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Spectrum of Hemoglobinopathies Diagnosed by High-Performance Liquid Chromatography in Western Odisha

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Abstract
Hemoglobinopathies are the most common genetic disorders reported in the World. The aim of our study was to establish the spectrum of the different haemoglobinopathies presenting in a tertiary medical college in western Odisha to help develop management, prevention and control programmes in the community. Patients who had any form of haemoglobinopathy underwent HPLC analysis, With regard to the different haemoglobin fractions our study revealed that HbSS (sickle cell disease) is the most common abnormal haemoglobin in Western Odisha, followed by HbAS (Sickle cell trait).Both beta thalassemia major and minor were associated with sickle cell haemoglobinopathy, more commonly with sickle cell disease. Castes like kulita, harijan, aghariya and chasa presented with a higher incidence of different haemoglobin-opathies. HbE was found to be very uncommon. HbF was found to have a higher level in all haemoglobinopathies
Keywords: *Haemoglobinopathies, western Odisha, high-performance liquid chromatography, sickle cell disease, sickle cell trait.*

Introduction

Hemoglobinopathies are the most common genetic disorders reported in the World[1,2]. Hemoglobinopathies constitute entities that are generated by either abnormal haemoglobin or thalassemias. While abnormal haemoglobin is caused by a qualitative structural abnormality of the haemoglobin molecule, thalassemias result by

diminished synthesis of the globin chain. Sickle cell disease (SCD) is one of the most common monogenic disorders globally with an autosomal recessive inheritance [3]

The occurrence has been reported to be the highest in the Middle East and the Indian subcontinent. Hemoglobinopathies are a significant problem in India and Sickle cell anaemia is a common haematological disorder, prevalent in many parts[4,5]. Odisha which has a varied genetic diversity of hemoglobinopathies presently faces a major genetic and public health problem from this aspect. More specifically western Odisha contributes to the major bulk of the prevalence recorded in the state. High-pressure liquid chromatography (HPLC) is one of the best methods for screening and detection of various hemoglobinopathies which was used for establishing the diagnosis of the patients [6].

The aim of our study was to establish the spectrum of the different haemoglobinopathies presenting in a tertiary medical college in western Odisha to help develop management, prevention and control programmes in the community.

Material & Methods

This study was carried out at a tertiary care centre in western Odisha between June 2004 to May 2006. 150 patients were included in the study. From among the patients admitted in the department of medicine or visiting the medicine OPD those who were detected to have anaemia clinically or were diagnosed with anaemia after investigation or had a family history or past history of any hemoglobinopathy were subjected to HPLC test. From among these patients, those who had any form of haemoglobinopathy were Included in the study. The results of the HPLC study with regard to the different haemoglobin fractions obtained were analysed.

Results

Table 1: Age distribution

Age (in yrs)	Male	Female	Total	Percentage
15-20	22	10	32	28.8
21-25	18	7	25	22.5
26-30	14	3	17	15.3
31-35	13	2	15	13.3
36-40	7	2	9	8.1
41-45	5	1	6	5.4
46-50	2	0	2	1.8
>50	4	1	5	4.5
	85	26	111	100

A maximum number of patients with hemoglobinopathies were in 15-20 year age group.

Table 2: Sex distribution

SEX	NO	PERCENTAGE
Male	85	76.05
Female	26	23.4
Total	111	100%

In our study, the males outnumbered the females by 3:1.

Table 3: Demographic distribution as per caste

CASTE	NO OF PATIENT	PERCENTAGE
Kulita	29	26.1
Harijan	27	24.3
Agharia	14	12.6
Chasa	14	12.6
Teli	7	6.3
Khyatriya & Khandayat	3	2.7
Dumal	3	2.7
Fisherman	2	1.8
Milk man	2	1.8
Others	10	9
Total	111	100%

In our study hemoglobinopathies are more common in Kulita (26.1 %), Harijan (24.3%), Agahria (12.6%) &Chasa (12.6%) but in other castes, it is less prevalent.

