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## International Journal of Biological & Medical Research

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### Original Article

# Disorders Of Haemoglobin Variants In Paediatric Patients Attending In A Tertiary Care Hospital Of North East India

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### ARTICLE INFO

#### Keywords:

Anaemia

Haemoglobinopathies

HPLC

Thalassaemia

### ABSTRACT

Haemoglobin Variants are genetic haematological abnormalities affecting millions of people all over the world. Hb E, Hb S and  $\beta$ -thalassaemia are the most prevalent Hb variants in North-Eastern region of India. Identification of these disorders is immensely important to prevent the occurrence of serious haemoglobin disorders. The objective of the study was to diagnose and find the occurrence of Hb variants and the commonly associated signs and symptoms among suspected anaemic paediatric patients. In this study various abnormal haemoglobin fractions on HPLC were observed in 522 cases (65.25%) out of the total 800 cases displayed. Among the 522 cases with Hb Variants, 268 (51.34%) were males and 254 (48.66%) were females. Hb E heterozygous is the most common form of Hb Variants (23.5%), followed by  $\beta$  thalassaemia trait (18.12%), Compound Hb E -  $\beta$  thalassaemia trait (9%), Hb E homozygous (6.5%), Hb S trait (3.25%),  $\beta$  thalassaemia major (2.12%), Hb S disease (2%),  $\alpha$ -thalassaemia (0.63%) and Compound Hb S-  $\beta$  thalassaemia (0.13%) respectively. Anaemia is the most common sign observed in all variant types. Other associated clinical manifestations are weakness, aches and pain, splenomegally, hepatosplenomegally, stomach pain, joint pain and fever.

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### INTRODUCTION:

Inherited abnormalities of haemoglobin synthesis include a myriad of disorders ranging from thalassaemia syndromes to structurally abnormal haemoglobin variants [1]. Variant haemoglobins (Hb) are genetically important haematological disorders affecting millions of people worldwide. The cumulative gene frequency of haemoglobinopathies in India is 4.2% [2, 3]. It has been estimated that around 50 million people in South East Asia alone carry the gene for Haemoglobin E (Hb E) [4, 5]. Hb E is mostly prevalent in North- East India, Bangladesh, Indonesia, Malaysia, Myanmar, Singapore and Thailand [4]. The prevalence of haemoglobinopathies varies with the geographic locations and ethnic groups. Among the common Hb variants, Hb E and  $\beta$ -thalassaemia are commonly found in the North-Eastern states of India i.e., Assam, Arunachal Pradesh, Nagaland, Manipur, Tripura and Meghalaya, and the average allele frequency of Hb E in North-

East region is 10.9% [3, 6]. Hb E occurs both in heterozygous (AE) and homozygous (EE) states and may co-inherit with alpha and beta thalassaemia [7] and sometimes also with Hb S.

People who have Compound Hb E-  $\beta$  thalassaemia inherit one gene for Hb E from one parent and one gene for  $\beta$  thalassaemia from the other parent. Compound Hb E-  $\beta$  thalassaemia is a severe disease and show severe clinical manifestation. Compound Hb S -  $\beta$  thalassaemia is also a clinically serious condition. Inherited disorders of haemoglobin variants are a serious public health problem. They place a large burden to the patients, their families and even their communities. These genetic diseases are not curable but can be prevented by proper genetic counselling, by screening population and couples at risk and by prenatal diagnosis. This study was undertaken to evaluate the haemoglobin variants among the suspected anaemic paediatric patients along with the commonly associated health problems in haemoglobin variant disorders.

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## MATERIALS AND METHODS:

This study has been cleared by the Institutional Ethics Committee of Gauhati Medical College & Hospital, Guwahati, Assam, India. This is a hospital based cross-sectional study in which a total of 800 anaemic paediatric patients along with their parents' blood samples were analysed for Hb variants who were referred from Department of Paediatrics of Gauhati Medical College and Hospital within a period of 25 months from June 2011 till July 2013 to our Laboratory for Hb typing. The patients came mainly with clinical manifestations like anaemia, splenomegally, hepatosplenomegally, weakness, fever, aches and pain etc. The other common causes of anaemia like worm infestation and malnutrition were not taken into account in this study. Detailed clinical history including ethnic origin, age, sex, blood transfusion etc. along with family history was recorded. The geographical distribution of all cases predominantly included parts of North-east India (Assam, Meghalaya, and Arunachal Pradesh). Figure I show the Map of India highlighting the states of North East India.

