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Original Article

Pathology Section

Haematophysiological Study of Transfusion Dependent Beta-thalassaemia Patients in a Tertiary Care Hospital of Odisha, India

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ABSTRACT

Introduction: The Transfusion Dependent Beta-thalassaemia (TDT) is an autosomal recessive disorder that affects the red blood cells, both in the decreased as well as absence in production of Adult Haemoglobin (HbA) and characterised by severe anaemia, splenomegaly and bone deformities and require lifelong transfusion therapy, iron chelation and/ or bone marrow transplantation for successful control.

Aim: To evaluate the haematophysiological status of various TDT patients.

Materials and Methods: This is a cross-sectional study conducted in the Hematology Department of SCB Medical College and Hospital Cuttack, Odisha, India. The study was done in the duration of 10 months, from April 2021 to January 2022. The blood samples were collected from 62 diagnosed cases of Beta-thalassaemia. Haematophysiological parameters were studied by asking research questionnaire developed for this purpose and analysing various clinical complaints and laboratory data. The haemoglobin variants were analysed by fully automated capillary zone electrophoresis to confirm the diagnosis of homozygous state of Beta-thalassaemia by decreased or absence of globin chain synthesis (HbA2) of the haemoglobin component. The data was anlysed using Statistical Package for Social Sciences (SPSS) version 19.

Results: In the present study majority of the patients 39 (62.9%) were below the age of 10 years. The clinical examination showed anaemia in all the patients with haepatomegaly and splenomegaly in 51 (82.25%) and 57 (91.93%) cases respectively. The mean haemoglobin was markedly decreased to 5.98±1.69 ranged from 3 gm% to 10 gm% followed by Foetal Haemoglobin (HbF) was raised up to 78.39±26.73% (normal absent to 0.02%) and adult haemoglobin (HbA2) was increased up to 8.00±5.72 (normal 95% to 98%).

Conclusion: TDT is one of the commonest haemoglobinopathy causing major health problem. Common sign and symptoms includes low-grade fever, severe anaemia and haepatosplenomegaly. The Present study helped the families having TDT beta thalassaemia for early detection and avail the facilities given by Government of Odisha.

Keywords: Anaemia, Haemoglobin variants, Beta-globin gene

INTRODUCTION

The thalassaemias are hereditary anaemias caused by mutations of the globin chain, the protein component of the haemoglobin. Thalassaemias produces a massive public health problems in many parts of the world [1]. These are the most common genetic diseases of mankind and have been encountered practically in every racial group and geographic locations in the world, however, the thalassaemias are most commonly reported in the Mediterranean, the equatorial, or near equatorial regions of Africa and Asia [2].

Thalassaemias are classified according to a particular globin chain(s). The production of these chains occured in a reduced amount and may lead to an imbalance in globin chains synthesis, ineffective erythropoiesis, hemolysis, and eventually to a variable degree of anaemia. The main types of thalassaemias are the alpha, beta and delta thalassaemia. The beta thalassaemias are more common and responsible for widely spread type which causes severe anaemia in the homozygous and compound heterozygous states [3].

Thalassaemias are clinically classified according to their severity, thalassaemia major requiring a regular blood transfusion throughout the life, thalassaemia intermedia characterised by anaemia but not of such severity as to require regular blood transfusion, and thalassaemia minor or trait which is the symptomless carrier state [4]. The severity of the clinical syndrome of Beta-thalassaemia depends on the type of mutation in the Beta-globin gene.

Beta-thalassaemia is caused by mutations resulting in a single nucleotide substitution, small deletions or insertions within the Beta-globin gene or its immediate flanking sequence, or in rare cases, gross deletions in chromosome no 11. These mutations result in decreased production of HbA component of Beta-globin chains. There are more than 350 Beta-thalassaemia mutations described in the literature. As per the severity it is classified as β+ mutations results in decreased production of Beta-globin chain synthesis and β0 mutations causing complete absence of Beta-globin chain [5]. Well known relationships have been reported between the hematological-clinical phenotype and the type of the Beta-thalassaemia mutation. Consequently, identifying the mutation in the patients is highly appreciated for a better management protocol [5].

