

Full length Research Paper

# Mutational Spectrum of $\beta$ -Thalassaemia of Northern part of Odisha, India

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Accepted 15 April, 2016

Prevention of  $\beta$  thalassaemia requires knowledge for diagnosing the molecular analysis in the population at risk. This knowledge is particularly necessary when prevention control is applied to a multiethnic population. For this purpose, we are analyzing different populations from northern part of Odisha, India. During the study, we encountered about 98 patients from District Head Quarter Hospital of Mayurbhanj and Balasore district of Odisha. Molecular analysis of  $\beta$  gene mutation were showing that population showing IVS 1-5(G-> C), cd 41/42(-CTTT), cd 8/9(+G), IVS 1-1(G->T), cd 15(G->A), cd 30(G->C) as well as 619 bp deletion. In most cases, we found the IVS 1-5(G-> C) mutation and cd 41/42(-CTTT) mutation. The novel 619 bp deletion is the first report being analyzed in northern part of Odisha. The patient's age group more prevalence in between 0 - 15 years and their hematological parameters were recorded.

**Keywords:**  $\beta$  thalassaemia,  $\beta$  gene mutation, IVS 1-5(G-> C), cd 41/42(-CTTT), cd 15(G->A) mutation.

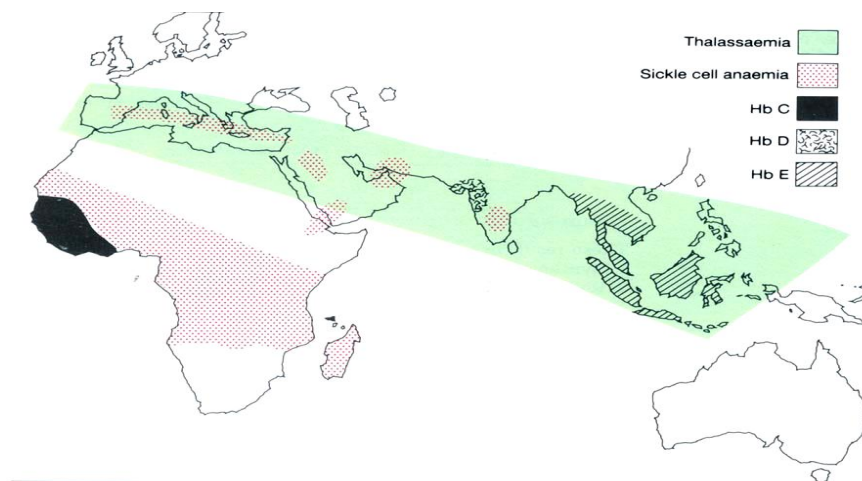
## INTRODUCTION

The term 'Haemoglobinopathies' is given to the inherited disorders of structure and synthesis of globin part of haemoglobin molecule and falls into several overlapping groups. It has been estimated that approximately 7% of the world population are carriers of such disorders and that 3, 00,000–4, 00, 000 babies with severe forms of these diseases are born each year. In developed countries it has been estimated that genetic disease constitutes upto 40% of the requirements for chronic care in pediatric practice. Inherited haemoglobin disorder falls into two main groups i.e. the structural haemoglobin variants (Sickle cell disease) and the thalassaemia. From a public health view point  $\alpha$  and  $\beta$  thalassaemia are sufficiently common to be of importance. According to World Health Organization (WHO) estimate about 7% of

World population carries an abnormal haemoglobin gene<sup>[1]</sup>. The average incidence of the  $\beta$  thalassaemia trait in India is 4% with one or two couples per 1000 being at risk of having affected offspring each year and annually at least 8000 – 10000 children with  $\beta$  thalassaemia major are born, constituting 10% of the total number born in the World every year<sup>[2]</sup>.

By convention, haemoglobinopathies are classified according to the qualitative nature of the resultant haemoglobin (i.e., sickle cell disease) and the quantitative amount of haemoglobin produced (i.e., thalassaemia). Several studies have reported occurrence of haemoglobinopathies at variable frequency in different states and caste population of India<sup>[3-10]</sup>. There is a paucity of information regarding the occurrence thalassaemia and abnormal haemoglobins other than sickle cell haemoglobin (HbS) in the state of Odisha which is inhabited by 36.7 million people comprising of 22.4% Scheduled Tribes and 16.2% Scheduled Caste population. The earlier studies from Odisha were from

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[Figure 1. shows the global representation of Haemoglobin Disorder. <sup>[1]</sup>]

**RESULTS**

Districts	Parameter	Hb(g/dl)	RBC( $10^{12}$ /l)	WBC( $10^9$ /l)	HbF %	HbE%	HbA <sub>2</sub> %	HbS%
Balasore	B thalassaemia(n – 51)	5.2±1.8	2.9±0.6	8.6±3.2	43.5±33.8	-----	14.8±21.7	-----
Mayurbhanj	B thalassaemia(n – 47)	5.8±2.6	3.2±0.5	8.3±3.1	42.7±33.0	-----	7.1±12.3	-----

**Table 1.** Mean (±SD) Haematological Parameters of β thalassaemia patients of Northern Odisha.

referred cases of Bhubaneswar and Burla regions only. The present report was based on the data of suspected cases of haemoglobinopathies belonging to northern region of Odisha to have an idea regarding the existence of abnormal haemoglobin based health problem in Odisha.

