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Research Article

HEMATOLOGICAL AND BIOCHEMICAL PROFILES IN BETA-THALASSEMIA PATIENTS WITH LITERATURE REVIEW

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ABSTRACT

Thalassemias are inherited haemoglobin disorders characterized by reduced synthesis of one or more globin chains of adult hemoglobin, HbA ($\alpha_2\beta_2$). The α and β thalassemia's are the most common classes, β-thalassemia is caused by deficient synthesis of β chains, whereas α-thalassemia is caused by deficient synthesis of α chains.This imbalance in globin chain synthesis causes varying degree of anemia, due to ineffective erythropoiesis and hemolysis. Red blood cell degradation and transfusion in thalassemic patients leads to excess iron deposits in vital organs like heart, liver, spleen, pancreas and endocrine organs. Iron overload, poor compliance to therapy and chronicity of the disease have contributed to a spectrum of complications like growth retardation, cardiac problems, liver failure, hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism and metabolic problems in adolescents. The aim of the present study is to evaluate and compare the haematological and biochemical parameters in β Thalassemia patients. Total 49 known cases of beta thalassemia were selected for the study. Detail clinical history and blood samples were collected from all the patients. The samples were subjected for haematological, biochemical and serological analysis. Complete blood picture was performed on Sysmex XN 350. Biochemical parameters like liver function tests and renal function tests were performed on ERBA EM 200. Thyroid function tests, serum ferritin estimation and viral screening (CMIA Method) were performed by Abbott Architect. The results were tabulated and compared to derive the correlation between the different parameter in thalassemic patients. The haematological and biochemical parameters of thalassemic patients showed much variation from the reference ranges in our study.

INTRODUCTION

Thalassemia's are a heterogeneous group of inherited haemoglobin disorders characterized by reduced synthesis of one or more globin chains of adult hemoglobin, HbA ($\alpha_2\beta_2$), leading to imbalance in globin chain synthesis, ineffective erythropoiesis, hemolysis, and eventually to a variable degree of anemia (Mahmood Asif *et al*., 2014). The hematologic consequences of diminished synthesis of one globin chain stem not only from hemoglobin deficiency but also from a relative excess of the other globin chain. The α and β thalassemia's are the most common classes, β-thalassemia is caused by deficient synthesis of β chains, whereas α-thalassemia is caused by deficient synthesis of α chains. The two α chains in HbA are encoded by an identical pair of α-globin genes on chromosome 16, while the two β chains are encoded by a single β-globin gene on chromosome 11 (Gira P *et al*., 2013). The studies on genetic basis of the disease have led to the identification of more than 200 mutations in the β-globin gene located at 11p15.5. Thalassemia syndromes are endemic in the Mediterranean basin, the Middle East, tropical Africa, the Indian subcontinent, and Asia, and in aggregate are among the most common inherited disorders of humans (Gira P *et al*., 2013; Nikita Tripathi *et al*., 2015). β Thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3-4 % is the most important and widely spread type which causes severe anemia in the homozygous and compound heterozygous states(Nikita Tripathi *et al*., 2015). Moreover, the anemia in this disease is microcytic and hypochromic in nature, with low Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) (Karim MF *et al*., 2016). Red blood cell degradation and transfusion in thalassemic patients leads to excess iron deposits in vital organs like heart, liver, spleen, pancreas and endocrine organs(Karim MF *et al*., 2016; Bushra Munir *et al*., 2013). Iron overload, poor compliance to therapy and chronicity of the disease have contributed to a spectrum of complications like growth retardation, cardiac problems, liver failure, hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism and metabolic problems

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in adolescents (Malik SA *et al*., 2010). The aim of the present study is to evaluate and compare the haematological and biochemical parameters in β Thalassemia patient.

MATERIALS AND METHODS

Total 49 known cases of beta thalassemia were selected for the study. Detail clinical history and blood samples were collected from all the patients. The samples were subjected for haematological, biochemical and serological analysis. Complete blood picture was performed on Sysmex XN 350. Biochemical parameters like liver function tests and renal function tests were performed on ERBA EM 200. Thyroid function tests, serum ferritin estimation and viral screening (CMIA Method) were performed by Abbott Architect. The results were tabulated and compared to derive the correlation between the different parameter in thalassemic patients.