Total 111 patient were detected with some hemoglobinopathy. From 85 (76.5%) were male & 26 (23.4%) were female. It is seen that most of the hemoglobinopathies come under age group 21-30 of age (37.8%) most of them are male (27.9%) Hemoglonopathy above 50 years in less frequent (4.5%).

Table 4: Distribution Of Hb Ao Level In Both Sex In Different Age Group

Age group	Now of Male	Mean Hb Ao	No of Female	…	Total	Percentage
10-20	23	3.32	9	3.4	32	3.3
21-30	31	2.8	11	2.3	42	2.5
31-40	20	3.3	4	4.9	24	4.2
41-50	7	1.04	1	2.2	8	3.1
>51	4	2.9	1	3.7	5	3.3

There was no significant difference in Hb Ao level as seen in either sex in different age group. The mean level of Hb in patients with HbAo was between 2-5 in most of the age groups.

Table 5: Distribution of Hb A2 level in both sexes in different age group.

Age group	No of Male	Mean Hb A0	No of female	Mean Hb A0	Total	Mean
10-20	23	2.01	9	2.25	32	2.1
21-30	31	2.76	11	2.4	42	2.55
31-40	20	2.69	4	2.85	24	2.7
41-50	7	2.69	1	4.4	8	3.5
>50	4	3.2	1	3.3	5	3.25
Total	85		26		111	

From the above table, it is seen that there is no significant difference between both the sexes in different age groups except it 41-50 yearage group.

Table 6: Distribution of Hb F Level In Both Sexes In Different Age Group

Age group	No of Male	Mean Hb F	No of female	Mean Hb F	Total No	Mean Hbs
10-20	23	19.9	9	18.7	32	19.3
21-30	31	21.1	11	21.1	42	21.1
31-40	20	16.65	4	10.1	24	13.38
41-50	7	13.9	1	0	8	13.9
>50	4	19.6	1	20.8	5	20.2
Total	85		26		111	

There is significant HbF fraction variation between both genders in theage group of 31-40 and 41-50 years. The males of the age group of 31-40 years had a HbF level of 16.65 and the females had a HbF level of 10.1. In the age group of 41-50 years, males had a HbF level of 13.9 in comparison to a low level in the of females of the same group.

The HbF level remains high (>10) in all age group. Mean fetal Hb in both sexes of 21-30 years is equal. In all age groups, males have higher HbF level than females

Table-7: Distribution Of HbS Level In Both Sexes In Different Age Group

Age group	No of Male	Mean HbS	No o female	Mean Hb S	Total No
10-20	23	60.57	9	59.1	32
21-30	31	60.8	11	62.8	42
41-50	20	51.4	4	51.65	24
>50	7	47.1	1	0	8
Total	4	39.3	1	63	5
	85		26		111

There is no significant variation of HbS level in both sexes of all age group except more than 50 years age where no case in the group.

Table-8: Distribution Of Patients With HbS Window <50 (Sickel Cell Trait) &>50 (Sickle Cell Diseases)

Age Group	Hbs>,50	Percntage %	Hbs <50	%
10-20	29	26.1	3	2.7
21-30	40	36.03	1	0.9
31-40	16	14.01	8	7.2
41-50	3	2.7	3	2.7
>50	4	3.6	0	0
Total	92	82.88	15	13.51

As shown in the table from the 111 patients who were having some hemoglobinopathies 92 (82.8%) patients were having Hbs window (>50%) so were acase of Sickell Cell Diseases & Are 15 Patient Were With Hbs Window <50 So Are Sickell Cell Trait Patients.

Out of 92 patients with sickle cell diseases, most of the patients (40) are in age group 21-30 (36.03%) followed by 10-20 years 29 (26.1%) only 3 patients (2.7%) remains in theage group 41-50 years. Age group in >50 years. Constitute also only 4 patients.

