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Effects of Hydroxyurea Treatment on Haematological Parameters and Neurological Functions in Patients of Sickle Beta Thalassemia

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Abstract
Background: *Sickle beta thalassemia (HbS-β-Thal) is a disorder which represents the double heterozygous state for the Sickle cell anaemia and the beta - thalassemia genes. It constitutes one of the major genetic haematological disorders in Odisha. The aim of the study is to determine the haematological profile before and after hydroxyurea treatment and its effects on neurological function.*
Methods: *Blood samples are collected from 45 diagnosed cases of sickle beta thalassemia after taking informed consent as well as due ethical Committee approval. Screening is done by Sickling test and Haemoglobin variants are analysed by fully automated capillary zone electrophoresis. Hydroxyurea is given in appropriate doses and its effect on haematological parameters and neurological functions are studied.*
Results: *In our observation the fetal hemoglobin is raised to 30.63% ranges from 27% to 36.7%. Adult haemoglobin (HbA> 3.5%) and Sickle cell haemoglobin (HbS > 67%) was taken as determinant for Sickle cell beta thalassemia. We observed high percentage of HbS ranging from 45.9% to 82.3% and the mean is being 69.45%. There are overall increases in HbF by 6% with reductions in the frequency of blood transfusion and no neurological deficit observed after hydroxyurea treatment.*
Conclusion: *In sickle beta thalassemia, there are overall increases in HbF with reductions in the frequency of blood transfusion after hydroxyurea treatment. Moreover molecular diagnosis is required for β-Globin Gene mutations.*
Abbreviations: *HbS-β-Thal -Sickle beta thalassemia, Hb A- Adult haemoglobin, HbS- sickle cell haemoglobin, HbF-foetal haemoglobin.*
Keywords: *β-Globin Gene, Sickle beta thalassemia, foetal haemoglobin.*

Introduction

Sickle cell beta thalassemia (Hb S/β-Thal) is a compound heterozygous state of sickle cell mutation and beta-thalassemia (β-Thal) gene mutation coined by Silvestroni and Bianco1944 [(1).] Sickle β thalassemia is a major hemoglobin disorder found in Odisha as well as India. [(2)] The sickle cell-β-thalassemia diagnosis is based on the findings of HbA, HbF, HbS and Hb A2 on electrophoresis under alkaline media. Elevated Hb

A levels (>3.5%) and the inverse relationship between Hb A and Hb F are pathognomonic of Indian sickle cell patients [3] 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 Sickle cell β++ thalassemia with high Hb A (20-30%).[4] In HbS/β-thalassaemia, the β-thalassaemia gene interacts with the HbS-gene to increase the level of HbF (>15%) and high HbS(>67%). [5] Sickle cell beta thalassemia(Hb S/β^0-Thal) is often clinically indistinguishable from sickle cell anemia in which the production of Hb A is abolished. [6,7] The beta thalassemia gene is acting on sickled red blood cells to induce microcytosis, hypochromia, and increased level Hb F.[9] Increased Foetal haemoglobin (HbF) causes an improvement of the circulatory competence of these cells, reduction of hemolysis, and a small increase in haemoglobin as well as packed cell volume. It is observed that the higher level of HbF in this double heterozygous condition may be useful by decreasing HbS polymerization and prevents crisis.[10] The net phenotypic expression of the interaction of two genes is remarkably variable: completely asymptomatic condition at one end while at the other end of the spectrum, the severity can be that of SCD or β+ Thalassaemia.[11, 17] The haematological parameters include sickle red cell, 60-90% of HbS, 0-30% of HbA, 1-20% of Hb.[12]The type of β-thalassaemia gene which is co-inherited with HbS gene may explain such variations which need to be corroborated by further study. The high incidence of iron deficiency and α-thalassaemia gene in our state may alter the haematological parameters significantly which is useful as differential diagnosis and treatment. Treatment using HU in S/Beta thalassemia is well documented. Patients are showing clinical improvement and increase in hematological values after six months of Hydroxyurea (HU) treatment.[13,14] The most commonly reported neurological complications are overt stroke, silent infarction, leukoencepha-lopathy and cerebral atrophy can happen in the course of the disease. [19] Orissa is a state where there is higher percentage of Hb F in Sickle Cell Disease (HbSS). There is also high prevalence of both HbS and β-thalassaemia genes, which culminates Sickle Beta Thalassemia (HbS-β-thalassaemia). These haemoglobinopathies have a tremendous importance for physical and social health of the state. This study reinforces the importance of molecular studies in the observed population to enhance further knowledge about the disease, which will improve genetic counseling, follow up, and treatment.