OBSERVATIONS & RESULTS

The haematological and biochemical parameters of thalassemic patients showed much variation from the reference ranges. Among 49 cases, 37 cases were thalassemia major and 12 cases were thalassemia intermedia with slight female predominance. In our study, 37 cases were in 0-10 year's age group, with 21 females and 16 males, 12 cases were between 11-20 years with equal sex distribution (Table 1). The peripheral smear in most of the cases showed target cells, nucleated RBC's with high reticulocytes (Figure 1).

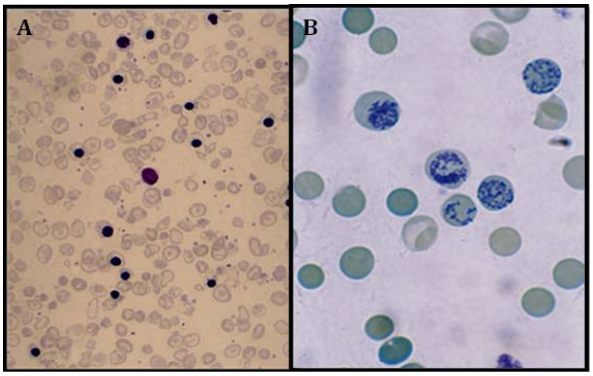

Figure 1: A, Peripheral smear with many target cells and nucleated RBC's; B, Methylene blue staining shows increased reticulocytes on peripheral smear.

Table 1 Age and sex wise distribution of cases.

Age (Years)	Females	Males	Total
0-10	21	16	37
11-20	6	6	12
Total	27	22	49

The haemoglobin levels of most patients were between 5-10 gm% in 34 (70%) cases, 12 (25%) cases had value more than 10 gms% and only 3(5%) cases had less than 5gms%.

Table 2 Hemoglobin ranges in patients.

Haemoglobin(gm%)	No of cases
0-5	3
5-10	34
>10	12

The RBC was <3.5 million/µl in all patients and lowest recorded RBC count was 0.82 million/µl. MCV in 33 (67.3%) patients was below the lower normal limit and remaining 16(33%) patients were within normal range, the lowest recorded MCV was 47.4fl. Similar findings were observed in MCH and near normal ranges in MCHC (Table 3).

Table 3 Haematological parametersin Thalassemia cases.

Parameter	Units	Mean	Lowest	Highest
HB	g/dl	8.0	2.3	12
RBC	10^6/µl	3.0	0.82	3.5
HCT	%	28	6.6	36
MCV	fl	78	47.4	90.3
MCHC	g/dl	31	21	40
MCH	pg	31	21	41

Serum iron was in normal range (28-170µg/dl) in 18 (36%) cases and remaining 31(64%) had significant increase (>170µg/dl) in iron levels. Serum ferritin was increased in all cases with 31(64%) cases having levels >2000ng/dl and 18(36%) cases having levels >200 ng/dl, indicating higher iron and ferritin levels in most cases (Table).

Table 4 Iron and ferritin levels in Thalassemia cases.

Serum ferritin (ng/dl)	Serum iron (ug/dl)	No of cases
> 200	28-170	18
> 2000	>170	31

Liver function tests showed increase in indirect bilirubin levels in 25 (51%) cases and remaining 24 (49%) cases were in normal range. Direct bilirubin was normal in 47 (95%) cases and mild elevation in 2 (5%) cases. Both SGPT and SGOT were elevated in all 49 cases. ALP levels were increased in 10(20%) cases and in remaining 39(80%) cases were within normal limits. Total proteins and albumin levels were normal in all cases, calcium and phosphorous levels was abnormal in eight and three cases respectively (Table 5 & 6). Renal parameters and viral screening was normal in all cases.

Table 5 Liver enzyme levels in thalassemia cases.

Parameters	Units	Mean	Minimum	Maximum
ALT/SGPT	U/L	119	55	550
ALP	U/L	150	30	680
AST/SGOT	U/L	77	60	131

Table 6 Total cases with normal and abnormal liver parameters.

