Spectrum of Hemoglobinopathies in the State of Orissa, India: A Ten Years Cohort Study

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Abstract

Objectives: i) To determine the pattern of spectrum of hemoglobinopathies in the state of Orissa, ii) To find the ethnic groups at high risk of hemoglobinopathies, iii) Geographical distribution of hemoglobinopathies, and iv) To know epidemiological aspects of hemoglobinopathy cases in Orissa.

Material and Methods: One thousand fifteen cases of anemia were analysed referred from different peripheral hospitals and Medical Colleges and Hospitals of Orissa state for diagnosis and counseling during 1994 to 2003. About 2-3 ml. intravenous blood samples were collected after obtaining informed consent from each individual. Hematological indices were measured using MS4 Cell Counter. Background data of each individual were recorded like age, sex, caste, place of origin, consanguinity, etc. Hemoglobin electrophoresis was carried out on CAM in Tris-EDTA-Borate buffer at pH 8.9 and quantification of A2 fraction of hemoglobin by elution method. The value more than 3.5% of A2 fraction of hemoglobin was taken as cut off point for β−thalassemia trait and more than 10% as Hb E. Hb electrophoresis in acidic medium (pH 6.2) was also carried out to confirm Hb D or E band. Estimation of fetal hemoglobin was done. Family studies were carried out to confirm the diagnosis.

Results: Most common hemoglobinopathies observed out of 1015 cases were: sickle cell trait (29.8%), sickle cell disease (7.5%), sickle cell-β−thalassemia (1.7%), β−thalassemia trait (18.2%), thalassemia major (5.3%), thalassemia intermedia (0.9%), Hb E trait (0.9%), Hb E disease (0.3%), E-β−thalassemia (0.7%), Hb D trait (0.2%) and SD disease (0.2%). Sickle cell disorders with high level of fetal hemoglobin were common in general castes (0.3-20.7%), scheduled castes (0-8.9%) and scheduled tribes (0-5.5%). Transfusion dependent β−thalassemia syndrome was prevalent in Brahmin, Karan, Khandyat, Teli, etc. Most of the cases belong to Anugul district, followed by Khurda, Nayagarh, Phulbani, Cuttack, Jaipur, Dhenkanal, Ganjam, Keonjhar, Mayurbhanj, etc.

Conclusions: The heterogeneous population is harbouring almost all major hemoglobinopathies in general castes, scheduled castes and tribes, belonging to Coastal and South-Western regions of Orissa. This study provides for the first time a comprehensive database on the pattern of spectrum of hemoglobinopathies in Orissa. ©

INTRODUCTION

Thalassemia and other structural hemoglobinopathies are the major erythrocytic genetic disorders prevalent in certain parts of the world including India. While the general incidence of thalassemia trait and sickle cell hemoglobinopathy in India varies between 3-17% and 1-44%, respectively because of consanguinity and, caste and area endogamy, some communities show a very high incidence, making the disease as a major public health problem in our country.1,2 Inherited disorders of hemoglobin synthesis are an important cause of morbidity and mortality worldwide. They place a large burden on the patients, their families and even their communities. They are generally not curable but can be prevented by population screening, genetic counseling and prenatal diagnosis.2

The state of Orissa inhabits 36.7 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 affect their breeding structures and vulnerability towards hereditary diseases in Orissa.3

The Regional Medical Research Centre (ICMR) at Bhubaneswar caters facilities for population screening, provides the diagnosis to referral cases from all over the state, and genetic counseling to the affected persons and their families of Orissa. Since the suspected cases are

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referred from all over the state, representing vulnerable communities, ethnic groups, etc. and they could very well represent the local population and the pattern of hemoglobinopathies in the state. This paper presents the pattern of hemoglobinopathies amongst the referral cases of anemia for the period from 1994 to 2003. The present study was designed with the following aims and objectives in mind: i) To determine the pattern of spectrum of hemoglobinopathies in the state of Orissa, ii) To find the ethnic groups at high risk of hemoglobinopathies, iii) Geographical distribution of hemoglobinopathies, and iv) To know the epidemiological aspects of cases of hemoglobinopathies in Orissa.

**Material And Methods**

A total of 1,015 cases were referred to the Division of Human Genetics, Regional Medical Research Centre (ICMR), Bhubaneswar during the period from 1994 to 2003 as cases of anemia from different peripheral hospitals and Medical Colleges and Hospitals of different regions of Orissa. They were suspected to be suffering from hemolytic anemia.

