International Journal of Medical and Health Sciences

Journal Home Page: http://www.ijmhs.net ISSN:2277-4505

Original article

Prospective Study of Sickle Beta Thalassemia in A Tertiary Care Hospital

Dharma Niranjan Mishra[1*], Rabindra Kumar Jena[2]

[1]Assistant Professor, Department of Anatomy, [2]Professor & Head, Department of clinical Haematology, S. C. B. Medical College Cuttack 753007, Orissa.

ABSTRACT

Background: Sickle cell beta thalassemia (Hb S/β Thal) is an inherited form of sickle cell disease that affects red blood cells both in the production of abnormal hemoglobin as well as the decreased synthesis of beta globin chains. It constitutes one of the major genetic haematological disorders in Odisha. The aim of the study is to determine the haematological profile of patients suffering from sickle beta thalassemia. **Methods:** After obtaining informed consent and ethical committee approval, blood samples were collected from 45 diagnosed cases of sickle beta thalassemia. Screening was done by Sickling test and haemoglobin variants were analysed by fully automated capillary zone electrophoresis. **Results:** In our observation, the fetal hemoglobin (HbF) was raised to 21.63% (SD± 5.24) ranging from 11% to 32.7% and adult haemoglobin (Hb A2) was also raised with mean value 4.12 (SD±1.00) which is >3.5%, suggestive of sickle cell-β-thalassemia. We observed high percentage of sickle cell haemoglobin (HbS) ranging from 45.9% to 82.3%, the mean was being 69.45% (SD±4.35). **Conclusion:** The present study highlights the co-inheritance of β-thalassemia and sickle cell gene. Molecular diagnosis is required for β-Globin Gene mutations in this state.

KEYWORDS: Anaemia, haemoglobin variants, sickle cell beta thalassemia, β-Globin Gene

INTRODUCTION

The combination of the sickle cell mutation and beta-thalassemia (β-Thal) mutation gives rise to a compound heterozygous condition known as Sickle cell beta thalassemia (Hb S/β-Thal), which was first described in 1944 by Silvestroni and Bianco[1]. Sickle β thalassemia is a major hemoglobin disorder responsible for most of the symptoms and complications of sickle cell disease [2]. Patient's heterozygous sickle cell haemoglobin (HbS) & β Thalassemia (HbS-β-Thal) may suffer sickle cell disease but their symptoms are less severe than the homozygous sickle cell disease. Sickle cell β thalassemia is classified according to severity as sickle cell β° thalassemia in which β Globin production is zero, Sickle cell β^{+} thalassemia, where β globin production is less than normal and the milder form is designated as Sickle cell β^{++} thalassemia with high Hb A (20-30%)[3].

The symptomatology of Sickle cell disease and Sickle cell β° thalassemia is almost similar involving irreversible Sickle shape of red blood cell, severe anaemia and frequent vaso occlusive crisis [4]. Some forms of β thalassemia in which Hb A2 level is normal in heterozygotes are classified into Type 1 (Silent β Thal) having no haematological change and in Type 2 (Typical), the haematological changes are of Beta Thalassemia with increased Hb A2. In HbS/β-thalassaemia, the β-thalassaemia gene interacts with the HbS-gene to increase the level of HbF (usually>15%) and HbS from above 50% to a level near that which is observed in HbSS individuals [5].

Relatively higher level of HbF in this double heterozygous condition may be beneficial by decreasing HbS polymerization while adding a new detrimental effect by aggravating the mild haemolytic components of HbS gene [6]. The net phenotypic expression of the interaction of two genes is remarkably variable from completely asymptomatic condition at one end to severe form of sickle cell disease (SCD) or β+-Thalassaemia [7]. Some of the unique complications of SCD like vitreous haemorrhage and asceptic necrosis of femoral head etc. may be associated with certain genotype-phenotype variations [8] .Changes in haematological parameters include microcytic red cell, target cell, 60-90%of HbS, 0-30% of HbA, 1-20% of HbF. The type of β-thalassaemia gene that is co-inherited with HbS gene may partly explain such variations which need to be corroborated by further study. Moreover, the high

incidence of iron deficiency and α-thalassaemia gene in our population may alter the picture significantly which is relevant to their management [9].

The genotype-phenotype expression of HbS-β-thalassaemia of our patients may be also different from different haplotypes seen in other countries. Few published study regarding vascular phenotypes of SCD (HbSS) and genotype-phenotype expression of HbS –β-thalassaemia are available from Odisha as well as from the whole country. Odisha is a state where there is higher percentage of HbF in SCD (HbSS) and high prevalence of both HbS and β-thalassaemia genes and thus HbS-β-thalassaemia. These haemoglobinopathies have a tremendous importance for physical and social health of our state. The aim of the study is to determine the haematological profile of patients suffering from sickle beta thalassemia

MATERIALS AND METHODS

Study Design: Cohort Study (Prospective Observational study) with asking research questionnaire developed for this purpose.