Parameter (Reference range)	Normal	Abnormal
DB (0-0.2)	47	2
IB (0.2-0.8)	25	24
SGPT (<45)	0	49
SGOT (<35)	0	49
ALP (30-300)	39	10
TP (6.4-8.3)	49	0
ALBUMIN (3.8-5.4)	49	0
GLOBULIN (2.3-3.5)	49	0
CALICIUM (8.4-10.4)	41	8
PHOSPHORUS (3.5-5.9)	46	3

In our study, 11(22%) cases showed hypothyroidism, among these 8 cases were males and 3 cases were females. Mean ferritin levels were significantly high in patients with hypothyroidism (Table 7).

Table 7 Comparison of Thyroid & Ferritin levels.

Thyroid status	Patients		Mean Ferritin(ng/dl)
	Males	Females	
Euthyroid	20	18	2890
Primary Hypothyroidism	5	1	3280
Subclinical	3	1	2950
Secondary Hypothyroidism	-	1	3560

This data show that there are significant differences in haematological and biochemical parameters in thalassemia patients.

DISCUSSION

Thalassemia was first described by Cooley in 1925, hence also termed as Cooley's anemia (Ali D *et al*., 2008; Mehrnoosh Shanaki *et al*., 2016). Clinical classification of β-thalassemia is based on the severity of the anemia. First severe and transfusion dependent anemia called β-thalassemia major (Sherief LM *et al*., 2017), second mild asymptomatic microcytic anemia referred to as β-thalassemia minor or β-thalassemia traitand a third genetically heterogeneous variant of moderate severity is called β-thalassemia intermedia. The clinical presentation of the severity depends on the genetic defect (β^{+} or β^{0}) and the gene dosage (homozygous or heterozygous). In general, individuals with two β-thalassemia alleles have severe form; heterozygotes with one β-thalassemia gene and one normal gene usually have a milder disease, with intermediate disease falling in-between. Signs and symptoms of thalassemia include pallor, weakness, fatigue, dyspnea, jaundice, facial bone deformities, irritability, slow growth, protruding abdomen, dark urine, and these symptoms depend on type and severity of thalassemia (Nihad Ahmed Ameen, 2016).

Thalassemia major presents in early age as observed by other studies, in our study, 37 cases were in 0-10 years age group, which was in concordance with literature and 12 patients were between 11-20 years and only 2 patients were recorded in adolescent age group, which may be due to the less severe form of disease and good compliance to the treatment. The mean haemoglobin level in our study was 8.0g/dl, with few cases having less than 5.0 g/dl. Increased haemolysis as a result of abnormal haemoglobin leads to lower level of RBC in these patients; In our study the RBC count was <3.5 million/µl in all patients and lowest recorded RBC count was 0.82 million/µl. MCV was below the lower normal limit in 67 % patients and 33% patients were within normal range, Similar findings were observed for MCH, with MCHC falling in the normal range for all cases. The management of thalassemia major is one of the urgent concerns for the patients and require regular blood transfusion to keep the hemoglobin levels close to normal, which inturn helps to maintain normal growth and development and reduce extramedullary hematopoiesis. Without blood transfusion the majorities of patients with thalassaemia suffers from growth retardation and die at an early age from the effects of anemia. In those who survive long enough, the cheek bones and other bony prominences are enlarged and distorted. Though regular transfusions improve the anemia, suppress complications related to excessive erythropoiesis and prolong survival into adolescence or early adult life, they lead to complications of their own (Letsky EA *et al*., 1974).

Frequent blood transfusions, peripheral hemolysis, increased intestinal iron absorption and ineffective erythropoiesis are inevitably associated with iron overload and which cause iron accumulation in various organs, notably in heart, liver, kidney and endocrine glands (Mehrnoosh Shanaki *et al*., 2016; Rasool M *et al*., 2016). Hemosiderosis and secondary hemochromatosis, the two manifestations of iron overload, occur in almost all patients. Serum ferritin was increased in all cases in our study with 31 cases having the value >2000ng/dl. Serum iron ranged between 28-170µg/dl in 18(36%) patients and remaining 31(64%) had >170µg/dl showing significant increase in iron levels in 64% of patients, which is due to the repeated transfusions. Serum ferritin is used as an indirect measurement for estimating iron overload in recurrent transfused patients (Munir B *et al*., 2013), but several studies showed discordance. Hepatic iron concentration (HIC) is the most useful method for estimating iron load in chronically transfused patients (Mazza P *et al*., 1995). No clear relationship exists between age and hematological parameters of thalassemic patients.