About 2-3 ml. intravenous blood samples were collected after obtaining the informed consent using ethylene diamine tetra acetic acid (EDTA) as anticoagulant by disposable syringes and needles from each individual free of at least one month’s blood transfusion. Hematological indices were measured using MS4 Cell Counter (Melet and Schloesing Laboratories, France), which was calibrated with commercially available controls (Bio Rad calibrations, Australia). Laboratory investigations were carried out following the standard procedures after cross checking for quality control from time to time. Data of each individual pertaining to age, sex, caste, place of origin, consanguinity, etc. were recorded.

The sickling test was performed by using freshly prepared sodium metabisulphite solution as reducing agent. The routine hemoglobin electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-Borate buffer at pH 8.9 and quantification of A2 fraction of hemoglobin by elution method. The value more than 3.5% of A2 fraction of hemoglobin by elution method was taken as cut off point for determining the β-thalassemia trait and more than 10% was assumed to be hemoglobin E. Electrophoresis in acidic medium (pH 6.2) was also carried out to identify and confirm the presence of hemoglobin D or E band. Estimation of fetal hemoglobin was done by Betke and Coworkers’ method as described by Weatherall. Family studies were carried out to confirm the diagnosis, wherever it was necessary; and the results of relatives also included in the analysis.

**Results**

During the period of 1994 to 2003, a total of one thousand fifteen cases of anemia were referred to Regional Medical Research Centre (ICMR), Bhubaneswar for further diagnostic investigations and counseling and 354 families were screened. Out 1,015 cases, 348 (34.3%) were found normal and 667 (65.7%) had one form or the other of hemoglobinopathies. Out of 667 abnormal cases, 349 (52.3%) were males and 318 (47.7%) females, thus giving preponderance of males over females. Due to socio-cultural factors, more health care is generally taken for males over females, which is reflected in this referral cases cohort study.

Table 1 gives the spectrum of hemoglobinopathies encountered during 1994 to 2003 period. It is interesting to note that the sickle cell trait is the most common hemoglobinopathy (29.8%), followed by β-thalassemia trait (18.2%), sickle cell disease (7.6%), thalassemia major (5.3%), sickle cell-β-thalassemia (1.7%), δβ-thalassemia (0.9%), hemoglobin E trait (0.9%), E-β-thalassemia (0.7%) and so on in the decreasing sequence. Two cases each of hemoglobin D trait and SD disease were encountered. Hemoglobin abnormalities have been detected in both male and female populations. Since these cases represented the local population, it was indicative of the high morbidity, vulnerability and incidence of abnormal hemoglobins in the state of Orissa. Moreover, all major hemoglobinopathies prevalent all over India have been encountered in Orissa showing thereby the population admixture in this Central-East part of India.

Age and sex-wise distribution of cases of different hemoglobinopathies is presented in Table 2. As expected it is apparent from the table that a majority of the cases of hemoglobinopathy belong to reproductive age group, i.e. 16 to 45 years, followed by neonatal to childhood period (0-15 years), and only a few cases of old age (46+ years) are being detected when they face clinical complications. Most of the cases of hemoglobinopathy in India in general are detected accidentally when the couple is advised by the physician to go for laboratory investigations to find the cause of anemia. The results of

<table>
<thead>
<tr>
<th>Types of hemoglobinopathies</th>
<th>Males No.</th>
<th>Females No.</th>
<th>Total No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>184</td>
<td>164</td>
<td>348</td>
<td>34.3</td>
</tr>
<tr>
<td>Sickle Cell Trait</td>
<td>171</td>
<td>131</td>
<td>302</td>
<td>29.8</td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>41</td>
<td>36</td>
<td>77</td>
<td>7.6</td>
</tr>
<tr>
<td>Sickle Cell-β-Thalassemia</td>
<td>4</td>
<td>13</td>
<td>17</td>
<td>1.7</td>
</tr>
<tr>
<td>β-Thalassemia Trait</td>
<td>91</td>
<td>94</td>
<td>185</td>
<td>18.2</td>
</tr>
<tr>
<td>Thalassemia Major</td>
<td>29</td>
<td>25</td>
<td>54</td>
<td>5.3</td>
</tr>
<tr>
<td>δβ-Thalassemia</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>0.9</td>
</tr>
<tr>
<td>Hemoglobin E Trait</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>0.9</td>
</tr>
<tr>
<td>Hemoglobin E Disease</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>E-β-Thalassemia</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.7</td>
</tr>
<tr>
<td>Hemoglobin D Trait</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>SD Disease</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>533</td>
<td>482</td>
<td>1,015</td>
<td>100.0</td>
</tr>
</tbody>
</table>
the present study are consistent to this notion.