From the 15 (13.51) patients detected on sickle cell trait maximum no belongs to age group 31-40 years (7.2%). There is no patient in more than 50 year age group.

Table-9: Sex Distribution In Patients With HbS Window

SEX	<50	%	>,50	%
M	10	9.09	74	67.27
F	5	4.54	18	16.36

The above table gives the sex distribution of the sickle cell trait &sickle cell diseases (>50) patient. In sickle cell traits males (10) outnumbered female (5) by 2:1.

In sickle cell diseases patients male predomination is the observation. 74 (67.27%) males has outnumbered female (18) by 4:1

Table-10: Distribution Of HbF In Patients With Sickle Cell Disease (Hbs>,50)

Age group	HbF<18	%	HbfF> 18	%
10-20	8	7.2	21	18.91
21-30	11	9.9	28	25.22
31-40	2	1.8	14	13.51
41-50	2	1.8	2	1.8
>50	0	0	4	3.6
Total	23	20.72	60	62.16

Table-11: Sex Distribution In Persons With Sickle Cell Disease (Hbs>, 50)

SEX	HbF< 18	%	HbF> 18	%
M	19	17.11	56	50.45
F	4	3.6	14	12.61

As shown in table 10& table 11 from the 92 patients with sickle cell diseases 69 (62.16%) have HbF>18. Max no of patients belongs to age group 21-30 (25.22%) with HbF> 18. There isa wide variation in HbF level in thedifferent age groups. 41-50 years constitute the least no of patients of (2) with HbF>18.

Sex distribution showed that the no of males is quite high than females (14) in patients with HbF>18. Also, maleshave ahigh incidence (11 patient)in theage group 21-30. With patients of HbF<18 total no of male patients19 (17.1%) in compassion to female 4 (3.6%)

Table-12: Age Distribution Of Patients With Hb A_2 Fraction >4 (B-thal)

Age group	A_2<4	%	A_2>, 4	%
10-20	32	28.82	0	0
21-30	43	38.73	1	0.9
31-40	23	20.72	2	1.8
41-50	5	4.5	1	0.9
>50	3	2.7	1	0.9
Total	106	95.49	5	4.5

Table-13: Sex Distribution Of Patients With Hb A_2>,4 (B-thal)

Sex	A_2<4	%	A_2>4	%
M	83	74.7	3	2.7
F	23	20.7	2	1.8
Total	106		5	

From the above tables, it is shown that 5 patients (4.5%) Hb A_2 level >, 4 so B-thalassemia and max are in age group 31-40 years. Males have ahigher incidence than females in this group (3:2).

Table-14: Distribution Of Patient With Hb A_2>, 4 & Presence Of S Window (Scd & Sct)

Age group/ S Window	Present	%	Absent	%
10-20	0	0	0	0
21-30	1	0.9	0	0
31-40	2	1.8	0	0
41-50	0	0	1	0.9
>50	0	0	1	0.9
Total	3	2.7	2	1.8

Table 15: sex distribution

Age group/ S Window	M	F	T	M	F	T
10-20	0	0	0	0	0	
21-30	0	0	0	0	1	1
31-40	2	0	2	0	0	0
41-50	0	0	0	0	0	0
>50	0	0	0	0	0	0

In our study out of the patients with Hb A_2> 4 (B thalassemia) 3 of (2.7%) of them have S window, so they were categorised as having sickle cell haemoglobinopathy with beta thalassemia.

Both the SCT with B thalassemia belongs to 31-40 years of age. The sickle cell diseases with b thalassemiabelong to 21-30 years of age.