Material & Method

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 Haematology S.C.B. Medical Collage Hospital, Cuttack.

This study is based on 45 cases of sickle beta thalassemia selected from the Out Patient Department (OPD) cases in the clinical haematology, S.C.B. Medical Collage Hospital, Cuttack from 2014 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 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 automated blood cell counter and haemoglobin electropho-resis. The analysis of levels of haemoglobin variants i.e, HbA, HbF, HbS and HbA2 analysed by fully automated capillary zone electrophoresis. Sickling test was done by sodium matabisulphite solution as a reducing agent for the presence of sickle cell haemoglobin.

Adult Hb> 3.5% was taken as the important parameter for beta thalassemia trait. Patients having high levels HbA (>3.5%) as well as HbF

(67%) are determinant for Sickle cell beta thalassemia (Weatherall). The diagnosis was based on clinical examination family history and findings of HbA, HbF, HbS and HbA2.

Inclusion Criteria: All patients who diagnosed or suspected to have a sickle cell beta thalassemia and confirmed by haemoglobin electrophoresis data and positive sickling test.

Exclusion criteria: Healthy people who suspected to have sickle cell beta thalassemia 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. [18]. 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 analysed using the Graph Pad's web site. 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.

Observation

There were 45 cases of sickle cell beta Thalassemia having 37 male and 08 female patients, the Male: Female ratio was being 4.6:1.There were 15 cases in the age group 0-10yrs, 11 cases in 11-20yrs, 15 cases in 21-30yrs and 04 cases in 31-40 yrs (Table 1).

Table 1 Age: Sex Distribution

Age/sex	0-10yrs	11-20yrs	21-30yrs	31-40yrs	Total
Male	14	08	13	02	37
Female	01	03	02	02	08
Total	15	11	15	04	45
Percentage	33.33%	24.44%	33.33%	8.8%	

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 shows that maximum 41cases (91%) has been seen below 30 years of age Table 1. Male patients were observed to be more than female. There were two peak incidences in the age group of 0-10 years and 21-30 years.

Table No 2. Percentage of Haemoglobin

Hb%	No of cases	Percentage %	Mean		P Value
0-5 gm%	3 cases	6.56%			
6-10gm%	28 cases	62.33%			
>10 gm%	14 cases	31.11%			
Total	45 cases	100%	10.2gm%	±1.9	<0.0001

There were three cases having haemoglobin percentage less than 5 gm%(6.6%), 28 patients between 6-10gm% (62.33%) and 14 cases (31.11%) more than 10 gm%. (Table No 2).The mean haemoglobin concentration of all the cases was 10.2 gm% SD ± 1.9, which showed moderate anaemia in our observation. Table No 2.

Table No 3. Sickle Cell Trait and Beta Thalassemia Trait

Trait	Sickle cell trait(SCT)	Beta thal trait (BTT)	Total
Paternal	6 cases +SCT	7cases +BTT	13 cases
Maternal	7 cases +SCT	9 cases +BTT	16 cases
Both parents	8 cases + SCT	8 cases+ BTT	16 cases
Total	21	24	45 cases
Percentage	46.66%	53.34%	

We observed 21 (46.66%) cases as sickle cell trait(SCT) positive and 24(53.33%) cases as beta thalassemia trait (BTT) positive and 16cases (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 3

Table No 4. Physical and general Examination

General features	Number (n)	Mean	Standard deviation(SD)	P Value	S Error
Height cms	45	134.44	± 34.67	<0.0001	5.168
Weight	45	38.48	± 16.00	<0.0001	2.385
Order of birth	45	1.73	± 1.29	<0.0001	0.192
BT Requird	18	9.6	±11.94		

Out of 45 cases age between 4 to 34yrs the median age being 14yrs.Table No 4 There were 37 male & 8 female patients, physical examination shows remitted-low grade fever, Pallor & bone pain R-VOC is present in 15 out of 45 cases (33.33%), At the time of diagnosis Splenomegally was invariable present 29 out of 45 cases (64.44) in moderation i.e. 2-4 cms in an average only in two cases >12cm was being observed. Recurrent B/T is required in 18 cases out of 45(53.33).and one case is presented with cholilithiasis. The mean height in centimeters was 134.44 cms(± 34.67) and weight in kilograms is 38.48 kg (±16.00).

