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SICKLE CELL DISORDER: A FAMILY STUDY WITH EIGHT HOMOZYGOUS AND TWO HETEROZYGOUS CHILDREN

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ABSTRACT

Sickle cell anaemia is a universal haemoglobin genetic disorder exhibiting segregation in many families but according to our find, this large family of eight homozygous cases, is the largest single family reported in the world literature.

Keywords: Sickle cell disorder, rare family with many sicklers; need for counselling centres; Tribals need regular study; sickle ell syndrome; largest number with homozygotes in a family.

INTRODUCTION

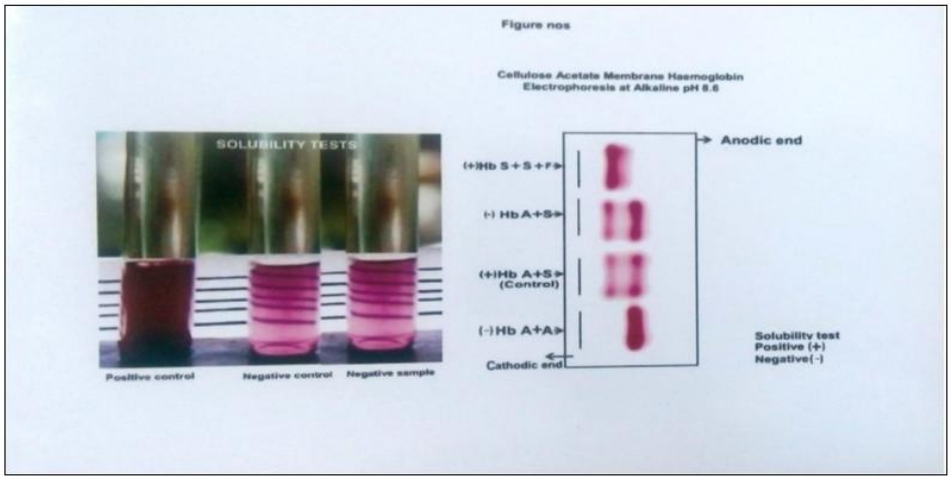
Sickle cell disease is a common clinical problem in most part of India. However it is found with high prevalence in central and south India, being mostly confined to scheduled tribes (ST), scheduled caste (SC) and other backward communities (OBC) (Roshan *et al.,* 2014). Highest prevalence is recorded among ST population from Gujarat, Maharashtra, Madhya Pradesh, Chattisgarh, Odisha and Kerala states (Rao 1988, Mohanty *et al.,* 2002). One of us (KSL) has conducted population genetics surveys in different parts of Maharashtra and found high prevalence in Bhill and Pawara tribal population groups from Satpuda hilly ranges of northern Maharashtra (heterozygous 1 in 5). The common clinical symptoms found are anaemia, mild jaundice and severe joint pains. Vaso-occlusive crisis remains a common casue of mortality. All symptoms recorded in medical literature except priapism and leg ulcers are found in these populations (Kate *et al.,* 2002). High prevalence of alpha thalessemia and increased amount of fetal hemoglobins are likely to ameliorate the features of sickle cell disease (Mukherjee MB *et al.,* 2000).

MATERIALS AND METHODS

We established a community control centre popularly known as “Sickle Cell Dawakhana” in the high risk area of Satpuda Hilly Ranges (Roshmal Budurk, Tal Dhadgaon Dist. Nandurbar. Maharashtra 23.5 N – 75 E) and provided the following facilities to the local tribal Bhil and Pawara Communities.

- Diagnostic facilities
- Physical examination, possible treatment & follow-up of patient.
- Population genetics-screening program.
- Health education.
- Improvement in quality of life of homozygous patients.
- Family studies.
- Marriage counselling, family planning.
- Guidance for Prenatal diagnosis.
- Training Program.

3253 patients are taking regular treatment and more than 2.5 lakh tribal people have received counselling at the centre.



