SPECTRUM OF HAEMOGLOBINOPATHIES IN PAEDIATRIC POPULATION IN SOUTHERN ODISHA- AN INSTITUTIONAL STUDY
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ABSTRACT

BACKGROUND
Haemoglobinopathies are one of the major public health problems in the world with an estimated 7% of the world population being carriers of thalassemia and haemoglobinopathies and that 3,00,000-4,00,000 babies with severe forms of these diseases are born each year. Haemoglobinopathies are prevalent in south Odisha as evidenced from the fact that there are 511 transfusion dependent patients registered in the blood bank of MKCG Medical College and Hospital, Berhampur. They also cause significant morbidity and mortality in the population. A plethora of variant haemoglobin’s have been described in the multi-ethnic Indian population. Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem. We wanted to determine the spectrum of haemoglobinopathies in paediatric population diagnosed by High Performance Liquid Chromatography (HPLC) Bio-Rad Variant II.

METHODS
This study was a prospective study done in the Department of Pathology, MKCG Medical College and Hospital, Berhampur from October 2015 and September 2017. A total of 435 paediatric cases of suspected haemoglobinopathy were subjected to detailed clinical and routine haematological evaluation followed by HPLC using BIO-RAD ‘VARIANT II’.

RESULTS
Sickle cell anaemia including both disease and trait constituted the most prevalent form followed by beta thalassemia trait. The rare variants encountered were Lepore, haemoglobin E- beta thalassemia double heterozygous, haemoglobin D - beta double heterozygous, HPFH and Alfa thalassemia.

CONCLUSIONS
Out of 435 cases studied, 257 were of haemoglobinopathy (~59.1%). 78.5% were of sickle cell anaemia. Multi-disciplinary approach along with screening, creating public awareness by counselling and mass education can reduce both mortality and morbidity of haemoglobinopathies.

KEYWORDS
Haemoglobinopathies, HPLC, Paediatric


BACKGROUND
Haemoglobin comprises of four globin chains: foetal haemoglobin (Hb F) has two α and two gamma chains (α2γ2) and adult haemoglobin (Hb A) has two α and two β chains (α2β2). Genes in the α-globin and β-globin gene clusters (on chromosomes 16 and 11) control globin-chain production.¹ They fall into two broad groups - structural variants that change the amino acid sequence and produce an unusual haemoglobin, and thalassemia that lower or abolish production of globin chains.² The clinical spectrum of these disorders varies from asymptomatic conditions (beta-thalassemia minor) to serious disorders such as thalassemia major that require regular blood transfusions and extensive medical care.³

It has been estimated that approximately 7% of the world population are carriers of thalassemia and haemoglobinopathies and that 3,00,000-4,00,000 babies with severe forms of these diseases are born each year.⁴ The prevalence of beta-thalassemia trait and sickle cell in various regions of India is around 3%-17% and 1%-44%, respectively, because of consanguinity, caste, and area endogamy.¹ Every year, around ten thousand children with beta-thalassemia major are born in India, which constitutes

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about 10% of the total global load of beta-thalassemia. The frequency of carriers of haemoglobinopathies varies from 3 to 17% in different population groups of India.

Haemoglobinopathies are one of the major public health problems in the state of Odisha, India. They are generally not curable but can be prevented by mass screening, genetic counselling and prenatal diagnosis. Accurate and timely detection of various known and unknown Hb variants can prevent the occurrence of serious Hb disorders such as thalassemia major in the newborns.

Haemoglobinopathies are prevalent in the southern region of Odisha as evidenced from the fact that there are 511 transfusion dependant patients registered in Blood bank of MKCG Medical College and hospital (MKCG MCH), Berhampur, which is the premiere tertiary care hospital in this region. This paper presents the pattern of haemoglobinopathies amongst the 435 referral paediatric cases that came to the Department of Pathology, MKCG MCH for the period from October 2015 to September 2017.

METHODS
A total of 435 paediatric cases of suspected haemoglobinopathy referred to department of pathology, MKCG MCH between October 2015 and September 2017 were subjected to detailed clinical and routine haematological evaluation including complete blood count, reticulocyte count and sickling test, followed by HPLC using BIO-RAD ‘VARIANT II’.

2 ml. EDTA intravenous blood samples were collected after obtaining informed consent from each patient. Complete blood count using Sysmex XT-2000i and peripheral smear examination was done. Sickling test was performed by using freshly prepared sodium metabisulphite solution as reducing agent. The instrument, known as BIO-RAD ‘VARIANT II’ (beta thalassemia short program) utilizes the principle of HPLC. An Hb A2/F calibrator and two levels of controls (BIO-RAD) were analysed at the beginning of each run. The total area acceptable was between 1 and 3 million. The software delivers a printed report showing the chromatogram, with all the haemoglobin fractions eluted. The integrated peaks are assigned to manufacturer-defined "windows" derived from specific retention time (RT). This RT is the time that elapses from the sample injection to the apex of the elution peak, of normal haemoglobin fraction and common variants.

RESULTS
A total of 435 clinically suspected paediatric cases of haemoglobinopathies were included in the study. 257 cases (59.1%) showed different abnormal haemoglobin variants. The overall incidence in male was 58.2% and that in female was 41.8% with the male female ratio of 1.4:1. Maximum number of cases were seen in 1-6-year age group (47.1%) followed by 39.7% cases in the age group of 6-14 years and 13.2% cases were less than 1 year of age. The youngest patient was 3 months old. Criteria for suspecting haemoglobinopathy in these cases included: results of screening tests such as various discriminant functions so obtained on haematology cell counters, findings obtained from peripheral smear examination, family history, and relevant clinical signs and symptoms suggestive of haemoglobinopathy. The different haemoglobinopathies found are as shown in Table 2.