Referred cases were classified according to their caste affiliation. It is apparent from Table 3 that a majority of the patients of hemoglobinopathy belong to general castes for sickle cell disorders (64.6%), \(\beta^-\)thalassemia syndrome (79.6%) and other hemoglobinopathies (91.3%), respectively in the state of Orissa. These results are contrary to the findings reported elsewhere in India where the problem of hemoglobinopathy is stated to confine only to scheduled tribes or scheduled castes and the general castes are not affected. However, a considerable number of people belonging to scheduled castes in Orissa also suffer from hemoglobinopathies (Table 3), but the frequency is comparatively low (8.7-27.4%). Hereditary disorders of hemoglobin among the tribal population (0-8%) are very uncommon in Orissa. This may be due to breeding isolation of the people from the general stream and strictly following the tribal endogamy.

Table 4 provides the distribution of sickle cell disorders and \(\beta^-\)-thalassemia syndrome cases in different ethnic groups of Orissa. The geographical distribution of sickle cell disorders and \(\beta^-\)-thalassemia syndrome cases in Central-East India, this is to point out that there are certain
pockets or regions in Orissa (Fig. 1), which are more vulnerable to hemoglobinopathies than the others (Table 5). For example, Anugul, Phulbani, Khurda, Cuttack, Dhenkanal, Keonjhar, Nayagarh, Ganjam, Sundargarh and Kalahandi districts are having the highest number of cases of sickle cell disorders in Orissa. The β-thalassemia syndromes are common in Khurda, Puri, Jajpur, Nayagarh, Cuttack, Keonjhar, Mayurbhanj, Jagatsinghpur, Kendrapara and Bolangir districts of Orissa. These results clearly indicate that the sickle cell disorders are generally confined to Central, Western and Southern Orissa and β-thalassemia syndromes are generally limited to coastal districts or region of Orissa. Further interpretations of the results suggest the migration or gene flow of sickle cell abnormality from Central India (Gondwana Land) towards Western and Southern Orissa and that of β-thalassemia syndrome from Northern and Eastern India towards Eastern Coastal and Southern region of Orissa.

Table 6 gives the glimpses of distribution of other abnormal hemoglobins in different districts or regions and communities of Orissa. Hemoglobin E and E-β-thalassemia syndrome is generally confined to communities and coastal region of Orissa due to gene flow from North and Eastern region of India.

**DISCUSSION**

A large number of hemoglobin variants prevalent in the populations of Orissa indicate that hemoglobinopathies are not uncommon at birth and also their related complications. The inherited disorders of hemoglobin synthesis are one of the important public health problems in Central Eastern part of India. This scenario of hemoglobinopathies reflects that the population of the state of Orissa is genetically heterogeneous one and so many ethnic elements have absorbed into the main stream of people along with the original inhabitants with varied genetic heritages, resulting in population diversity with the passage of time. The findings of different hemoglobinopathies prevalent in the state are in agreement with the population admixture in the state.

Historical accounts regarding the gene flow or migrations of different waves of people from different corners of the state are obscure due to intriguing nature of the people. However, the scanty account in this regard available points out that there were at least three waves of people who entered through Northern, Eastern and Western corridors of the state. The Northern waves (for example, Brahmins of Orissa have been said to be migrated from the state of Uttar Pradesh) have brought the abnormal β-thalassemia gene, the Eastern migrants from West Bengal and Assam came with hemoglobin E, and the Western people bestowed with sickle cell gene in Orissa from Gondwana Land (presently a part of

<table>
<thead>
<tr>
<th>District</th>
<th>Community</th>
<th>Abnormal Hemoglobins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puri</td>
<td>Brahmin, Khandyat</td>
<td>Hb AE=7; Hb EE=1; Hb E-β-thalassemia=4</td>
</tr>
<tr>
<td>Khurda</td>
<td>Brahmin</td>
<td>Hb AE=1; Hb EE=1; Hb E-β-thalassemia=1</td>
</tr>
<tr>
<td>Nayagarh</td>
<td>Gauda, Brahmin</td>
<td>Hb AE=1; Hb EE=1; Hb E-β-thalassemia=2</td>
</tr>
<tr>
<td>Anugul</td>
<td>Barber, Chasa</td>
<td>Hb AD=2; Hb SD=1</td>
</tr>
<tr>
<td>Sundargarh</td>
<td>Khandyat</td>
<td>Hb SD=1</td>
</tr>
</tbody>
</table>