*Study Location***:** This study was undertaken in the Out Patient Department Clinical of Haematology S.C.B. Medical Collage Hospital, Cuttack from 2013 to 2016. Their family history, name, age, sex, caste, native place, pedigree chart and clinical sign symptoms were rerecorded after taking written consent.

About 3-4 ml intravenous (IV) blood samples were collected using EDTA as anti coagulant by disposable syringe from each patient. Clinical sign and symptoms related to haemoglobinopathy and laboratory investigations were done by fully automated blood cell counter and haemoglobin electrophoresis. The analysis of levels of haemoglobin variants i.e, HbA, HbF, HbS and HbA2 by electrophoresis and clinical data were diagnostic of Sickle beta thalassaemia. Sickling test was done by sodium matabisulphite solution as a reducing agent for the presence of sickle cell haemoglobin.

Inclusion Criteria: All patients who diagnosed or suspected to have a sickle cell haemoglobinopathies and confirmed by positive sickling test. *Exclusion criteria*: Healthy people who suspected to have sickle cell haemoglobinopathies with negative sickling test.

*Ethical issues:*This study confirms to the ethical principles of medical research developed by the World Medical Association Declaration of Helsinki. Ethical clearance was given by the Research Committee Department of Skin and Venereal Diseases S.C.B Medical College Cuttack.

Data Analysis: All data obtained with questionnaire and biochemical analysis were analyzed using the Graph Pad's web site. The chi square test was used to test distribution of categorical variables. Statistical significance was accepted when P value is ≤ 0.0001.The two-tailed P value is less than 0.0001 by conventional criteria and this difference is considered to be extremely statistically significant in all data given in the following table with the respective standard errors.

RESULTS

The physical and clinical examination of patients suffering from sickle beta thalassemia showed that, there were 45 cases of sickle cell beta Thalassemia having 37 male and 08 female patients' ranges from 4yrs to 34yrs. The median age was being 14yrs with Male: Female ratio was 4.6:1. There were 15 cases in the age group 0-10yrs , 11 cases in 11-20yrs , 15 cases in 21-30yr and 04 cases in 31-40 yrs. In our study 15(33.33%) cases were below 10 years, 11 cases (24.33%) in the age group of 11-20 years, 15 cases (33.33) between 21 to 30 years and 4 cases (8.8%) were seen in 31 to 40 years, the mean age was being 17.16± 8.7(P Value <.0001 and Standard Error 1.297) . The present study showed maximum 41cases (91%) below 30 years of age [**Table 1]**.

Table 1: Age and Sex Distribution of the study subjects

Age/sex	0-10yrs	11-20yrs	21-30yrs	31-40yrs	Total cases
Male	14(93.33%)	08(72.73%)	13(86.67%)	02(50%)	37
Female	01(6.67%)	03(27.27%)	02()13.33%	02(50%)	08
Total	15(100%)	11(100%)	15(100%)	04()100%	45

We observed 21 (46.66%) cases as sickle cell trait (SCT) positive and 24(53.33%) cases as beta thalassemia trait (BTT) positive and in 16 (35.55%) cases both father and mother are carrier of either sickle cell treat or beta thalassemia trait indicating that the percentage of beta thalassemia trait was greater than the percentage of HbS trait [**Table No 2].** Physical examination showed remitted-low grade fever, Pallor, bone pain and recurrent vasoocclusive crisis (R-VOC) was present in 15 out of 45 cases (33.33%).

At the time of diagnosis, Splenomegally was invariably present in 29 out of 45 cases (64.44) in moderation, i.e. 2-4 cms below the left costal margin. Only in two cases showed spleenic enlargement more than 12cm. Recurrent blood transfusion (BT) was required in 18 cases out of 45(53.33) and one case was presented with cholilithiasis. The mean height in centimeters was 134.44 cms(± 34.67) and weight in kilograms was 38.48 kg (±16.00) which indicates growth retardation **[Table No 3].**

Table 2: Sickle Cell Trait and Beta Thalassemia Trait among study subjects

Trait	Sickle cell trait(SCT)	Beta thal trait (BTT)	Total
Paternal	6 (28.56%)	7 (29.17%)	13 (28.8%)
Maternal	7 (33.33%)	9 (37.5%)	16 (35.6%)
Both parents	8 (38.1%)	8 (33.33%)	16 (35.6%)
Total	21(100%)	24 (100%)	45 (100%)

Table 3: Physical and general Examination of (n=45) study subjects

General features	Mean ± SD
Height in centimeters	134.44± 34.67
Weight in kilograms	38.48 ± 16.00
Order of birth	1.73 ± 1.29
Blood transfusion	9.6 ±11.94