Prevention of β thalassaemia requires knowledge for diagnosing the molecular analysis in the population at risk. This knowledge is particularly necessary when prevention control is applied to a multiethnic population. For this purpose, we are analyzing mutation of different populations from northern part of Odisha, India.

**METHODOLOGY**

Venous blood samples (5-10 ml) were collected in EDTA after informed consent from individuals prior to transfusion. Data pertaining to age, sex, caste and place of origin were also recorded. Both suspected patients and parents were screened by performing solubility/sickling test <sup>[11]</sup>, nestroft <sup>[12]</sup>, and Hb electrophoresis both in

alkaline (pH 8.9) and acidic conditions (pH 6.2). Foetalhaemoglobin (HbF) quantitation by alkali denaturation method and HbA<sub>2</sub> estimation by eluting the fraction after the electrophoresis on cellulose acetate membrane (CAM) at alkaline pH were carried out <sup>[13,14]</sup>. Genomic DNA was extracted by DNA Extraction kit(Qiagen,Germany) and was analysed for six common thalassaemia mutations [IVS1-5 (G-C), IVS1-1 (G-T), CD8/9 (+G), CD41/42 (-CTTT),CD 30(G-C) and CD15 (G-A)] by ARMS- PCR-based methods in selected cases <sup>[15]</sup>.The -619 bp deletion was explored by PCR amplification and size analysis by gel electrophoresis <sup>[9]</sup>.

**DISCUSSION**

We have investigated the molecular basis of βthalassaemia in different communities in Northern Orissa and detected six different βthalassaemia mutations (CD 41/42, CD 8/9, IVS I-1, CD30, CD15, IVS I-5). Amongst

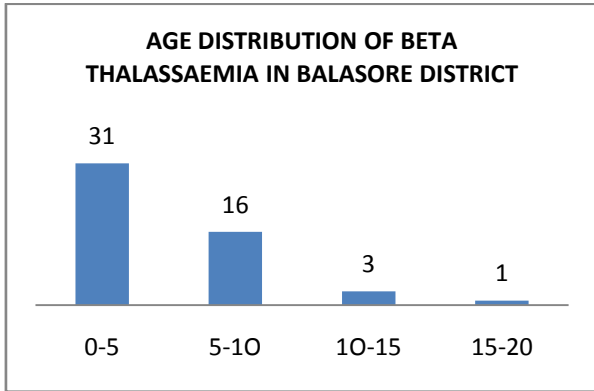


Figure 2: Age wise distribution of  $\beta$ Thalassaemiadisease of Balasore district, Odisha

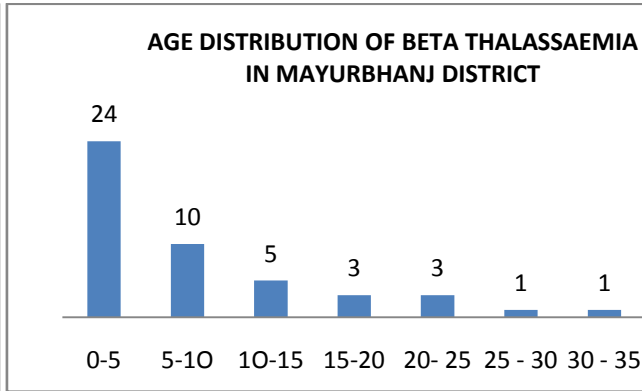


Figure 3: Age wise distribution of  $\beta$ Thalassaemiadisease of Mayurbhanj district, Odisha