Blood samples were collected by finger prick method (about 5-6 drops) in 3 ml tubes, containing EDTA saline solution. The packed cells are obtained by centrifuging the tube. Solubility test is performed by using 20 µl of the packed cells. Remaining cells are lysed by half ml of distilled water to obtain haemoglobin solution. Electrophoresis is performed on haemoglobin solution at alkaline pH (8.6) by using cellulose acetate membrane as a supporting medium. Since this is a very remote area only these two tests were possible in this area.

The diagnostic criteria used for the identification of cases are as follows.

Solubility test	Electrophoresis pattern	Sickle cell status
Negative	A+A	Normal
Positive	A+S	Heterozygous (Carrier)
Positive	S+S	Homozygous

In June 2002 index patient (II_1) attended the clinic with the complaints of moderate Anaemia, yellowish sclera and joint pains. After physical examination, followed by laboratory tests, he was found to be suffering from sickle cell anaemia. He was given medications and counselling.

A visit to his residence was organised for collecting the blood samples of all possible family members. While collecting demographic data, we found 12 family members (parents and 10 children). Blood samples of all the 12 members were collected by finger prick method.

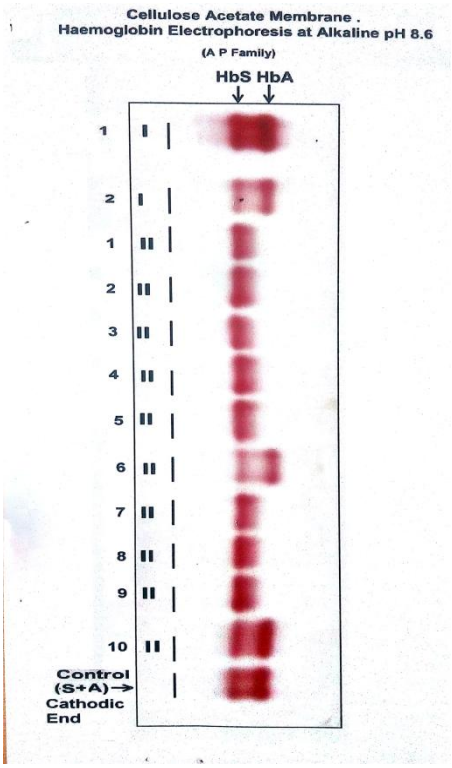
Second visit to the family members was organised for counselling. After a written consent, 2 ml venous blood sample of each family member was collected in K_2EDTA. These samples were brought to Pune and analysed on Coulter AcT diff automated blood cell counter for complete blood counts and were subjected to High performance liquid Chromatography (Bio-Rad Variant) for the separation of haemoglobins.

Observations

The family studied comprised of parents and ten children. Father 44 yrs, and mother 38 yrs. The 10 children were from age 4 yrs to 22 yrs. There were 6 males and 4 females.

After performing the screening laboratory tests we found that both the parents were carriers for Hb S, eight

children were found to be homozygous and two children were carriers for sickle cell disorder (Table 1).



FAMILY ELECTROPHOROTOGRAM

The haematological parameters studied in the family are shown in Table 2. The haemoglobin range observed was 8.0 gm% to 13 gm%. Thus all the cases had low Hb. The father had Hb of 13.0 gm% and the mother had Hbof 10.8 gm%. The two heterozygous children had Hb of 12.3 gm% and 8.0 gm% respectively. Thus the HbS heterozygous cases had a mean Hb of 11.02 gm%. The eight Hb S homozygous children had Hb range from 8.1 gm% to 10.0 gm% with a mean Hb of 9.5 gm%, which was lower than the Hb S heterozygous cases.

While studying the type of anaemia it was found that all the cases had microcytic anaemia with the MCV range of 64.8 fl to 72.3 fl. with a mean MCV of 67 fl.

The mean RBC count of the HbS heterozygous cases was 5.4 m/cmm with a range of 4.0 to 6.13 m/cmm, whereas the HbS homogygous cases had mean RBC count of 4.2 m/cmm and the range of 4.1 to 6.4m/cmm.

The total WBC count of the HbShetrozygotes ranged from 4,800 to 8,700/cmm, with a mean of 7,000/cmm. The HbS homozygous cases had a higher mean TLC of 10,100/cmm with a range of 6,200 to 18,500/cmm. The total WBC count of three HbS homozygotes was high, all the rest had normal total WBC counts. Interestingly the three HbS homozygotes who had high WBC also had high platelet counts as well, which was probably the effect of auto splenectomy.

