ABSTRACT: OBJECTIVES: The study was carried out for creating a profile in cases of hemoglobinopathies coming to our Hospital and comparing the results with other Indian studies.

SETTING: The study was carried out at a premier tertiary care center in Orissa. A total of 820 referred cases of Anemia were examined between March 2010 to July 2015.

METHODS: Hematological indices and hemoglobin HPLC with quantification of the bands was done in all cases. Hematological indices were measured on SYSMEX Cell Counter (XT-1800i) and Hemoglobin HPLC was performed on BIORAD D10.

RESULTS: Out of 820 referred cases 453 was found to be normal and 367 had one or other form of haemoglobinopathy. The data shows the prevalence of Sickle cell Trait to be 18.6%, β Thalassemia -Trait 10.8% & Sickle cell disease 8.7% in the study population.

CONCLUSIONS: The prevalence of hemoglobinopathy is found to be very high. It is present in a proportion of 1:2.25 in patients coming for anemia for investigation or clinically suspected cases of hemoglobinopathies. Hence, all the cases of anemia should undergo HPLC screening in this part of the country. There should be an initiative towards population screening, genetic counseling and prenatal diagnosis to counter the magnitude of problem.

KEYWORDS: Anemia; Hemoglobinopathies; Thalassemia.

INTRODUCTION: Inherited hemoglobin disorders fall into two main groups; structural hemoglobin variants (HbS, HbE, HbD etc.) and Thalassemia (Alpha and beta thalassemia), which are caused by defective globin production.[1] Of the several structural abnormal hemoglobins,[2] there are three variants – sickle cell (Hb S), hemoglobin E (Hb E) and hemoglobin D (Hb D), which are predominantly prevalent in India. The sickle cell disease is wide spread in tribal as well as nontribal communities especially in the Central-East India. Hb E is widely distributed in north-eastern states of India.[3,4] Hb D is predominantly seen in Punjab, Uttar Pradesh, Gujarat and Jammu and Kashmir.[5,6] In India, β-thalassaemia comprises about 80–90% of the total thalassaemias reported. Beta-thalassaemia is detectable in almost every Indian population, however, it is seen with highest frequency in north-west and far east.[7] Alpha-thalassaemia is most widely prevalent in the tribal population with a frequency of 1-40% in Andhra Pradesh and Gujarat.[8]

The state of Orissa inhabits 42 million of population, comprising 22.4% scheduled tribes and 16.2% scheduled caste people. They have their own socio-cultural customs, traditions, breeding practices and life-styles quite distinct from each other, which influences their vulnerability towards hereditary diseases in Orissa.[9] The presence of thalassaemia and Hb E is seen in the eastern coastal part of Orissa and that of Hb S in the central, western and southern Orissa.
The present study was undertaken to create a profile of hemoglobinopathies referred to this hospital and comparing the results with other hospital based studies. As this is a Tertiary care Hospital which caters patients from all over the state the study represents the pattern of hemoglobinopathies in the state.

MATERIALS AND METHODS: Hospital based retro prospective data analysis was conducted between March 2010 to July 2015. A total of 820 cases of anaemia were referred for further diagnostic investigations and counseling. Using ethylene diamine tetra acetic acid (EDTA) as anticoagulant, 3 ml. intravenous blood samples were collected by disposable syringes. Age, Sex and clinical history of each individual were obtained. Haemoglobin HPLC was carried out on Biorad D.\textsuperscript{10} Hematological indices were measured using SYSMEX Cell Counter (XT-1800i), which was calibrated with commercially available controls (SYSMEX e-check XE). The lab is NABL accredited and the above tests are under the scope of accreditation.

HPLC is an excellent, powerful diagnostic tool and is suitable for the routine investigation of hemoglobin variants, hemoglobinopathies, and thalassemia. The value more than 4.0% of A2 fraction of hemoglobin was taken as cut off point for determining the β-thalassemia trait. An HbF-value of more than 2.0 was considered abnormal. The diagnosis of sickle cell-β-thalassemia was based on the findings of hemoglobin Hb- A, F, S/D and A2 on HPLC.

RESULTS: Out of 820 cases examined 421(55.8%) were found to have a normal HPLC pattern and 367(44.2%) were found to have one or the other form of haemoglobinopathy.