Table No 5.Hematological finding

Blood test	Min	max	Number(n)	Mean	Standard deviation (SD)	P Value	S Error
Hb % gm%	4.6	13.6	45	10.2 gm%	(SD ±1.9)	<0.0001	0.283
TLC K	6000	12000	45	9815 K	(SD±1649.6)	<0.0001	245.9
TPC L	160	210	45	184.3 L	(SD± 20.76)	<0.0001	3.095
Rericulocyte count %	1.9	11.2	45	5.51	(SD± 2.32)	<0.0001	0.346
MCVfl	58.9	70	45	63.06	(SD± 2.63)	<0.0001	0.392
MCH pg	14.8	23	45	19.35	(SD± 2.16)	<0.0001	0.322
MCHC	24.4	32.1	45	27.14	(SD± 1.73)	<0.0001	0.258
S. ferritin/ ng	116	434	13	297.94	SD±99.62	<0.0001	27.630

We were observed in Table No 5 that all the 45 cases were sickling positive with mean haemoglobin percentage is 10.2(SD ±1.9) with minimum 4.6gm% to maximum 13.6 gm% , the mean of TLC and TPC was 9815 (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, 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 MCHCg/dl value were below the normal range.

Table No 6.Electrophoresis data

Hb Variants	Number (n)	min	Max	Mean	Standard deviation(SD)	P Value	S Error
HbA	45	1.3	33.2	3.69	(SD± 2.94)	<0.0001	0.438
HbF	45	11	32.7	21.63	(SD± 5.24)	<0.0001	0.781
HbA2	45	1.8	5.8	4.12	(SD±1.00)	<0.0001	0.194
HbS	45	45.9	82.3	69.45	(SD±4.35)	<0.0001	0.648

Analysis of electrophoresis in Table No 6 showed the 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 30.63% ranges from 27% to 36.7% 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.Patients having high levels HbA (>3.5%) as well as HbS (67%) are determinant for Sickle cell beta thalassemia (Weatherall D.J Clegg J.B)

Table No. 7. After Hydroxyurea treatment M- month

Observation	Before Hydoxyurea Treatment	After Hydoxyurea Treatment
HbF%	30.63 ± 5.24	33.99±6.81
Blood Transfusion Requird	9.6 ± 11.94 (1-2 transfusion/M)	2.58 ± 2.09 (1 transfusion/2-3M)
Recurrent Vaso-occusive Crisis	15 cases	3 cases
Avascular Necrosis	1	0

In Table No 7 Hydroxyurea was administered orally at doses between 10 and 20 mg/kg per day [13]. There were overall increases in HbF in most of the cases with reductions in the frequency of VOC & AVN [14]. There were marked reduction in requirement of blood transfusion from 9.6 ± 11.94 units to 2.58 ± 2.09 units. There was increase in HbF from 30.63 ± 5.24 to 33.99±6.81 and reduction of vasoocclusive crises from 15 to 3 before and after treatment. The overall incidence of avascular necrosis was reduced to normal in the present study.

Table No. 8. Neurological assessment (NAD: No Abnormality Detected)

Observation	Before Hydoxyurea Treatment	After Hydoxyurea Treatment
Somato-sensory Headache and Bone pain +	39 (86.66%)	09 (20%)
Sensory dysfunction	NAD	NAD
Motor dysfunction	1	NAD
Higher function	Normal	Normal
Cranial nerves	Normal	Normal

Assessment of neurological function in (Table No 8) revealed headache and bone pain, which was somatosensory in most of the cases 39 (86.66%) during diagnosis but there was marked reduction in 09 (20%) after hydroxyurea treatment. We observed one case having avascular necrosis of femoral head with motor dysfunction of lower limb. Other parameters like higher neurological function and cranial nerves were found to be normal.

