Research Article

Spectrum of hemoglobinopathies in Odisha—an institutional study by CE-HPLC

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Abstract

Background: Hemoglobinopathies are common genetic disorders and represent a significant load of anemic patients coming to a hospital with various complaints.

Objective: To assess the different types of hemoglobinopathies prevalent in Odisha in relation to hematological profile and district-wise distribution.

Materials and Methods: This study was done on all the patients who had undergone high performance liquid chromatography (HPLC) study in Hi-Tech Medical College and Hospital, Bhubaneswar, Odisha, India, from 2010 to March 2015 whose complete history and data required for this study were available. Bio-Rad dual cation exchange high performance liquid chromatography (CE-HPLC) machine was used.

Result: Although it was not a screening study, of the 331 cases, 105 were found to be normal. Along with SCD and beta-thalassemia (β-thalassemia), hemoglobin E (HbE), and hemoglobin E-beta thalassemia (HbE/β-thalassemia) cases were found in significant numbers even though this region is not known for its endemicity of HbE hemoglobinopathies. Four cases of HbD-Punjab carriers were also encountered.

Conclusion: This study revealed that HbS is the most common abnormal hemoglobin in Odisha, followed closely by β-thalassemia. As in West Bengal, Odisha is also found to be endemic for HbE.

KEY WORDS: hemoglobinopathies, cation exchange high performance liquid chromatography (CE-HPLC), Odisha

Introduction

In recent days, the world harbors 269 million people with hemoglobinopathies constituting 4.5% of the world's population.[1] Recent survey suggests that between 300,000 and 400,000 babies are born with serious hemoglobin disorder each year.[2] India, an ethnically diverse country, holds an approximate population of 1.2 billion. A range of <1% to 17% and an average of 3.3% have been reported with regard to the frequency of beta-thalassemia trait (βTT).[3] This study gives a present day scenario of the spectrum of hemoglobinopathies in Odisha and highlights the differences that occur in a screening study and a study done on symptomatic patients.

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Materials and Methods

Study Design

An institutional, cross-sectional study was carried out on 331 subjects who were tested on cation exchange high performance liquid chromatography (CE-HPLC) for hemoglobinopathies owing to anemia, other hemolytic features, or relevant signs and symptoms to indicate hemoglobinopathies in the central laboratory of Hi-Tech Medical College and Hospital, Bhubaneswar, Odisha, India. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. The institution serves mostly the people from Bhubaneswar, Cuttack, Puri, Khurda, Jajapur, and several other districts of Odisha. Data from only those subjects whose complete history and data could be acquired from MRD during the study period of four-and-a-half years. Data could also be retrieved from the software Windows NT, which is inbuilt in the machine Bio-Rad D-10. Exclusion criteria included cases whose relevant history could not be retrieved from our MRD. Routine hematological parameters were measured with an automated hematology analyzer (XS800i Sysmex). Red cell

morphology and platelet counts were cross-checked with well-prepared peripheral films. In addition, hematological profile and the district-wise distribution of the subjects were done.

HPLC System and Blood Sampling

The machine used for evaluation was Bio-Rad D-10 Hb electrophoresis and HbA_{1C} dual system. The dual system provides comprehensive fully automated hemoglobin testing in a compact footprinting. Combining diabetes and thalassemia testing on a single platform, the system offers simple and efficient operations for clinics and clinical laboratories. When in doubt, the cases were confirmed with capillary electrophroresis. Sickling test was performed whenever S window had to be confirmed.

Result

All the 331 cases from 2010 to March 2015 suspected for having hemoglobinopathies were selected for this study, which included all age groups. Although it was not a screening study, of the 331 cases, 105 (31.81%) of them were found to have normal hemoglobin levels, in spite of being symptomatic. Sixty-two (18.73%) cases showed βTT. Four (1.21%) cases revealed β-thalassemia minor. Four (1.21%) cases showed α-thalassemia trait (αTT), and seven (2.11%) cases presented β-thalassemia major. Therefore, 77 (23.26%) cases were thalassemia cases.

Fifty-one (15.40%) cases revealed sickle cell trait (SCT), and 34 cases (10.27%) showed sickle cell disease (SCD). Therefore, 85 (25.67%) cases presented with sickle cell disorder.

HbS/β-thalassemia constituted 23% (43) of cases [Table 1]. Thalassemia is prevalent more in Bhubaneswar, Cuttack, Khurda, and Puri, while SCD is more prevalent in Bhubaneswar, Cuttack, Khurda, and Angul [Table 2]. We have to accept the bias caused owing to our institution getting most of its cases from these regions. Moreover, population in Bhubaneswar and Cuttack are cosmopolitan in nature, and inhabitants have come from all over Odisha and India. Interestingly, a relative high prevalence of HbE was found in this study. Five (1.51%) cases showed HbE trait, three (0.91%) cases HbE disease, and nine (2.71%) cases HbE/β-thalassemia.

About 83% of hemoglobinopathy cases in this study presented a normal or slightly subnormal mean corpuscular hemoglobin concentration (MCHC), while only 17% of cases of hemoglobinopathy presented a low MCHC.