Figure 1 gives the spectrum of hemoglobinopathies encountered in 367 positive cases. In our study, the commonest disorder was Sickle cell Trait (18.6%), followed by β Thalassemia-Trait (10.8%) & Sickle cell disease (8.7%), α-Thalassemia (1.7%), compound heterozygous for sickle cell-β-thalassemia (1.4%), β-thalassemia major (1.2%), HbE heterozygous (1.2%) and δβ - Thalassemia (0.5%) in decreasing frequency.

Table 1 shows the comparative analysis of hemoglobinopathies with other studies.\textsuperscript{[10-13]} Due to the regional distribution of hemoglobinopathies one can find large difference in the studies.

Table 2a, b, c. shows a comparative analysis of different hematological parameters for the three common hemoglobinopathies at different centers in India. Though there are regional variation in the prevalence of different hemoglobinopathies, yet the hematological indices remains almost the same for the three common hemoglobinopathies encountered at our hospital.

DISCUSSION: Our study showed Sickle cell trait to be 18.6%, β–thalassemia trait 10.8%, Sickle cell disease 8.7% and β-thalassemia major 1.2%. Our study differed in the pattern of distribution of hemoglobinopathies in other studies.\textsuperscript{[10-13]} due to regional variations. The incidence of β–thalassemia trait was similar to AIIMS, New Delhi and Army Hospital, New Delhi. However the hematological indices for various hemoglobinopathies remain the same.

This study shows all variants of β-thalassemia & Sickle cell being prevalent in Orissa. This scenario of hemoglobinopathies reflects that the population of the state of Orissa is genetically heterogeneous.
The physicians should advice all cases of anemia to go for HPLC screening. The result of the present study is an indicator towards the same. All the hereditary disorders are preventable, treatable and curable. There should be an aggressive attempt to improve the environmental and socio-economic conditions, provide better public health care and medical screening facilities. The health care personnel and the government should join hands to alleviate and ameliorate the sufferings of the affected masses in the state.

**Figure 1**: Illustration shows Spectrum of hemoglobinopathies (n=367 positive cases)

| Hemoglobinopathy Screening by HPLC | Apollo Hospitals, Bhubaneswar | AIIMS New Delhi | Apollo Hospitals, Chennai | CIMS Bilaspur Chatisgarh | Army Hospital (R&R), New Delhi |
|-----------------------------------|-------------------------------|----------------|--------------------------|-------------------------|-------------------------------|
| β Thalassemia –Trait              | 10.8                          | 18.1           | 37.9                     | -                       | 17.0                          |
| β Thalassemia –Major              | 1.2                           | 2.9            | 2.3                      | -                       | 0.4                           |
| Sickle cell Trait                 | 18.6                          | 1.4            | 5.3                      | 35                      | 2.3                           |
| Sickle cell Disease               | 8.7                           | 0.5            | 1.4                      | 4.7                     | 1.7                           |
| Compound heterozygous for sickle cell-β−thalassemia | 1.4 | 0.8 | 2.5 | - | 0.6 |

| Hemoglobinopathy Screening by HPLC | Apollo Hospitals, Bhubaneswar | AIIMS New Delhi | Apollo Hospitals, Chennai | CIMS Bilaspur Chatisgarh | Army Hospital (R&R), New Delhi |
|-----------------------------------|-------------------------------|----------------|--------------------------|-------------------------|-------------------------------|
| β Thalassemia –Trait              | 10.8                          | 18.1           | 37.9                     | -                       | 17.0                          |
| β Thalassemia –Major              | 1.2                           | 2.9            | 2.3                      | -                       | 0.4                           |
| Sickle cell Trait                 | 18.6                          | 1.4            | 5.3                      | 35                      | 2.3                           |
| Sickle cell Disease               | 8.7                           | 0.5            | 1.4                      | 4.7                     | 1.7                           |
| Compound heterozygous for sickle cell-β−thalassemia | 1.4 | 0.8 | 2.5 | - | 0.6 |

The physicians should advice all cases of anemia to go for HPLC screening. The result of the present study is an indicator towards the same. All the hereditary disorders are preventable, treatable and curable. There should be an aggressive attempt to improve the environmental and socio-economic conditions, provide better public health care and medical screening facilities. The health care personnel and the government should join hands to alleviate and ameliorate the sufferings of the affected masses in the state.