The mean haemoglobin concentration of all the cases was 10.2 gm% SD ± 1.9, which showed moderate anaemia in our observation. All the 45 cases were sickling positive with mean haemoglobin percentage 10.2gm%(SD ±1.9) with minimum 4.6gm% to maxmum 13.6 gm% , the mean of total leucocytes count (TLC) and total platelet count (TPC) was 9.815 thousand (SD±1649.6) and 184.3 lakhs (SD± 20.76) respectively. The reticulocyte count was also found to be increased indicating haemolytic anaemia. We measured serum ferritin in 13 cases and the mean was being 297.94 ng/ml (SD±99.62). Our observation showed large range of variation in hemoglobin levels (4.6-13.6 gm %), but the majority had moderate anemia, the total leucocyte count on the higher side of the normal range and the total platelet count was found to be normal. There were increased level of reticulocyte with mean being 5.51% ±2.32. The mean corpuscular volume (MCVfl), MCH pg and MCHC% value were below the normal range **[Table No 4]**.

Table 4: Hematological findings of study subjects

Blood test	Min	Max	Mean± Standard Deviation	P. Value	S. Error
Hb(%)	4.6(%)	13.6(%)	10.2 ± 1.9	<0.0001	0.283
TLC(K)	6000(K)	12000(K)	9815 ± 1649.6	<0.0001	245.9
TPC(L)	160(L)	210(L)	184.3 ± 20.76	<0.0001	3.095
R.C(%)	1.9(%)	11.2(%)	5.51± 2.32	<0.0001	0.346
MCV(fl)	58.9(fl)	70(fl)	63.06± 2.63	<0.0001	0.392
MCH (pg)	14.8(pg)	23(pg)	19.4± 2.16	<0.0001	0.322
MCHC(%)	24.4(%)	32.1(%)	27.1± 1.73	<0.0001	0.258
S.F (ng)	116(ng)	434(ng)	297.9 ±99.62	<0.0001	27.630

*Hb%-haemoglobin percentage TLC- total leukocyte count in thousands(K) TPC-total platelet count in lakhs(L), RC- reticulocyte count, MCV- mean corpuscular volume femtolitre (fl) , MCH-mean corpuscular haemoglobin in picogram (pg), MCHC-mean corpuscular haemoglobin concentration in percent(%) ,SF –serum ferritin in nanogram(ng)

Analysis of electrophoresis showed that mean value of HbA was 3.69(SD± 2.94), HbF 21.63 (SD± 5.24), HbA2 4.12 (SD±1.00) and HbS 69.45 (SD±4.35). In our observation the fetal hemoglobin was raised to 21.63, ranging from 11% to 32.7% and Hb A2 was also raised with mean value 4.12 which is >3.5%, suggestive of sickle cell-β-thalassemia. We observed high percentage of HbS ranging from 45.9 to 82.3, the mean was being 69.45 [**Table No 5].**

Table 5: Haemoglobin Electrophoresis data of study **subjects**

Hb Variants	N	Min	Max	Mean± Standard Deviation	P Value	Std Error
HbA(%)	45	1.3 (%)	33.2(%)	3.69 ± 2.94	<0.0001	0.438
HbF(%)	45	11(%)	32.7(%)	21.63 ± 5.24	<0.0001	0.781
HbA2(%)	45	1.8(%)	5.8(%)	4.12 ±1.00	<0.0001	0.194
HbS(%)	45	45.9(%)	82.3(%)	69.45 ±4.35	<0.0001	0.648

Hb- haemoglobin, SD-standard deviation, HbA-adult haemoglobin 1, HbF-foetal haemoglobin, HbA2 – adult haemoglobin 2, HbS- sickle haemoglobin.

DISCUSSION

The high prevalence of sickle cell disease and beta thalassaemia in state of Odisha culminates the sickle beta thalassemia as a major health problem and has considerable morbidity and mortality. The combination of the sickle cell mutation and beta-thalassemia (β-Thal) mutation gives rise to a compound heterozygous condition known as Sickle cell beta thalassemia (Hb S/β-Thal) as described by Silverstroni E et.al [1]. There are more male as compared to females in the present study, which may be due to the fact that male child gets more attention and as compared to female child, the mean age is being 17.16± 8.7(P Value <.0001 and Standard Error 1.297), we observed maximum 41 cases (91%) has been seen below 30 years of age comparable to similar study done by Saurav Banerjee et.al [2].

Total hemoglobin (Hb) is low in patient more so in females as compared to males although this is not statistically significant. This may be due to hemolysis, blood loss due to hematuria, repeated infections, and nutritional deficiencies because of low socio-economic status similar to the study of Sanjeev Shyam Rao et.al [3]. There are two peak age incidences in this study in the first and third decade which might be due to lack awareness about the disease. The persistence splenomegaly is higher in the present study probably due to the raised HbF level found in Indians. The mean haemoglobin concentration of all the cases is 10.2 gm% SD ± 1.9, which shows moderate anaemia [3, 10].