Four cases of another hemoglobin variant, the HbD-Punjab trait, were detected.

Discussion

This study gives an important insight to the present day scenario of hemoglobinopathies in symptomatic patients in Odisha in relation to hematological profile. Hence, together SCD and thalassemia constitute 61.93% of cases with SCD, being the most common hemoglobinopathy.

Several other studies have been done in different states, which include, a study done by Madan et al.,[3] which reported the frequency of β thalassemia to be 5.47% and 2.68% in school children of Delhi and Mumbai, respectively. A study was done primarily with patients of West Bengal and nearby areas, in which βTT was the commonest disorder (9.62%), followed by HbE trait (4.92%), HbE/β-thalassemia (3.67%), and persistent fetal Hb (1.9%). Other hemoglobinopathies were relatively rare (<1%).[4] The average frequency of *HbE* gene in Indian population is about 10.9%.[5] HbE allele was detected in heterozygous and homozygous forms in Delki Kharia tribe for the first time in Sundergarh district of Odisha. However, the cases of HbE had earlier been reported in other caste populations in the coastal region of Odisha, West Bengal, and northeastern parts of India (Balgir 2004, 2005b). The presence of HbE among the Delki Kharia tribe suggests the admixture with other tribes and/or nontribal populations of West Bengal or northeastern India among whom this trait is quite frequently observed (Balgir 1995, 2003).[6] Overall, the prevalence of βTT and SCT in South Gujarat was 4.4% and 1.3%, respectively.[7] Moreover, β-thalassemia was the commonest hemoglobinopathy in Uttar Pradesh.[8] An excellent study was done on the spectrum of hemoglobinopathy in Odisha, the study period of which was from 1994 to 2003 by Balgir.[9]

Knowing West Bengal is endemic for HbE, the study surprised us to see a strikingly high prevalence of HbE in Odisha. This raises a question if these cases were truly from Odisha or were they immigrants from neighboring state West Bengal. On tracing back the history, of the 17 cases, six were immigrants from Medinipur, while others were originally from Odisha. Hence, indicating that HbE is not very uncommon even in this region.

Four cases of HbD-Punjab or HbD-Los Angeles were found. It was prevalent in the Punjab region. The heterozygous state is clinically asymptomatic and its homozygosis is the rarest form of inheritance.[10]

Districts in Odisha, which are endemic for malaria, are also endemic for SCT and αTT. The carriers of α-thalassemia are more difficult to identify than β-thalassemia carriers, because they do not show typical HbA_2 and HbF levels.

When compared with screening studies in this region, this study on symptomatic patients suggested a higher prevalence of hemoglobinopathies. The prevalence of SCD/β-thalassemia was much higher in symptomatic cases (23%) than in asymptomatic cases (1.7%).[10] Patients with $HbS\beta^0$-thalassemia show a slightly higher Hb level, greater HbA_2 level (4%–6%), and a smaller mean corpuscular volume (MCV) (65–75 fL) than those with HbSS. $HbS\beta^+$-thalassemia show higher Hb levels and lower reticulocyte count than those with $HbS\beta^0$-thalassemia.

Cases of HbE disorders were nearly double in symptomatic cases (5.13%) than in asymptomatic cases (2.80%).[9]

The lowest average MCV and MCH were found in thalassemia major and then in TT, while the MCHC was relatively not low, as seen in iron deficiency anemia.

The lowest Hb concentration was found in thalassemia major (2.6 gm/dL). Surprisingly, a case of HbD-Punjab