**Figure 1**: Illustration shows Spectrum of hemoglobinopathies (n=367 positive cases)
### Table 1: Comparison with other Hemoglobinopathy studies at various centers. (in %)

| Phenotype                          | Apollo Bhubaneswar | AIIMS New Delhi | Apollo Chennai | CIMS Bilaspur |
|------------------------------------|--------------------|-----------------|----------------|--------------|
| HbA2 (gm/dl)                       | 1.84               | 3.34            | 2.73           | 2.24         |
| HbC (gm/dl)                        | NA                 | NA              | NA             | NA           |
| HbE (gm/dl)                        | 0.8                | 0.8             | 0.8            | 0.8          |
| HbF (gm/dl)                        | 9.8                | 9.8             | 9.8            | 9.8          |
| HbS (gm/dl)                        | 6.8                | 6.8             | 6.8            | 6.8          |
| MCHC (%)                           | 32.4               | 32.4            | 32.4           | 32.4         |
| RDWC                               | 19.8               | 19.8            | 19.8           | 19.8         |
| HbA2 (%)                           | 3.34               | 3.34            | 3.34           | 3.34         |
| HbC (%)                            | NA                 | NA              | NA             | NA           |
| HbE (%)                            | 0.8                | 0.8             | 0.8            | 0.8          |
| HbF (%)                            | 9.8                | 9.8             | 9.8            | 9.8          |
| HbS (%)                            | 6.8                | 6.8             | 6.8            | 6.8          |
| MCHC (%)                           | 32.4               | 32.4            | 32.4           | 32.4         |
| RDWC                               | 19.8               | 19.8            | 19.8           | 19.8         |

### Table 2: Comparative Hematological Profile in major Hemoglobinopathies at various centers in India.

| Study Centre   | Cases | Hb (gm/dl) | RBC (mill/mm³) | PCV (%) | MCV (fl) | MCHC (%) | RDWC | HbF | HbA2 | HbS |
|----------------|-------|------------|----------------|---------|----------|----------|------|-----|------|-----|
| Apollo Bhubaneswar | 153   | 11.2       | 4.4            | 33.5    | 76.4     | 54.1     | 18.3 | 1.4 | 3    | 32  |
| AIIMS New Delhi   | 11    | 11.6       | 4.45           | NA      | 84.3     | 31.7     | NA   | 2.3 | 3.3  | 36.3|
| Apollo Chennai    | 29    | 11.3       | 4.5            | NA      | 77.2     | 32.3     | 15.6 | 1.34| 3.3  | 30.9|
| CIMS Bilaspur     | 441   | 10.3       | 3.87           | 31.48   | 89.4     | 30.7     | NA   | NA  | NA   | NA  |

### Table 2a: Sickle Cell Trait

| Study Centre   | Cases | Hb (gm/dl) | RBC (mill/mm³) | PCV (%) | MCV (fl) | MCHC (%) | RDWC | HbF | HbA2 | HbS |
|----------------|-------|------------|----------------|---------|----------|----------|------|-----|------|-----|
| Apollo Bhubaneswar | 72    | 8.8        | 3.3            | 26.4    | 81.4     | 27.5     | 18.8 | 1.5 | 5.6  | NA  |
| AIIMS New Delhi   | 4     | 8.3        | 2.8            | NA      | 90.5     | 32.7     | NA   | 1   | 5.5  | NA  |
| Apollo Chennai    | 8     | 7.8        | 3.3            | NA      | 77.4     | 31.5     | 24.8 | 8   | 2.7  | 79.9|
| CIMS Bilaspur     | 56    | 7.9        | 2.92           | 30.18   | 93.9     | 31.4     | NA   | NA  | 2.24 | NA  |

### Table 2b: Sickle Cell Disease

| Study Centre   | Cases | Hb (gm/dl) | RBC (mill/mm³) | PCV (%) | MCV (fl) | MCHC (%) | RDWC | HbF | HbA2 | HbS |
|----------------|-------|------------|----------------|---------|----------|----------|------|-----|------|-----|
| Apollo Bhubaneswar | 89    | 9          | 4.6            | 28.8    | 67.4     | 21.5     | 22.4 | 1.5 | 5.6  | NA  |
| AIIMS New Delhi   | 145   | 10.3       | 5.06           | NA      | 68.6     | 28.3     | NA   | 1   | 5.5  | NA  |
| Apollo Chennai    | 206   | 10.4       | 5.2            | NA      | 65.5     | 30.5     | 16.5 | 1.4 | 5.4  | NA  |
| CIMS Bilaspur     | NA    | NA         | NA             | NA      | NA       | NA       | NA   | NA  | NA   | NA  |

### Table 2c: β- Thalassemia Trait
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Date of Submission: 06/08/2015.
Date of Peer Review: 08/08/2015.
Date of Acceptance: 17/08/2015.
Date of Publishing: 20/08/2015.