In the present study beta thalassemia trait is greater than the percentage of HbS trait [7]. We observed the mean height in centimeters is134.44 cms(± 34.67) and weight in kilograms is 38.48 kg (±16.00) which indicates growth retardation compared with data from disabled world. The key contributing factors to stunted growth in patients with Sickle Beta Thalassemia include chronic anemia, transfusion-related iron overload, and chelation toxicity [7]. The majority of the sickle cell-β -thalassemia cases showed reduced values of red cell indices like MCV, MCH, MCHC and increased percentage of reticulocyte count suggestive of hypochromic and microcytic anaemia [8].

The percentage of HbS ranges from 45.9 to 82.3 and the mean is being 69.45. Patients having high levels HbA2 (>3.5%) as well as HbS (67%) are determinant for Sickle cell beta thalassemia [11]. We conclude that sickle cell β Thalassaemia is seen predominantly in male sex below 30 years with moderate anaemia , growth retardation and significant elevations of Hb F, Hb A2, Hb S and low HbA percentage.

CONCLUSION

A large number of Sickle cell beta thalassemia cases remain undiagnosed or misdiagnosed and mismanaged leading to premature death without proper treatment in the state of Orissa. Differentiation of sickle cell anaemia and the sickle beta thalassemia syndromes has to be done carefully due to close similarity of symptoms and laboratory findings. The Hemoglobin Electrophoresis pattern is the key to diagnosis of the sickle-beta thalassemia. Molecular diagnosis of Hb D, HbE or Hb S gene is required along with characterization of β-thalassemia mutations in this region. The prenatal diagnostic facilities and services, genetic/marriage counseling are the ultimate aims to be achieved in the state of Orissa.

Competing interest: The authors declare that they have no competing interests.

REFERENCES

1. Silverstroni E, Bicano,Granziani B, Carboni C et.al. Heterozygous beta thalassaemia with normal haemoglobin pattern. Haematologic, haemoglobin and biosynthesis study of 4 families. Acta Haematol 1978; 59(6):332-40.

2.Saurav Banerjee, Rabindra Kumar Singh, Ramesh Kumar Shrivastava, Sunil Kumar Mahto et.al. Study of Haemoglobinopathies in Patients of Anaemia Using High Performance Liquid Chromatography (HPLC). J. Evolution Med. Dent Sci. 2016;5(46):2929-2933

3. Sanjeev Shyam Rao, Jagdish Prasad Goyal and Vijay B. Shah et.al. Hematological profile of sickle cell disease from South Gujarat, India. Hematol Rep 2012; 4(2):e8.

4. Balgir R S. Division of Human Genetics, Regional Medical Research Centre (ICMR), Bhubaneswar, Orissa. Aberrant Heterosis In Hemoglobinopathies With Special Reference To Thalassemia And Structurally Abnormal Hemoglobins E and S In Orissa, India,Journal of Clinical and Diagnostic Research 2007; 3:122-130.

5. R. S. Balgir, Division of Human Genetics, Regional Medical Research Centre (ICMR), Chandrasekharpur, Nandan Kanan Road, Bhubaneswar 751 023, India. The burden of haemoglobinopathies in India and the challenges ahead .Current Science 2000; 79:1537-1547.

6.R. S. Balgir, R. K. Mishra and B.Murmu. Clinical and Hematological Profile of Hemoglobinopathies in Two Tribal Communities of Sundargarh District in Orissa, India. Int J Hum Genet 2003; 3(4): 209-216.

7. A. Eman Ajjack.Hiba A. Awooda,Sana Eltahir Abdalla et.al. Haemoglobin Patterns in Patients with Sickle Cell Haemoglobinopathies, International Journal of Hematological Disorders 2014; 1(1) :8-11.

8. Fabia Neves, Osvaldo Alves Menezes and Ivan Lucena Anglo.et.al. Hematological differences between patients with different subtypes of sickle cell disease on hydroxyurea treatment, Hematol Hemoter. 2012; 34(6):426-429.

9. Patel, Shailesh M et.al. Electrophoresis Pattern In Clinically And Hematologically Suspected Cases Of Haemoglobinopathies. NJIRM 2012; 3(3) :24-27.

10.Maria Stella Figueiredo et.al. The compound state: Hb S/beta-thalassemia, Brazilian Journal of Hematology and Hemotherapy 2015; 37(3): 150–152.

11. Weatherall D.J Clegg J.B et.al. The thalassaemia syndromes. Am J Hum Genet, 2004; 74(3): 385–392.

*Corresponding author: Dharma Niranjan Mishra
E-Mail: dharmaniranjan.mishra08@gmail.com