About 2ml intravenous blood samples were collected in EDTA (Ethylene Diamine Tetra Acetic acid) coated vacutainers from each individual free of blood transfusions after obtaining written informed consent. The haematological analysis was performed on pocH-100i (Sysmex) to obtain the Red cell indices (RBC, Hb%, HCT, MCV and MCHC). The method for counting the blood cells is based on the electric resistance detection principle and the haemoglobin concentration is determined by a photometric measuring method. Then the Hb Variants analysis were carried out on BIO RAD D10 Dual Program (Extended Program) which is based on chromatographic separation of the analytes by ion-exchange high performance liquid chromatography (HPLC). The samples are automatically diluted on the D10 and injected into the analytical cartridge where a programmed buffer gradient of increasing ionic strength is delivered and the haemoglobins are separated based on their ionic interactions with the cartridge material. The separated haemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415nm are measured and chromatographic data of each patient are obtained. The statistical analysis of all data was carried out using Microsoft Office Excel.

## RESULTS AND DISCUSSION:

Results: Abnormal haemoglobin fractions on HPLC were seen in 522 cases (65.25%) out of the 800 cases displayed. Among 522 cases with Hb Variants, 268 (51.34%) were males and 254 (48.66%) were females. Commonly associated clinical signs and symptoms helping in the clinical diagnosis are shown in the Table I. Anaemia is the most common sign observed in all variant types. Other observed sign and symptoms are weakness, aches and pain, splenomegally, hepatosplenomegally, stomach pain, joint pain and fever. Table II represents haematological findings with mean values  $\pm$  SD associated with different Haemoglobinopathies and thalassaemias. Figure II shows the occurrence of different Haemoglobin Variants. Hb E heterozygous is the most common form of Hb Variants (23.5%), followed by  $\beta$  thalassaemia trait (18.12%), Compound Hb

E -  $\beta$  thalassaemia trait (9%), Hb E homozygous (6.5%), Hb S trait (3.25%),  $\beta$  thalassaemia major (2.13%), Hb S disease (2%),  $\alpha$  - thalassaemia (0.63%) and Compound Hb S -  $\beta$  thalassaemia (0.12%). Figure III shows chromatograms of patients with different Hb Variants.

**Discussion:** Thalassaemias and haemoglobinopathies are inherited blood disorders, primarily affecting the globin moiety of the haemoglobin molecule. These disorders, which were mainly confined to certain areas, religions, castes and tribes particularly with endogamous norms of marriages, are now widely prevalent all over the world. This is because of the ever increasing migration of people from one place to another and the mixing of different communities through marriages. Hb E was mainly prevalent in the North eastern states of India with only few case reports from other parts of the country including Uttar Pradesh and Orissa [3, 5, 8]. But now the frequency of Hb E and thalassaemia is increasing in Uttar Pradesh and its neighbouring areas [3, 9, 10]. In our study 3 forms of Hb E variants namely Hb E heterozygous or Hb E trait, Hb E homozygous or Hb E disease and Compound Hb E-  $\beta$  thalassaemia were observed.

In our study it is observed that 39.8% of the Hb E trait patients are anaemic. Other presenting clinical findings of Hb E trait patients are weakness, splenomegally, hepatosplenomegally, stomach pain, joint pain and fever. Malnutrition and worm infestation may be the associated factor of anaemia, as our hospital is a government one and mostly patients from low socio-economic strata attend this hospital. Splenomegally and hepatosplenomegally may be a manifestation of chronic malaria as these patients hail from malaria endemic area. Haematological investigations of these individuals reveal mild microcytosis, hypochromia and erythrocytosis as seen with the  $\beta$  - thalassaemia trait. However, identification of these individuals with Hb E trait is of crucial importance as they may be transmitters of the abnormal gene, giving rise to various combinations of haemoglobinopathies and thalassaemias in their progeny. Most patients with Hb E disease show clinical symptoms by the age of 10 years. In this study the most common presentation of Hb E disease is anaemia, weakness, fever, abdominal pain, splenomegally, hepatosplenomegally. Compound heterozygous for Hb E-  $\beta$  thalassaemia present with anaemia, weakness, splenomegally, hepatosplenomegally, stomach pain and joint pain. Although most of the clinical sign and symptoms are the same in all Hb variant patients, the percentage of occurrence of different features varies in different variant types (Table I).