The manifestations of TDT are widely variable and mainly depends on the anaemia of haemolytic etiology and organ dysfunction due to iron toxicity. For survival, patients require regular blood transfusion and holistic medical evaluation. Hence, the phenotype of TDT varies widely from place to place depending upon many social factors like financial burden, education and health care facilities etc. There is a paucity of studies in the state of Odisha in documenting these features among TDT. The present study was undertaken to evaluate the haematophysiological status of various TDT patient to help in diagnosis and treatment of TDT Beta-thalassaemia.

MATERIALS AND METHODS

A cross-sectional study was done on various TDT major patients, conducted at the Department of Haemtology of SCB Medical College and Hospital Cuttack, Odisha, India. Duration of the study was 10 months, from April 2021 to January 2022. Permission to conduct the study was obtained from Instiutional research committee and Instiutional Ethics committee (IEC No 674/20-08-18). Clinically and haematologycally diagnosed Beta-thalassaemia patients were included in the study where as Alpha-thalassaemia and other haemoglobin disorders are excluded.

Study Procedure

The cases were confirmed by haemoglobin variant analysis by High Performance Liquid Chromatography (HPLC) Bio-rad for adult haemoglobin and foetal haemoglobin estimation. The other haematological parameters analysed in the present study are Hb %, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC) counts, serum ferritin level, liver function tests, gonadal hormone estimation, thyroid and pararhyroid hormone assay. Sixty two patient's transfusion dependant Beta-thalassaemia patients were studied from hematology Outpatient Department (OPD) of SCB Medical College Cuttack. The hematological and clinical parameters like Low-grade fever, Abdominal distension, Dyspnoea, Loose motion, Dizziness, Pallor, Spleen enlargement, Haepatomegaly and Jaundice were studied. Five milliliter of Venous blood samples with EDTA anticoagulant were used for Complete Blood Count (CBC) by automatic cell counter and HPLC for haemoglobin variants with due informed consent was obtained for the study. Haematophysiological parameters were studied by asking a research questionnaire prepared by Head of Department, Haematology for the present study for analysis of various clinical complaints and laboratory data obtained about the family history, past history and chronic illness leading to early death.

STATISTICAL ANALYSIS

Data were analysed using QuickCalcs Graph Pad Software and SPSS version 19. p-value <0.05 was considered significant.

RESULTS

In the present study M:F ratio was being 2.1:1 (ranged from 1 year 10 months to 23 years). The mean age was being 9.21±4.36. Maximum cases were seen in the age group of 6-10 years. 25 (40.32%) in male patient and 11 to 15 years 18 (29.03%) followed by 0 to 5 years 14 (22.58%). Hence most of the cases were till 15 years 57 (91.93%). There were only 5 (08.06%) cases at the age of sixteen and above [Table/Fig-1].

Age in years	Male	%	Female	%	Total	Percentage
1 to 5	9	21.4	05	25	14	22.58
6-10	18	42.85	07	35	25	40.32
11-15	13	30.95	05	25	18	29.03
16-20	02	4.7	02	10	04	06.45
≤21	00	00	01	05	01	01.61
Total	42	100	20	100	62	100

[Table/Fig-1]: Age and sex distribution of the study population.

The sign and symptoms were studied. Pallor 60 (96.77%), splenomegaly 57 (91.93%) and haepatomegaly 51 (82.25%) were seen in maximum percentage of cases followed by low-grade fever in 18 (29.02%), Abdominal Distension 3 (4.8%), dyspnoea 5 (8.06%), Loose motion 4 (6.45%) and dizziness 7 (11.29%) cases were also observed [Table/Fig-2].

Parameters		Values
Symptoms	Low-grade fever	18 (29.02%)
	Abdominal distension	3 (4.8%)
	Dyspnoea	5 (8.06%)
	Loose motion	4 (6.45%)
	Dizziness	7 (11.29%)
Signs	Pallor	60 (96.77%)
	Spleen enlargement	57 (91.93%)
	Hepatomegally	51 (82.25%)
	Jaundice	9 (14.51%)

[Table/Fig-2]: Signs and symptoms of the study population.