Figure 4: This figure shows Cellulose Acetate electrophoresis

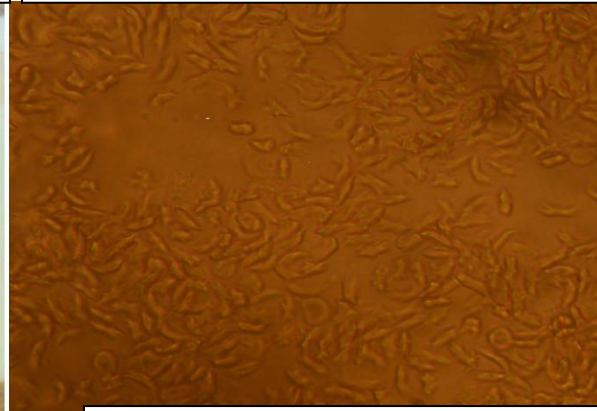


Figure 5: This figure shows sickling test of Sickle Cell Disease

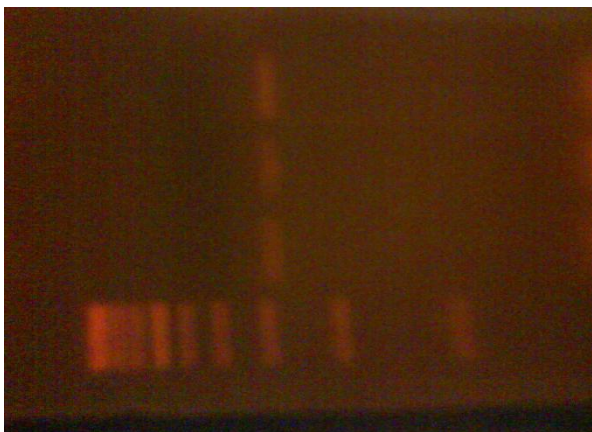


Figure 6: This figure shows IVS1-5(G-C)mutation at 285bp

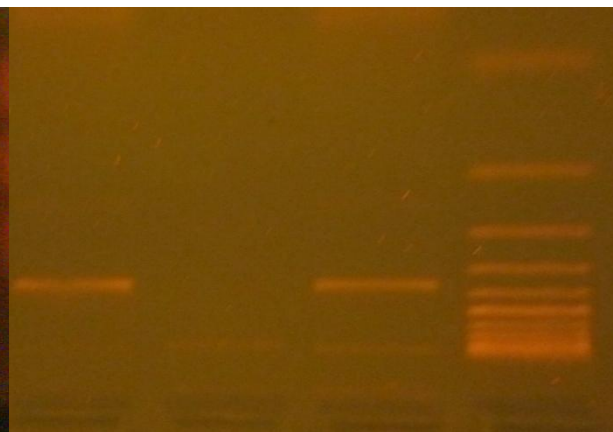
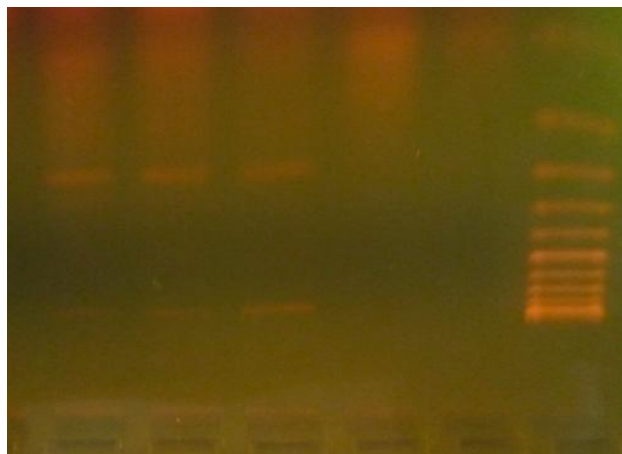


Figure 7: This figure shows CD41/42 (-CTTT) mutation at 439 bp.

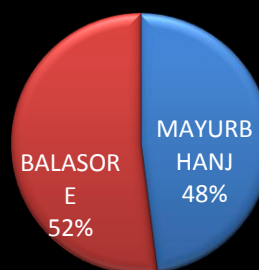
these different mutations, IVS I-5 was found to be the most common mutation in the present population as reported earlier in other populations of India <sup>[15,16,17]</sup>. The

traditional marriage system within each ethnic group, that is, the strict monogamy of the caste system might be the cause of the restricted community variation in the distri-



**Figure 8:** This figure shows CD 30(G-C) mutation at 280 bp

#### DISTRIBUTION OF BETA THALASSAEMIA AT NORTHERN ODISHA



**Figure 9:** Distribution of  $\beta$ thalassaemia patients of Northern Odisha

bution of the  $\beta$ thalassaemia mutations in Orissa <sup>[18]</sup>. The hematological profile showed the abnormal changes than the normal cases. The most prevalence IVS I-5(G-C), CD 41/42 and CD 30(G-C) mutation reported in the northern part of Odisha. The correlation can add to the existing knowledge to widen our understanding of the varied clinical presentation, phenotypic diversity and genotypic heterogeneity. This will help early intervention on patients at high risk and promote prevention by genetic counseling. Our data suggest that prediction of mild phenotypes may avoid unnecessary transfusion <sup>[19]</sup>. We believe that a prenatal diagnosis plan, or at least newborn screening for molecular markers, can lead to better outcomes in developing countries with thalassaemia endemicity.

#### ACKNOWLEDGEMENT

The authors are highly indebted to the Department of Science and Technology, Govt. of Odisha for providing financial support for the project work. Our thanks also to the Vice Chancellor of Fakir Mohan University, for his encouragement and support to carry out the project work in the department. Special thanks to all the thalassaemic and sickle cell patients and health centers that voluntarily provided us the blood samples for the study.

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