Pathophysiology is complex for compound heterozygous states. Hb E trait may be co-inherited with either  $\beta^0$  - thalassaemia or  $\beta^+$  - thalassaemia [11, 12]. The compound heterozygous state for Hb E-  $\beta$  is common in Thailand and occurs in Southeast Asia stretching from Indonesia to Sri Lanka, Northeast India and Bangladesh [11, 13, 14, 15]. The  $\beta$  - thalassaemia major and the Compound Hb E-  $\beta$  thalassaemia patients are anaemic and transfusion dependent.

Common Sign& Symptoms	ET <sup>1*</sup>	ED <sup>2*</sup>	Haemoglobin variants							
			βT3*	βM <sup>4*</sup>	Eβ <sup>5*</sup>	ST <sup>6*</sup>	SD <sup>7*</sup>	αT <sup>8*</sup>	Sβ <sup>9*</sup>	
Anaemia	39.8	84.37	46.6	100	97.22	35.7	100	80	100	
Weakness	50	87.5	62.5	100	91.66	71.43	100	80	100	
Aches &Pain	36.11	65.62	40.62	30	61.11	42.85	62.5	-	-	
Splenomegally		5.55	12.5	2.08	100	63.89	-	50	20	-
Hepatosple- -nomegally	3.7	6.25	0	0	11.11	-	12.5	-	-	
Massive sple- -nomegally	0	3.12	-	-	-	-	-	-	-	
Stomach pain	3.7	9.37	1.04	10	11.11	-	-	-	100	
Joint pain	2.77	3.12	2.08	10	11.11	7.14	37.5	20	-	
Fever	4.63	6.25	4.16	20	27.77	-	-	-	-	
<b>Abbreviations:</b> ET <sup>1*</sup> - Hb E trait, ED <sup>2*</sup> -Hb E disease, βT3*-Beta thalassaemia trait, βM <sup>4*</sup> -Beta thalassaemia major, Eβ <sup>5*</sup> -Compound heterozygous for Hb E & Beta Thalassemia, ST <sup>6*</sup> -Sickle cell trait, SD <sup>7*</sup> - Sickle cell disease, αT <sup>8*</sup> - Alpha thalassaemia, Sβ <sup>9*</sup> -Compound heterozygous for Hb S and beta thalassemia										

**Table II: Haematological features of different types of haemoglobinopathies and thalassaemias in the study subjects**

Variable	$\beta M^1$	$\beta T^2$	$E T^3$	$E D^4$	$S T^5$	$S D^6$	$E\beta^7$	$\alpha T^8$	$S\beta^9$	No Hb/Thal
N	17	145	188	52	26	16	72	5	1	278
HGB(g/dl)	4.2± 1.8	10.8± 1.92	10.8± 3.11	9.11± 2.11	11.78± 2.19	6.18± 1.88	4.86± 2.19	6.16± 4.5	3.4	10.49± 3.6
RBC( $10^6/\mu l$ )	1.88± 0.86	5.16± 0.93	4.35± 1.09	4.62± 0.86	4.46± 0.74	2.28± 0.75	2.62± 1.07	3.0± 1.73	1.17	3.96± 1.56
HCT(%)	12.76± 5.74	34.12± 5.58	32.4± 8.91	27.59± 5.91	35.06± 5.53	19.25± 5.11	16.27± 6.63	19.76± 14.7	10.7	31.51± 9.9
MCV(fl)	67.98± 5.5	66.63± 7.47	74.86± 9.05	59.4± 6.22	78.41± 9.27	85.6± 9.39	63.72± 8.27	70.28± 14.2	91.5	80.77± 11.82
MCH(pg)	23.15± 4.52	21.36± 2.78	26.11± 17.12	19.55± 2.5	26.39± 3.97	27.21± 3.68	18.6± 2.96	19.76± 6.62	29.1	27.19± 6.46
MCHC(g/dl)	34.03± 5.79	32.17± 1.61	33.18± 1.98	32.85± 1.68	33.37± 1.9	31.83± 2.41	29.22± 3.09	27.94± 3.81	31.8	33.92± 18.62
HB Ao (%)	5.64± 3.27	80.98± 9.49	59.21± 8.97	7.17± 7.7	55.52± 11.53	2.5± 0.43	6.85± 4.67	85.44± 3.08	5.9	84.01± 7.91
HB A2(%)	3.62± 2.68	6.72± 6.6	35.42± 9.82	100± 15.67	3.09± 0.60	2.0± 0.63	65.64± 14.21	1.56± 0.37	4.7	2.98± 1.17
HB F(%)	71.05± 6.97	1.18± 0.88	1.44± 1.4	5.33± 3.67	1.31± 0.65	14.14± 7.13	28.66± 10.56	0.92± 0.2	30.5	1.24± 2.22
HB S (%)					31.43± 4.16	75.52± 7.8			49.2	

