed 29 fold higher values in the case group as compared to controls. Ferritin also showed a positive correlation with age, signifying that it is the regular blood transfusions that add up to the iron overload.

The excessive morbidity and mortality in βthalassemia patients is caused by this iron overload condition. At neutral and alkaline pH of our body, the ferric (Fe3+) state of iron is favoured in aqueous environment. In this state iron slowly forms large polynuclear complexes with hydroxide ion, water, and other anions that may be present. These large complexes precipitate in different body organs like liver, kidney, heart, with pathological consequences such as cirrhosis, liver fibrosis, heart disease and endocrine abnormalities. Iron also can bind to and influence the structure and function as well as cause oxidative damage to various macromolecules with deleterious results [16].

Liver dysfunction from iron toxicity has come forward as a major cause of morbidity and mortality in several studies done on βthalassemia patients [17,18]. In the present study the liver enzymes in both the case subgroups are found to be significantly raised as compared to controls. Mean value of AST was 95.89± 65.75U/L in βthal major while 91.56± 48.53U/L in HbE-βthal patients. ALT in thalassemia major was 73.64± 58.36U/L and in HbE-β thal patients 63.61± 44.10U/L. Though biochemical studies in HbE-βthal are not many, but same pathological events also occur in this category of patients as they also need blood transfusion resulting in iron overload. The liver enzymes however did not show any significant rise with increase in the duration of transfusion. A positive correlation has been found between serum ALT(r=.008) and AST(r=.103) concentration and serum ferritin levels in the case group. Similar findings have been repoted by different studies, confirming the iron overload aetiology of liver dysfunction in this category of patients. Other markers of liver dysfunction, namely total bilirubin and ALP have also been found to be significantly elevated in the case group.

Apart from the liver, the kidney is another important organ which suffers damage from iron overload. Kidney function tests on βthalassemic patients receiving blood transfusion have reported significant elevation in urine protein and high levels of protein/creatinine ratio [19-21]. These findings suggest oxidative damage to proximal tubules .In the present study however the creatinine level in the case group was lower than the control group,(0.5±0.11 vs 0.46±0.14).But a p-value of 0.0078 suggesting very significant difference in creatinine levels between thalassemia major and HbE-βthal patients was found with a higher level in the former group. Creatinine levels continued to rise with increasing duration of blood transfusion resulting in a significant difference (p-value<.05) between the two groups, one with blood transfusion ≤5years and other >5 years. Creatinine levels however did not show a positive correlation with ferritin levels. Urea level was however significantly high in the case group as compared to the control group in contrary to findings of other similar studies where no significant difference was found in the urea level between β thalassemia cases and controls [19, 20, 22].

Uric acid level was found to be significantly elevated in the case group, in concurrence with other similar studies [18, 22]. High uric acid level may be a result of high marrow activity and nucleic acid turnover [23, 24]. Rise in uric acid levels in thalassemic patients may also be a way of counteracting the oxidative damage by iron, uric acid being an important antioxidant in plasma [22]. Increase in the number of transfusions received did not give rise to significant rise in the uric acid level.

Hypocalcemia, an important marker of hypoparathyroidism resulting from iron accumulation in the anterior pituitary, was not found in this study. The mean calcium in the case group was found to be in the normal range of 9-11mg/dl; though the level was not so significantly low as compared to controls.

CONCLUSION

The quality of life of β thalassemic patients have improved with blood transfusion and chelation therapy. But treating physicians should always be alert and look out for any adverse effects of iron overload. Liver is one important organ which is seen to be adversely affected by regular blood transfusions. Thalassemia major though may not overtly damage the

kidneys, but long term effect on the kidneys has to be considered when treating such patients. Regular monitoring of the biochemical profile is an effective way of detecting any organ dysfunction at an early stage. HbE and HbE-β thal patients are mainly concentrated in this North Eastern part of the country, but studies on these two groups are limited. More such studies need to be undertaken for a better understanding of the disease and adverse effects of therapy. Thalassemia major, HbE-βthal along with other hemoglobinopathies and congenital disorders are proving to be a major health care problem in India. As prevention is better than cure, premarital genetic testing needs to be made compulsory. Also fetal medicine units need to be set up in all the tertiary care centres for prenatal diagnosis and management of these inherited genetic disorders.

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Conflict of interest: No conflict of interest.

Abbreviations

ALT: Alanine amino transferase,
AST: Aspartate amino transferase,
ALP: Alkaline phosphatase,
Tbil: Total bilirubin,
TP: Total Protein,
Fe: Iron,
TIBC: Total iron binding capacity,
Hb: hemoglobin,
thal: thalassemia,
LFT: Liver function test,
KFT: Kidney function test.

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