Repeated transfusion also increases the risk of transfusion associated infections, where hepatitis B and hepatitis C are the most common infections detected (Salama KM *et al*., 2015); in our study we have not detected any infections in patients. Peripheral hemolysis is so rapid that it exceeds the liver capacity to metabolize the bilirubin, leading to increased bilirubin (Palit S *et al*., 2012). The free α-globin accumulates, and unpaired α–chains aggregate and precipitate to form inclusion bodies, leading to destruction of immature developing erythroblasts within the bone marrow leading to ineffective erythropoiesis. Long lasting anemia, iron overload, toxicity of deferoxamine (an iron-chelating agent) and chronic hypoxia lead to renal dysfunctions in patients with β-Thalassemia major. Studies of the renal involvement in thalassemic syndromes have shown interstitial iron deposition and hemosiderin deposits in tubule resulting in renal abnormalities (Vinita Belsare *et al*., 2015). Thalassemia major patients had higher BUN and creatinine levels and lower calcium and phosphate levels, but in our study renal parameters were normal with only few cases showing mild decrease in calcium and phosphorous levels.

In our study direct bilirubin levels were normal in 47 cases and only two cases showed mild elevation of the levels, whereas indirect bilirubin levels were increased in 25(51%) patients and remaining 24 (49%) patients were in normal range. Both SGPT and SGOT were elevated in all 49 patients and 10(20%) patients had elevated ALP levels, remaining 39(80%) patients ALP levels were within normal limits, similar finding were observed in other studies. Repeated transfusions also lead to low levels of thyroid hormone. It has been demonstrated that thyroid abnormalities in these patients are related to iron overload. In present study 11 cases showed hypothyroidism and all cases were of thalassemia major category. According to some studies significant association was found between ferritin levels and thyroid functional status. Mean ferritin levels were significantly high in hypothyroid patients in our study, hence, it is postulated that higher serum ferritin levels predispose to a greater risk of developing endocrinopathies like hypothyroidism. The precise mechanism by which iron overload causes tissue damage is not completely understood. Iron has a catalytic role to produce powerful reactive oxidant species (ROS) and free radicals which lead to oxidative damage, which on deposition in organs act at the cellular level and cause damage via free radical formation and lipid peroxidation resulting in mitochondrial, lysosomal and sarcolemmal membrane damage. Therefore evaluation of oxidative stress can be useful in protecting β- thalassemia patients from more serious complications of the disease followed by iron deposition in different parts of body (Mehrnoosh Shanaki *et al*., 2016; Palit

S *et al*., 2012). Deferoxamine, an iron-chelating agent is used in patients with severe iron overload.

With this enormous burden and no effective treatment available, the focus in thalassemia patients has shifted in early diagnosis and prevention. The studies on genetic basis of the disease have led to the identification of more than 200 mutations in the β-globin gene located at 11p15.5. Carrier detection and prenatal diagnosis are the most feasible modes of prophylaxis available (Bashyam MD *et al*., 2004). Prenatal diagnosis and carrier status detection are the most appropriate preventive measures to contain the disease and reduce the load of the mutant alleles in the gene pool. Therefore, carrier status detection and genetic counseling is highly significant.

CONCLUSION

β Thalassemia is one of the most common single gene disorders in India, causing the enormous burden on society. Without treatment the majorities of patients with thalassaemia suffers from growth retardation and die at an early age from the effects of anemia. The management of thalassemia major is one of the urgent concerns for the patients and requires regular blood transfusion to keep the hemoglobin levels close to normal, which in turn helps to maintain normal growth and development, but they are associated with complications due to excess iron deposition, causing damage to the vital organs. The present study showed that thalassemic patients had significant abnormalities in haematological and biochemical profiles, which may be due to ineffective erythropoiesis, haemolysis and iron overload due to repeated blood transfusion. The haematological parameters like MCV, MCH were low in patients when compared to reference group. Serum ferritin and serum iron levels were significantly high in these patients due to repeated transfusions, which further lead to liver, renal and endocrine abnormalities. With this enormous burden and no effective treatment available, carrier detection and prenatal diagnosis are the most feasible modes of prevention available. Thus all suspected cases should undergo screening and genetic counseling as a preventive measure.

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