**Note:**

□ **Data are Mean ±SD**

□ HB Ao: Adult Haemoglobin, HB A2: small percentage of Adult Haemoglobin, HB F: Foetal haemoglobin, HB S: Sick cell

□ HGB: Haemoglobin, RBC: Red Blood Corpuscles, HCT: Haematocrite, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentration.

□  $\beta M^1$ -Beta thalassaemia major,  $\beta T^2$ -Beta thalassaemia trait,  $E T^3$  - Hb E trait,  $E D^4$ -Hb E disease,  $S T^5$  -Sickle cell trait,  $S D^6$ - Sickle cell disease,  $E\beta^7$ -Compound heterozygous for Hb E & Beta Thalassemia,  $\alpha T^8$ - Alpha thalassaemia,  $S\beta^9$  -Compound heterozygous for Hb S and beta thalassemia, No Hb/ Thal - No Haemoglobinopathies or thalassaemia.

□ In  $S\beta^9$ : As there is only one sample so no Mean± SD is calculated.

□ HB Ao+ HB A2+ HB F+ HB S does not sum to 100%, as other fractions like A1a, A1b and Unknowns are not shown in the table.

Figure I: Map of India highlighting the states of North East India.

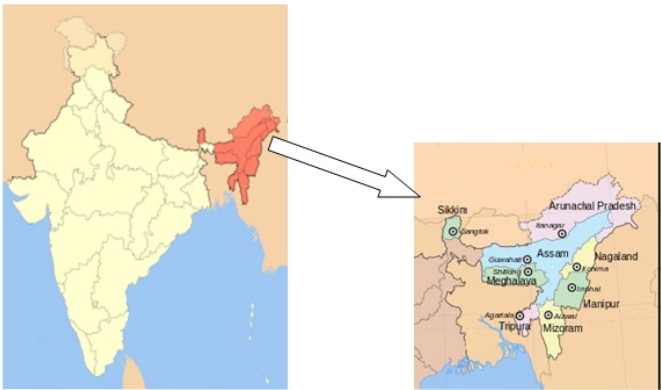


Figure II: Pie diagram illustrating the percentage of occurrence of different Hb variants