* Sickle Cell Disorders include Sickle Cell Trait, Sickle Cell Disease and Sickle Cell-β-Thalassemia. ** β-Thalassemia syndrome includes Thalassemia Major, Thalassemia Trait and δβ-Thalassemia Cases
Similar results were obtained by Garewal and Das\textsuperscript{15} and Haddi, Gonda, Ghasi and Dhoba of the state (Table 4). Gauda, Teli, etc. and scheduled castes, namely, Pana, Chasa, Khandyat, Brahmin, Karan, Gau, Teli, etc. and scheduled castes, namely, Pana, Haddi, Gonda, Ghasi and Dhoba of the state (Table 4). Similar results were obtained by Garewal and Das\textsuperscript{15} and Kar.\textsuperscript{16} It is quite interesting that among the isolated tribal populations of Orissa, these defective genes have not so vigorously penetrated (Oraon, Saora and Santal) as evident from the present study.

The geographical scatter of sickle cell disorders, $\beta$-thalassemia syndromes, and other hemoglobinopathies in Central Eastern part of India shows the differential migration pattern of the people in the state of Orissa. The populations nearer to the place of parent origin have the higher frequency of sickle cell, $\beta$-thalassemia syndromes or abnormal hemoglobin E gene than those who are far away. For example, Hb E is often encountered in cases referred from Eastern Coastal part of the state (Puri, Khurda, Balasore and Bhadrak districts), a region exposed to South East Asia through West Bengal, Assam, Manipur and Nagaland, where this abnormal hemoglobin E is highly prevalent.\textsuperscript{15,17} Hence it is reasonable to postulate that its occurrence in Orissa is essentially through gene flow from these areas. Similarly, the gradual inflow of sickle cell gene from Gondwana land towards Western and Southern Orissa is clearly evident in the populations of that region.\textsuperscript{18} The gradual movement of people like this is evident from the distribution of hemoglobin E and sickle cell disorders in the present study (Table 5).

Rapid proliferations of hemolytic genetic disorders in a given geographical pocket or region due to comparatively small population size, caste and area endogamy, consanguinity, and virtually lack of medical facilities and natural barriers like geographical landscape, forests and ecological niches, river, etc. have further compounded the complexity of sickle cell disorders and $\beta$–thalassemia syndromes in the Central East part of India. The bioenvironmental and socio-cultural factors further put constraints on the viability, proliferation and growth of the population under the threat of endemicity of dreadful malaria.\textsuperscript{19} The harbouring of spectrum of hemoglobinopathies perhaps provides the heterozygote advantage for protection against the dreadful malaria in the agricultural and wet environmental conditions of the state.

The prevention and control of spectrum of hemoglobinopathies in the state is an uphill task for the planners, policy makers and medical and health care machinery of the state with political will of the people.\textsuperscript{20} The results of present study would be cost effective to combat the hereditary disorders of hemoglobin in different high risk communities of Orissa. How far this genetic disease burden is eluded in the society is a matter of concern for all of us for the betterment of future generations.

**CONCLUSIONS**

Hemoglobinopathies are the most common monogenic disorders of erythrocytes. India is the home of several hemoglobin variants causing much suffering to afflicted individuals and impose considerable financial, genetic and psycho-social burden on family, society and nation at large. Most common hemoglobinopathies observed out of 1,015 cases were: sickle cell trait (29.8%), sickle cell disease (7.5%), sickle cell-$\beta$–thalassemia (1.7%), $\beta$–thalassemia trait (18.2%), thalassemia major (5.3%), thalassemia intermedia (0.9%), Hb E trait (0.9%), Hb E disease (0.3%), E-$\beta$–thalassemia (0.7%), Hb D trait (0.2%) and SD disease (0.2%). Male preponderance was seen with age of presentation varying from early childhood to adult age group in sickle cell disorders, followed by $\beta$–thalassemia trait, thalassemia major in Orissa. Sickle cell disorders with high level of fetal hemoglobin are common in general castes (0.3-20.7%), scheduled castes (0-8.9%) and scheduled tribes (0-5.5%). Transfusion dependent $\beta$–thalassemia syndrome is prevalent in Brahmin, Karan, Khandyat, Teli, etc. Hemoglobin E was detected in Brahmin and Khandyat Castes and hemoglobin D in Chasa and Khandyat castes. Most of the cases belong to Anugul district, followed by Khurda, Nayagarh, Phulbani, Cuttack, Jajpur, Dhenkanal, Ganjam, Keonjhar, Mayurbhanj, etc. The heterogeneous population of Orissa is harbouring almost all major hemoglobinopathies in general castes, scheduled castes and tribes, belonging to Coastal and South-Western regions. This study provides for the first time a comprehensive database on the spectrum of hemoglobinopathies in the state.

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