Present study showed the mean Haemoglobin percentage was 5.98±1.69 ranged from 3 gm% to 10 gm% resulting microcytic-hypochromic anaemia. It was observed that high WBC count 12.16±6.95 thousands responsible for repeated attack of common cold and upper respiratory tract infections in the study population. Serum Ferritin level was high 3173.23±2061.22 ng/L suggestive of haemolysis. There were increased levels of Aspartate Aminotrans ferase (AST), Alanine Transaminase (ALT) and serum bilirubin also in support of haemolysis. There were high Thoyroid Stimulating Hormone (TSH) level (12.75±9.13) followed by decreased level of parathyroid hormone (41.5±21.77), testosterone (176.71±96.49) and Ostradiol (15.26±7.99) responsible for hypothyroidism and gonadal dysfunction [Table/Fig-3]. There were marked increase in HbA- 8.00±5.72 ranged 1.1 to 15.5 normal should be (95-98%) and increased HbF- 68.39±26.73 ranged 0.8 to 98.3 (normal absent to 0.02) suggestive of Beta-thalassaemia.

Parameters	Minimum	Maximum	Mean±SD	Physiological range
Hb (gm%)	3 gm%	10 gm%	5.98±1.69	11.5-15
WBC (thousands)	3.92	24	12.16±6.95	4-10
MCV (fL)	55.9	94.1	69.66± 6.60	75-95
MCH (pg)	18.7	27.5	22.98±2.66	30-35
MCHC (g/dL)	28	37.2	32.69±2.42	30-35
S Ferritin (ng/L)	637.9	9000	3173.23±2061.22	10-120
AST (U/L)	24	126	59.22±19.72	7-45
ALT(U/L)	14	91	56.75±18.79	8-48
Bilirubin (T)- (mg/dL)	0.6	3.4	2.09± 0.96	0.3-1.0
Bilirubin (D)- (mg/dL)	0.2	1.8	0.82±0.55	0.1-0.3
TSH (mIU/L)	2.1	29.2	12.75±9.13	0.5 to 5.0
PTH (pg/mL)	15.2	75.5	41.5±21.77	10-55
Testosterone (ng/dL)	51.5	299.8	176.71±96.49	(300-1000)
Estradiol (pg/mL)	5.5	25.7	15.26±7.99	30-400
Hb A gm%	1.1	15.5	8.00±5.72	95-98 (%) 0.95-0.98
HbA2 gm%	1.4	5.3	3.01±1.19	2(%) 0.02-0.03
HbF gm%	0.8	98.3	68.39±26.73	0.8-2 (%) 0.008-0.02

[Table/Fig-3]: Comparison of Hematological parameters with Physiological range in the study population.
Hb: Haemoglobin; WBC: White blood cell; MCV: Mean corpuscular volume; fl: femtoliter; MCH: mean corpuscular haemoglobin; pg: picogram; MCHC: mean corpuscular haemoglobin concentration ; AST: aspartate aminotransferase ALT: alanine transaminase; TSH: thyroid stimulating hormone PTH-parathormone; HbA: adult haemoglobin and HbF-fetal haemoglobin

The most commonly affected organ due to iron overload is Heart i.e., (cardiomyopathy) seen in 15/57 (26.31) cases in <16 years age group in comparison to 80% of >16 age group p value is 0.014(<0.05), which is statistically significant. Other affected organs were Hypogonadism in both the age groups 29 (46.77%), followed by Parathyroid abnormality 13 (19.35%) and Thyroid dysfunction 7 (11.29%) cases and the p values were statistically insignificant [Table/Fig-4].

Complications detected	Dysfunction observed (n=62)	<16 years (n=57)	≥16 years (n=5)	p-value
Cardiomyopathy	Echo- cardiographs	15/57 (26.31)	4/5(80%)	**0.014**
Hypogonadism	Decreased testosterone increased FH & LH-29 (46.77%)	27/57(47.37%)	2/5(40%)	0.764
Parathyroid abnormality	13 (19.35%)	12/57(21.05%)	1/5(10%)	0.555
Thyroid dysfunction	7 (11.29%)	6/57(10.52)	1/5(10%)	1.000

[Table/Fig-4]: Frequency of organ complications seen in TDT Beta-thalassaemia patients.