Table 1: Assessment of hematological parameters in different groups of patients

Diagnosis (μ = 331)	No. of cases (%)	Hb (gm/dL)	Hematocrit (%)	RBC (×10^6/mm^3)	MCV (fL)	MCH (pg)	RDW (%)
Normal	105 (31.81)	12.3 ± 2.0	36.8 ± 5.8	4.50 ± 80	86.1 ± 10.8	27.9 ± 3.1	14.8 ± 5.0
Minimum		6.2	18.5	2.21	50.1	16.8	11.9
Maximum		16.3	50.2	6.47	110.1	34.6	33.6
βTT	62 (18.73)	10.6 ± 1.8	34.4 ± 5.9	5.30 ± 0.94	67.5 ± 5.8	20.5 ± 2.2	16.9 ± 4.0
Minimum		3.4	16.4	2.80	54.7	15.3	12.4
Maximum		14.5	43	6.68	89.4	31.2	38.2
SCT	51 (15.40)	12.1 ± 8	36.9 ± 8.8	4.50 ± 0.78	81.0 ± 6.5	26.6 ± 3.2	14.7 ± 1.8
Minimum		6.2	20.5	2.80	64.0	20.0	12.5
Maximum		13.9	39	6.68	91.2	30.4	19.0
HbS/βTT	43 (13)	8.1 ± 1.0	26.6 ± 4.1	3.44 ± 0.55	74.3 ± 4.2	22.8 ± 1.5	22.8 ± 5.2
Minimum		5.9	21.7	1.80	69.8	22.1	17.5
Maximum		11.8	32.7	4.38	78.9	25.4	27.2
HbS disease	34 (10.27)	8.0 ± 1.3	25.5 ± 3.2	3.00 ± 0.33	82.2 ± 7.3	26.0 ± 3.3	21.3 ± 7.0
Minimum		3.9	11.0	2.4	70.1	22.0	16.4
Maximum		11.1	32.0	3.80	90.2	32.1	34.2
HbE/β-thalassemia	9 (2.71)	6.2 ± 1.7	21.5 ± 5.0	3.48 ± 0.88	64.4 ± 9.0	18.5 ± 3.0	29.3 ± 5.9
Minimum		5.5	16.0	1.90	50.9	16.4	12.5
Maximum		10.9	37.5	4.40	89.0	30.4	48.7
HbE trait	5 (1.51)	11.7 ± 1.9	35.5 ± 5.8	4.67 ± 0.66	77.0 ± 5.5	25.1 ± 2.3	15.8 ± 6.0
Minimum		4.7	16.4	1.55	50.2	20.1	12.1
Maximum		14.1	45.9	5.12	92.1	29.6	37.6
HbE disease	3 (0.91)	9.0 ± 2.3	28.8 ± 6.5	4.89 ± 1.05	61.7 ± 6.3	19.2 ± 2.5	19.7 ± 4.0
Minimum		5.8	24.3	2.54	60.3	16.7	15.2
Maximum		12.4	37.5	5.45	81.4	26.0	33.6
β-thalassemia major	7 (2.11)	6.6 ± 4.6	21.5 ± 14.5	2.96 ± 1.45	73.2 ± 11.4	22.1 ± 4.9	31.4 ± 7.1
Minimum		2.7	10.3	1.80	61.5	18.6	16.7
Maximum		6.9	21.3	3.98	82.1	34.0	37.5
β-thalassemia minor	4 (1.21)	10.8 ± 1.8	34.4 ± 5.9	4.68 ± .54	74.8 ± 10.7	25.2 ± 3.3	16.3 ± 4.2
Minimum		9.1	28.3	3.32	62.1	17.0	12.8
Maximum		13.0	40.3	4.98	84.0	30.2	16.4
αTT	4 (1.21)	10.7 ± 1.9	34.3 ± 5.8	4.65 ± 0.77	72.3 ± 11	22.6 ± 2.3	15.8 ± 4.0
Minimum		9.2	28	3.16	59.1	16.4	13.4
Maximum		Max13.5	39.8	5.24	87.8	32.1	Max21.4
HbD-Punjab trait	4 (1.21)	11.6 ± 2.4	21.5 ± 10.0	3.98 ± 0.94	85.6 ± 5.1	28.4 ± 1.6	19.5 ± 11.6
Minimum		2.8	10	1.26	56.8	18.5	13.7

Continuous variables presented as mean ± SD.
*Values may be unreliable owing to small number of observations.
Normal, normal hemoglobin analysis in HPLC.

revealed an apparently low Hb concentration of 2.8 gm/dL, suggesting an overimposed cause of anemia as well. On studying the case, it could have been a case of anemia of malnutrition, this case being open to further studies.

This study also emphasizes on a strong correlation between MCHC of normal or slightly subnormal levels and low MCV and MCH levels in hemolytic anemia, when compared with anemia owing to iron deficiency anemia, in which MCHC is also low along with low MCV and MCH values.

The strength of this study is that the results of CE-HPLC were confirmed with capillary electrophoresis and sickling test whenever needed.

Table 2: Distribution of the study population (*n* = 331) in the districts of Odisha

District	All	Normal	βT/βTT	SCD/SCT	SCBT	HbE	Others
Bhubaneswar	76	27	13	14	1	5	6
Cuttack	50	12	12	12	8	3	3
Khurda	38	9	12	1	5	1	1
Puri	33	13	8	6	4	1	1
Jajapur	19	10	3	3	3		
Kendrapara	10	3	2	3	2		
Jagatsinghpur	10	4	2	3	1		
Angul	16	4	3	7	1		
Bauda	11	2	5	3	1		
Sambalpur	16	6	4	4	2		
Sundergarh	14	4	3	5	2		
Kalahandi	10	3	2	4	1		
Balasore	12	5	2	4	1		
Mayurbhanj	9	3	2	3	1		
Medinipur (WB)						6	

Limitation of this study would be that Bhubaneswar is a cosmopolitan city; hence, as our institution is based in Bhubaneswar, a true representation of the population of Odisha may not be reflected.

Conclusion

This study revealed that HbS is the most common abnormal hemoglobin in Odisha, followed closely by β-thalassemia. Hence, all women coming for antenatal checkup and presenting microcytic and hypochromic anemia with normal MCHC should be screened for thalassemia, as it would prevent complications in the newborn owing to hemoglobinopathies and provide them with proper counseling. Similarly, children attending pediatric outpatient department with anemia and normal or slightly subnormal MCHC should be subjected for HPLC to exclude hemoglobinopathies, so as to initiate better management with early diagnosis.

Similar to West Bengal, even Odisha is endemic for HbE; hence, expecting it is better than disregarding it as iron deficiency anemia on HPLC.

CE-HPLC is not only a useful tool in screening study but also reliable and laborsaving as a diagnostic procedure.

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