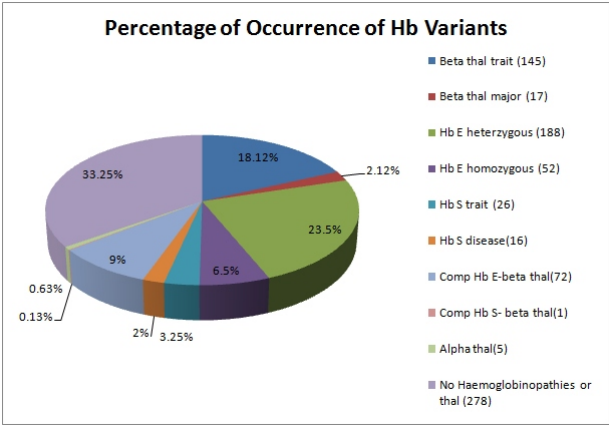
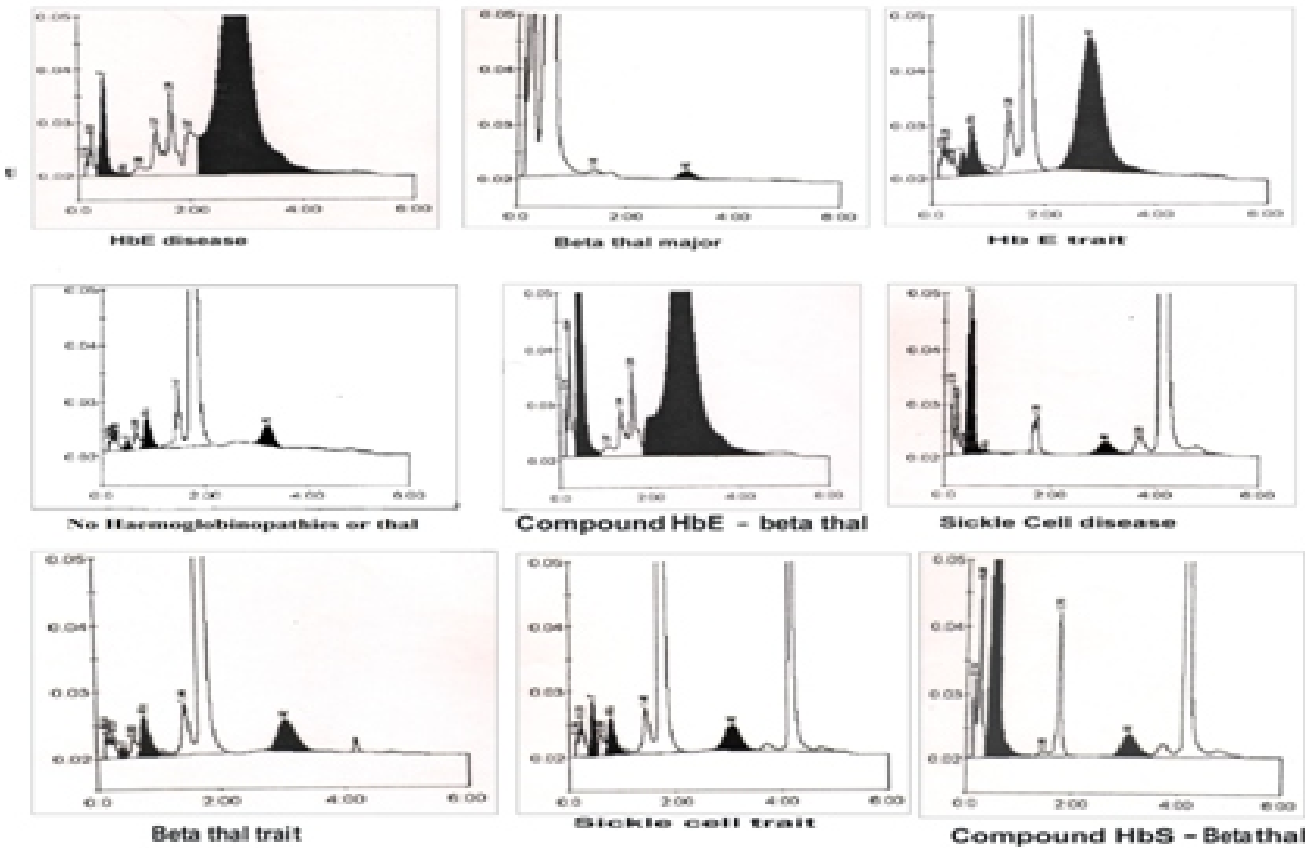


Figure III: Chromatograms of patient with different Hb variants



## CONCLUSION:

Genetic haemoglobin disorders with severe anaemia cause considerable pain and suffering to the patients and their families and are major drain on health resources in India. Frequencies found in the present study confirm that haemoglobinopathies and thalassaemia are public health problem in North East region of India, emphasizing the need for neonatal screening and genetic counselling programs. The HPLC based Haemoglobin Testing system forms a rapid and easy tool in early detection and management of various haemoglobin disorders. Nationwide Government sponsored programme can effectively reduce the occurrence of new cases of serious haemoglobin variants as well as thalassaemia major cases and thus making it possible to direct the available resources towards the optimization of treatment of the patients who are already present. Detection of these patients with abnormal haemoglobins will help in prevention of more serious Hb variant cases.

## ACKNOWLEDGEMENT:

The authors are thankful to Department of Biotechnology (DBT), Ministry of Science & Technology, Govt. of India for the financial support and are also grateful to Dr. Bindu Dey, Adviser and Dr. Pawan Sharma, Senior Consultant of DBT; DBT Nodal Centre, Tezpur University, Assam, India for their support.

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