Discussion

In the present study male patients are more than females, which may be due to the fact that male child gets more attention as compared to female child.(2) Total hemoglobin (Hb %) is low in female as compared to male, which is statistically insignificant. This may be due to hemolysis, repeated infections and nutritional deficiencies because of low socio-economic status (2). In our study the mean age is being 17.16± 8.7(P Value <.0001 and Standard Error 1.297). Most of cases

(91%) are seen below 30 years of age (Table 1). [2] There are two peak age incidences in the first and third decade (Table no 1) which might be due to lack awareness about the disease or patients suffering from sickle cell β++ thalassemia having mild symptoms reporting late. The persistence splenomegaly was higher in the present study probably due to the raised HbF level found in Indians.[3,4,5] The mean haemoglobin concentration of all the cases is 10.2 gm% SD ± 1.9 (Table no 5), which shows moderate anaemia. [11] In the present study beta thalassemia trait is greater than the percentage of HbS trait, Which is similar to the study done in USA Eman A et.al 2014. [7] The general incidence of thalassemia trait and sickle cell hemoglobinopathy in India varies between 3 -17% and 1-44% respectively. [15] We observed the mean height in centimeters is134.44 cms(± 34.67) and weight in kilograms is 38.48 kg (±16.00) (Table No 4) 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 by Vincenzo De Sanctis et.al 2013. [8] The majority of the sickle cell-β -thalassemia cases (Table No 5) showed reduced values of red cell indices like MCV, MCH, MCHC and increased percentage of reticulocyte count suggestive of hypochromic and microcytic anaemia, which is consistent with the studies carried out by Fabia Nerves et.al 2012. [9] There are high percentage of HbS ranging from 45.9 to 82, the mean is being 69.45. (Table No 6) Patients having high levels HbA (>3.5%) as well as HbS (67%) are determinant for Sickle cell beta thalassemia.[3,4,9,12] The study reflects that patients with sickle cell β Thalassaemia have shown significant elevations of (Hb F, Hb A and Hb S) with p value <0.0001. [7] Hydoxyurea is given in the recommended dose orally for two years.[13] There is overall increase in HbF (Table 7) in most of the cases with reductions in the frequency of VOC & AVN.[13,14] There are marked reduction in requirement of blood transfusion from 9.6 ± 11.94 units to 2.58 ± 2.09 units (from 1-2 transfusion per month to 1 transfusion per 2-3 months) as Hydroxyurea increases the red cells containing an increased amount of fetal hemoglobin, which inhibits HbS polymerization, and decrease of leukocytes, platelets, and reticulocytes, which significantly limits their adherence to the vascular wall.[13,14]

Assessment of neurological function in (Table No 8) revealed headache and bone in most of the cases during diagnosis but there is marked reduction after hydroxyurea treatment. Most of the cases reported with headache and bone pain, which is somatosensory in nature. There is absence peripheral neuropathy and central nervous system complication except one case of femoral head necrosis with motor deficit in right lower limb. [19]

Conclusion

Differentiation of sickle cell anaemia and the sickle beta thalassemia syndromes should be done carefully due to close similarity of symptoms and laboratory findings i.e. microcytosis, hypochromia, target cells and sickle cells in the peripheral smear. The Hemoglobin Electrophoresis pattern of the sickle-beta thalassemia consists of high HbS with an increase in HbF, HbA2 and low HbA value. The present study highlights the co-inheritance of β-thalassemia and Hb S gene, which is wide spread in Southern and Western Orissa. Further we assume that a large number of such double heterozygote cases remain undiagnosed or misdiagnosed leading to premature death without proper treatment. Molecular diagnosis of Hb D, HbE or Hb S gene is required along with characterization of β-globin gene mutations in this region. The prenatal diagnostic facilities and services, genetic/marriage counseling are the ultimate aims to be achieved. This is a preliminary study and we will carry out the Beta Globin gene mutation to establish the above facts in more detail.

Conflict of Interest: None

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