DISCUSSION

The manifestations of TDT are widely variable and it depends on the severity of anaemia of haemolytic etiology and organ dysfunction due to iron toxicity. For survival, patients require regular blood transfusion and holistic medical evaluation.

In the present study, beta-thalassaemia was seen maximum in the age group of 6 to 10 years 25 (40.32%) cases. The minimum and maximum age in the study population was 1 year and 23 year respectively. Only 2 cases were seen above 20 years. The male patients were almost 2.5 times higher than female patients in the total study population. So, most of the cases were seen <16 years 57 (91.93%) with male predominance. The present study corroborates with the observations of Kumar S et al., [6] Singh M et al., [7] Barua T et al., [8]. Beta-thalassaemia patients were presenting anaemia (pallor) 60 (96.77%) splenomegaly 57 (91.93%) and haepatomegaly 51 (82.25%) in maximum cases followed by low-grade fever in 18 (29.02%) cases. Anaemia commonly known as pallor was seen in 60 (96.77%) cases mostly hypochromic microcytic type due to iron deficiency caused by haemolysis. Haepatosplenomegaly with jaundice was also in the favour of haemolysis. Persistent low- grade fever explained lack of immune response and vulnerable to various infections. Toppo SM et al., [9], Meshram PM et al., [10] and Singh M et al., [7].

Hematological profile of Beta-thallasemia patients, Heamoglobin percentage was 5.98±1.69 which is almost 50% below normal value (11.5-15) gm% resulting from microcytic-hypochromic type of haemolytic anaemia. White blood cells were also increased from normal value (4-10) thousands to 12.16±6.95 thousands results in reoccurrence and sometimes life threatening infections. High Ferritin levels were invariably seen 3173.23±2061.22 (ng/L) suggestive of haemolysis followed by iron overload. Das Indrani [11]. Increased levels of AST, ALT and serum bilirubin were adding support to marked red cell destruction. Adult haemoglobin was increased to HbA- 8.00±5.72 (normal 95% to 98%) and marked increase in Foetal Haemoglobin HbF- 68.39±26.73 (normal absent to 0.02) suggestive of beta-thalassaemia as studied by Barua T et al., [8] Singh V et al., [12].

Cardiac abnormality was the most common complication showing decrease in systolic volume by 2D echo-cardiographs on TDT major in the age group ≤16 years with statistically significant value 0.014 in comparison to children [13,14]. Hypogonadism was observed 29 (46.77%) cases of children less than 16 years, which was 27 out of 57 (47.37%) of cases and outnumbered the adults with only 2 cases out of 5 patients amounting 40% in the present study. Hypogonadotrophic hypogonadism was resulting from iron deposition in the pituitary gonadal axis in transfusion dependant beta-thalassaemia major patients [15,16]. The other organs like parathyroid abnormality was seen in 13 (19.35%) cases and thyroid dysfunction 7 (11.29%) cases [17,18].

Limitation(s)

Smaller study group and shorter one year study period was the major limitation of the present study.

CONCLUSION(S)

Transfusion dependant Beta-thalassaemia cases were found to be maximum below the age of 10 years with male predominance. All the cases were presented with iron deficiency anaemia, low-grade fever and splenomegaly. There is invariably raised HbA2 and high HbF in all the cases. Organ abnormalities were also seen as cardiomyopathy, hypogonadism, parathyroid abnormality and thyroid dysfunction due to iron over load reflected by high serum ferritin. The clinical sign and symptoms with haematological parameters as per normal physiological ranges and their relationship with human Beta-globin gene mutation are still inadequate to reach definitive diagnosis. This study showed a platform for molecular genetics study on Beta-thalassaemia major, which is highly significant in the diagnosis and treatment of transfusion dependant beta thallasemia.

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