HPLC study

Of the 10 children 8 were homozygous for Hb S. (5 males and 3 females). The other two children one male & one female were Hb S heterozygous. The parents and the two heterozygous cases revealed HbA+S pattern with HbS range from26.2% to 26.4%. The eight homozygous cases had the HbS from 73.2% to 82.1%.

The foetal Hb of both the parents was 0.7%. The two heterozygous children had Hb F values of 0.6% and 1.7 % respectively, however the homozygous HbS cases had fetalHb ranging from 6.1 to 19.3%.

The HbA2 range in heterozygous cases was 3.4 to 3.9% and the homozygous cases had the range from1.1 to 6.2%.

Table 1. Family investigations for sickle cell disorder

Code	Relation	Age	Screening test	
			Solubility test	Electrophoresis
I 1	Father	44	Positive	A+S
I 2	Mother	38	Positive	A+S
II 1	Son	22	Positive	F+S
II 2	Daughter	20	Positive	F+S
II 3	Son	18	Positive	F+S
II 4	Daughter	16	Positive	F+S
II 5	Son	14	Positive	F+S
II 6	Son	12	Positive	A+S
II 7	Daughter	10	Positive	F+S
II 8	Son	8	Positive	F+S
II 9	Son	6	Positive	F+S
II 10	Daughter	4	Positive	A+S

Table 2. Sickle cell investigational quantitative data

Code	Hbgm%	RBC mill/ cmm	PCV%	MCV fl	MCH picogm	MCHC %	WBC/cmm	Platlet lac/cmm	HbA%	HbF%	HbS%	HbA2 %
I_1	13.0	6.13	41.0	67.0	21.0	32.0	4800	1.7	62.5	0.7	26.4	3.9
I_2	10.8	5.43	35.0	65.0	20.0	31.0	6700	1.28	62.9	0.7	26.3	3.4
II_1	8.1	4.27	27.0	64.8	19.0	29.4	18500	5.59	-	6.1	75.5	1.7
II_2	9.2	4.11	29.0	72.3	22.4	31.0	8900	1.76	-	12.9	73.2	3.4
II_3	9.2	6.41	21.0	66.0	20.2	30.6	9100	1.06	-	19.3	73.4	1.4
II_4	9.8	4.46	31.5	70.6	22.0	31.0	12300	7.37	-	13.1	75.0	3.2
II_5	10.0	4.69	33.6	70.3	21.5	30.6	11300	3.51	-	17.9	76.0	1.5
II_6	12.3	6.11	40.0	65.4	20.1	30.7	7800	2.6	62.7	0.6	26.2	3.7
II_7	9.8	4.9	32.6	66.5	20.8	31.3	7300	2.65	-	17.5	77.7	3.6
II_8	10.0	4.96	30.1	66.6	21.4	32.2	6200	3.17	-	8.8	82.1	1.1
II_9	10.0	4.6	31.0	69.2	21.9	31.6	7500	2.96	-	18.0	75.9	1.8
II_0	8.0	4.0	30.1	68.0	21.4	30.6	8700	2.6	62.3	1.7	26.2	3.7

Mutations Study

The family had mild to moderate phenotypic presentation. They were clinically quite stable and did not require any transfusion. We studied the mutations in the parents and three children. All the five had alpha thalassaemia mutation. Alpha genotype - 3.7 / - 3.7).

DISCUSSION

Sickle cell syndrome is seen worldwide with high incidence in certain populations in the world. While establishing the community centre in the high risk area of Satpuda hilly range, we came across a large family of 10 cases. Heterozygous parents had eight homozygous and two heterozygous children.

All the cases were anaemic with low MCV and were clinically stable without any transfusion support. Mutations analysis was done in five cases. Alpha gene deletion was found in all five cases, which is known for a milder phenotypic presentation.

CONCLUSION

As far as we know, this large family of eight homozygous cases which according to us, is the largest single family reported in the world literature. Dr G.R. Sergeant from Jamaica has seen maximum seven children homozygous in one family (As per personal communication).

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