Discussion

Among the 111 cases detected with haemoglobin-opathy, most of them belong to 15-20 years of age, followed by 21-25 years of age. Incidence gradually decreased with age with only 1.8% (in 45-50 years of age) and 4.5% (after 50 years of age).This can be explained in light of cooperation study [7] in which median survival forSS patients (which constitute the major fraction of our study) was estimated at 42 years for males and 48 years in females (platt et al[8] 1994).Similar studies in Jamaika suggests median survival of 53 years[9] (Weirenga et al). Fate of asian population i.e in india and Saudi arabia is better than their African counterpart [11](Kar et al 1986)

In our study males predominates the number 85 (76.5%) than females 26 (23.4%). The maximum no of males remain in 21-30 years of age (27.9%) and maximum no of females remain in 21-30 years of age (9.9%). This can be due to ahigher number of males coming to the hospital. However, Sergant[10] (1973) found male sex predominance in their series of sickle cell disease cases.

The hemoglobinopathies detected are more common in Kulita caste 29(26.1%) followed by Harijan 27(24.3%) other castes like Agharia 14(12.6%) and chasa 14(12.6%) have also high incidence. In other communities, the incidence is less frequent. However, kar B.C. et al[11] (1986) have found that incidence of sickle cell disease in more common in Kulita, Harijan, Agharia, Chasa and other backward castes. This can also be explained in light of thestudy by Rs Balgir et al.[12] (2004) describing the prevalence of hemoglobin-opathies in Orissa. He found that transfusion dependent B-thalassaemia syndrome is prevalent in castes like Brahmin, Karan, Khandayat, Teli etc.

The mean Hb Ao level remaining low (2-5) in all age group with amaximum of 4.9 in 31-40 years females & minimum of 2.2 in females of 41-50 years of age. There is no significant difference between both sexes in different age group. The normal HbAo level is 97% which is the adult Hemoglobin which is decreased in presence of

abnormal haemoglobins like Hbs, in sickle cell disease and sickle cell trait.

It is observed that the mean HbA2 level remains low between 2-4.4, with max mean HbA2 level in females of 41-50 years of age and minimum HbA2 level in males of 10-20 years. There is no significant difference in both sexes of different age group. HbA2 is a minor haemoglobin in anadult red cell with arange of 1.5 – 3.2% at Ist year of age. HbA2 elevation is a feature of some thalassemia and diagnosis of thalassemia done with HbA2 level >=4, as evident by a study was done by Tyagiet al[13] 2003 detecting 31 cases of β homozygous Thalassemia with high HbA2 level (>3.9%) and 8 cases of β heterozygous thalassemia. Study of sabo G et al. 1988 and Shafer et al. [14](1996) had also similar results.

It is seen that mean HbF level remains high in all age groups. The maximum mean HbF lies in theage group of 21-30 in both sexes. The HbF level remains higher in males of most age group except in females > 50 years of age ,this is in acordance to studies done by steinberg et al [15]. Sergeant et al.[10] (1985) suggested that the HbF levels are consistently higher in females the difference being significantly higher after theage of 10 years and may partly due to hormone effects of puberty. E1 – Hazmi MA (1994) showed fetal haemoglobin was higher in female population compared to males in same age group after theage of 30 years. The difference in thevalue of HbF in the male and female population becomes more apparent in sickle cell disease. The age, sex and genetic disorders of haemoglobin are factors that affect HbF level and indicates the possible involvement of an x – linked factor in thecontrol of HbF population.

The study done in Saudi arabia by Acquaye et al (1984) suggested avarageHb F level patients was between between 22-26.8%. kar et al[11] (1986) compared patients from odisha State(India) to Jamaican patients with sickle cell diseases. They showed theprotective level of HbF was on average 16.64% with arange of 4.6% to 31.5%. Saltzer et al (1992) reported on five black families with abnormally high levels of fetal haemoglobin (19-45%HbF). Koshy et. Al (1989) reported that fetal haemoglobin levels above 10% were associated with fewer chronic leg ulcer in patients with sickle cell diseases.

There is no significant different in HbS level in age group 10-20 & 20-30 years. All age group & sex Hbs S level remains high except a female of 41-50 years of age implying most of the patients with having some sickle cell disorder.