We found that a maximum number of patients were of sickle cell disease (39.3%) followed by sickle cell trait (30.3%). Sickle cell trait was considered in patients with Hb A 50-75%, Hb S 25-40% and Hb F <1%. They had mild normochromic normocytic anaemia with mild anisopoikilocytosis, and few target cells. The patients with Hb S 70-90%, Hb F 10-30% and Hb A 0-10% were diagnosed as sickle cell disease. (Figure 1 and 2)

Beta thalassemia trait (11.3% cases) was diagnosed based on high levels of Hb A2 (4-8%) Figure 3. These patients presented with mild anaemia, low mean corpuscular volume (MCV <80fl), and low mean corpuscular haemoglobin. Fetal Hb was not increased. Hb A2 of 3.5-3.9% was considered as borderline and were advised iron study with repeat HPLC after iron therapy.
13 cases (5.0%) of beta thalassemia major were reported. All these patients had raised Hb F values (75%-98%). Clinically they presented with severe pallor, blood transfusion dependency and moderate to marked splenomegaly. 3 cases of beta thalassemia intermedia were diagnosed. They had variable degree of anaemia with anisopoikilocytosis and microcytic hypochromic blood picture. Hb F were raised with a variable reduction in Hb A and patients were not transfusion dependent.

There were also 4 cases of hereditary persistence of foetal haemoglobin, 3 cases of alpha thalassemia and one case each of E-beta thalassemia, Delta beta thalassemia and Lepore.

**DISCUSSION**

Prevalence of wide range of haemoglobinopathy in the paediatric population of southern Odisha indicate that haemoglobinopathies and their related complications are not uncommon at birth and that a great deal of emphasis must be put on prevention and screening of at risk groups to avoid preventable morbidity and mortality.

257 out of a total of 435 paediatric cases sent to us were found to have some form of haemoglobinopathy. Moderate to severe degree of anaemia was seen in majority of our patients as this was a laboratory-based study with patients being sent as a workup for anaemia. Some of our patients included leukaemias, megaloblastic anaemia and other nutritional and non-nutritional causes of anaemia.

Appropriate laboratory tests are required for diagnosis and confirmation of these disorders. The identification of Hb variants by conventional techniques are often presumptive. HPLC offers the distinct advantage over classic Hb electrophoresis as it can more accurately identify and quantitate abnormal Hbs. We found the maximum number of cases were of sickle cell anaemia both heterozygous and homozygous followed by beta thalassemia trait. This calls for the need of antenatal screening and screening of marriageable age groups. This will help in the prevention of sickle cell disease and thalassemia major in the offspring.

The incidence of sickle cell haemoglobinopathies in India ranges from 1-44%. The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat (6.4%). But in light of population migration it is becoming a worldwide phenomenon.

78.5% cases in this study showed Sickle cell haemoglobinopathies. A study by Balgir et al., showed 39.3% cases showing Sickle cell haemoglobinopathies. This high rate in the present study was possibly because it was done in high prevalence zone for sickle cell haemoglobinopathies. Tambse et al showed 70.36% cases having sickle cell haemoglobinopathies in their study in northern Maharashtra, another high prevalence zone.

Sickle cell homozygous cases (39.3%) were more frequently encountered compared to Sickle cell heterozygous (30.3%). This could have been because this study was a hospital-based study in a low economic area.
where people visit a hospital only when they have severe symptoms. The same reason could also explain a high number of Beta thalassemia major and intermedia (6.2%) while Beta thalassemia trait cases were not that high (11.3%). We believe that if population-based studies is carried out it might have showed other haemoglobin disorders outnumbering the sickle cell disorders in particular.

HPFH was first documented in Ghana and has also been described in non-African populations. In HPFH, expression of the gamma-globin gene of Hb F persists at high levels in adult erythroid cells. Our study reported 4(1.6%) cases of HPFH. There were 3 cases (1.2%) of Alfa thalassemia trait, after excluding Iron deficiency and Beta thalassemia trait that had mild microcytic anaemia and a fast-moving peak in HPLC. There was also 1 case each of double heterozygous thalassemia. However, all these cases need genetic testing for confirmation. Hb Lepore constituted only a single case. Hb Lepore elutes in A2 window with concentration of 10%-15%. Hb Lepore shows a characteristic hump on the downward slope in comparison to Hb E.

HPLC is a valuable tool for diagnosis of various haemoglobinopathies. However, limitation of this technique, especially in budget constrained regions, is higher capital and reagent costs including high skill and experience required to interpret the results. Another limitation is various haemoglobinopathies elute similar retention time so cannot be ruled out by HPLC alone. A disclaimer should always accompany the report and findings must be supported by CBC finding, family history, haemoglobin electrophoresis if required, and sickling tests and advised for molecular studies.

CONCLUSIONS

HPLC is an ideal method for the routine diagnosis of haemoglobinopathies. We found a plethora of haemoglobinopathies in southern Odisha. Continuous awareness programmes, mass screening of the population especially of child bearing age and school going children will help in reducing the burden of disease. This can in turn with proper genetic counselling help in reducing morbidity and mortality.

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