From the 111 patients with hemoglobinopathies, 92 (82.88%) have Hbs window>,50 & 15 (13.51) having Hbs window<50 signifying presence of sickle cell diseases & sickle cell trait respectively. Out of 92 patients withsickle cell diseases most of them remain in 21-30 years age group 40 (36.02%) followed by 10-20 years of age 29 (26.1%). Incidence gradually decreases with increasing age. In sex distribution of sickle cell diseases of thetrait, most of them belong to themale group. In sickle cell trait male outnumbered female with 2:1, in sickle cell diseases patient males predominates 74 (67.27%) in comparison to females (16.3%). . Rs balgir et al 2004 found most common hemoglobinopathy to be sickle cell trait (29.8%) in a cohort study of 1500 cases in a span of 10 years. Other hemoglobinopathies were sickle cell diseases (7.5%), sickle cell βthalassemia (1.7%), βthalassemia trait (18.2%).Shafer et al. (1996) also screened 2 million infants and detected 492 with sickle cell diseases, 290 (58.9%) were with HbSS, 143 (29.0%) were with HbSC.& 47 (9.5%) were with S (bêta) thalassemia. This is also in accordance with the study done by kar et al. 1986 taking 627 patients.

The distribution of age & sex in patients with HbS window >,50 with HbF either <18 or 18 shows that majority 69(62.16%) of thepatientsbeing males are having Hbf F>18 level. The maximum position of patients with HbF >18 belongs to 21-30 years followed by 10-20 years (18.91%). All the patient above 50 years have HbF > 18 . Balgir RS et al 2004 showed that high level of fetal Hb

is common in Sickle cell hemoglobinopathy (0.3-20.78).

It is observed that 5 patients out of 111 hemoglobinopathies have Hb A_2 disorder (thalasemia) with maximum percentage remaining in 31-40 years of age (1.8%). The sex distribution shows that males predominate (3) in comparison in female (2).other anemia with high Hb A_2like in megaloblastic anemia can be excluded by doing MCV, vitamin B12 level. Tying et al 2003 detected βthal is 31 cases from 83 patients on the basis of High HbA_2 level (>3.9%). RS Balgir et al 2004 studied hemoglobinopathies in Orissa & detected of sickle cell B Thalassemia (1.7%), B Thalassemia trait (18.2%) thalassemia major (5.3%), thalassemia major (5.3%), thalassemia inter media (0.9%) .Kar et al 1986 had also similar results in his study.

The patients with sickle cell B thalassemia 2 of thanhaving sickle cell trait (Hbs >50) & 1 of them have sickle cell diseases (Hbs >,50). Sabo Get al 1998, Rs Balgir et al 2004,shaferetal 1996, David f keran et al 2003 &vonne. A Danniel had also similar observation of sickle cell with B thalassemia in their studies.

A single case of sickle cell diseases associated HbE was detected. Tyagi et al (2003), sabo G et al 1988, B Vonne. A Danniel et al 2005 had also detection HbE disorders but with lower prevalence .Balgir et al (2004) had a result of Hb E trait (0.9%), HbE disease(0.3%)

Conclusion

Our study revealed that HbS (sickle cell disease) is the most common abnormal haemoglobin in Western Odisha, followed by HbAS (Sickle cell trait). Beta thalassemia is also a common type of haemoglobinopathies. Both beta thalassemia major and minor were associated with sickle cell haemoglobinopathy, more commonly with sickle cell disease. Castes like kulita, harijan, aghariya and chasa presented with a higher incidence of different haemoglobinopathies. HbE was found to be very uncommon. HbF was found to have a higher level in all haemoglobinopathies. HPLC is a useful screening tool as well as a diagnostic procedure. Our study underlines the importance of HPLC both as a screening tool as well as a diagnostic procedure for detecting different hemoglobinopathy

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