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Epidemiology of Sickle Cell Disorder in the State of Maharashtra

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KEY WORDS Sickle cell anaemia; backward communities; Maharashtra.

ABSTRACT Sickle cell disease is a major genetic disorder amongst Scheduled Caste (SC), Scheduled Tribe (ST), and Other Backward Communities (OBC) population groups of Maharashtra. We modified diagnosis technique and developed simple laboratory technology to identify carrier (Hb SS) and sufferer (Hb AS) suitable for field work. In order to find out prevalence for sickle cell disorder we screened major communities from the state and found high prevalence amongst SC, ST and OBC. The overall prevalence amongst SC, ST and OBC is 10%. Severe joint pains and milder type of jaundice are peculiar symptoms amongst sicklers from the state of Maharashtra.

INTRODUCTION

Red blood cells of adult healthy human individual consists of mixture of three unique respiratory proteins known as hemoglobins. One major, with 96% concentration of the total, known as Adult hemoglobin (HbA) and other two minor with less than 2% or traces are Fetal hemoglobin (HbF) and Hemoglobin A_2 (HbA_2). The major function of hemoglobin is to transport oxygen from atmosphere to lungs and finally pass on to all vital organs. The property of combining reversibly with oxygen is unique wonder and interesting. Hemoglobin molecule is conjugated protein and is combination of four hemes and four polypeptide globin chains. Each globin chain is attached to one heme group. There are four different types of globin chains, which are Alpha (α), Beta (β), Gamma (γ) and Delta (δ). Each globin polypeptide chain is a polymer of different amino acids. α globin chain is a polymer of 141 amino acids while β, γ and δ chains each consists of 146 different amino acids. The sequence of amino acids in each globin chain is different and is very specific for that particular globin chain. The pair of α chain is common to all hemoglobins. However in adult hemoglobin (Hb A), the non α chain pair are β globin chains, in fetal hemoglobin (Hb F) pair of γ globin chains and in hemoglobin A_2 (Hb A_2) pair of δ chains.

It can be described as follows:

Hb A = $\alpha_2\beta_2$
Hb F = $\alpha_2\gamma_2$
Hb A_2 = $\alpha_2\delta_2$

The genes for α chains are located on short arm of chromosome number 16 and for β, γ and δ chains genes are located on chrom-osome number 11. The mode of inheritance is autosomal recessive type.

ABNORMAL HEMOGLOBINS

The alteration of sequence of amino acids in either of the four globin chains is termed as abnormal hemoglobin. Abnormal hemoglobins have similar structure of that of normal hemoglobin except slight alteration in the sequence of amino acids and hence may be designated as mutant or variant hemoglobin. A well known example of abnormal hemoglobin is sickle cell hemoglobin (Hb S) in which 6th amino acid (i.e. glutamic acid) is replaced by valine. (Hb S = $\alpha_2\beta_2^{\text{6-Glut-Val}}$). During last fifty years, more than 800 abnormal hemoglobins are reported in the literature. In 90 % of these there is altered sequence of single amino acid in any of the globin chains. Of these, single base mutation reported, 55 % result in β globin chain, 35 % in α globin chain and remaining γ and δ globin chain. In rest of 10 % there may be alteration of two or multiple amino acids or sometimes addition or deletion of amino acids in either of the globin chains. It is observed that β globin gene is most sensitive to single nucleotide base changes. Some of the prominent examples are shown in table number 1.

Abnormal hemoglobins with very high prevalence in world population are Hb C (West Africa); Hb D (North Western India); Hb E (West Bengal and North Eastern India) and Hb S (India, South Africa and Saudi Arabia). Most of the abnormal hemoglobins reported today are not associated with detectable clinical manifestation. Most common clinically relevant

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Table 1: Prominent examples of abnormal haemoglobin

Replacement of single amino acids	*Replacement of two amino acids*	*Deletion of amino acids*	*Addition of amino acids*
Hb C (β 6th Glut-Lys) Hb D (β 121st Glut-glutamine) Hb E (β 26th Glut-Lys) Hb S(β 6th Glut-Val) Hb Texas (γ 5th Glut-Lys) Hb Indonesia (δ 69th Glut-Arg) Hb Ananthraj (α 11th Lys-Glu) Hb Rampa (α 95th Pro-Ser)	Hb C Harlem (β 6th Glut-Val and β 73rd Asp-Asn)	Hb Gunhill (β chain - amino acid from 92nd to 96th position are deleted)	Hb Tak (β-8 extra amino acids added to C terminal end)

variants are Hb C, Hb D, Hb E, Hb O (Arab) and Hb S (all β chain variants) and occur in polymorphic frequencies in different geographical areas. Amongst all abnormal hemoglobins, Sickle Cell Hemoglobin (Hb S) is more deleterious, since in hypoxic condition it alter the shape of red cells leading to early destruction of the cells and sometime clogging the sickled red cell in microcapillaries producing tremendous, unbearable pain which does not respond to any pain killer. No other abnormal hemoglobin has such ability which ultimately leads to miserable life to patients suffering from sickle cell disease.

Abnormal Hemoglobins Amongst Indian Population

Of the genetic disorders prevalent in this country hemoglobinopathies have been most intensively studied both from case reports and population survey. There are more than dozen abnormal hemoglobins reported amongst different population groups from India. Some are sporadic confined to small community or family. Hb D, Hb E and Hb S are widely spread. Hb D is found amongst Sindhi, Punjabi and Gujrathi population groups with origin in North West India. Hb E amongst Bengali and Assami population groups while Hb S found amongst different population groups from south and central parts of India. Distribution represented in figure 1 (DESH).

Sickle Cell Hemoglobin (Hb S)

In 1904 Prof. Herrick observed that red cells of an African origin anaemic patient acquired sickle like shape instead of normal round shape. After 40 years of research it was found that hemoglobin inside the sickle red cell is mutant variant of normal hemoglobin in which 6th amino acid in β chain is replaced by valine. This was first abnormal hemoglobin reported in literature and labeled as hemoglobin B (Hb B), but because of its sickling property it is relabeled as Sickle Cell Hemoglobin (Hb S). The population survey conducted thereafter found high prevalence of sickle cell hemoglobin in different African tribal groups.

Prior to 1952, no information was available about existence of Sickle Cell Hemoglobin in India. In 1952 it was recorded for the first time simultaneously amongst tribal population groups of Nilgiri Hills and laborers in the tea gardens of Assam[1,2]. Now it is firmly established that this gene harbor amongst different caste groups but very high prevalence amongst Scheduled Caste (SC), Scheduled Tribe (ST) and Other Backward Communities (OBC).[3-15]

Sickle Cell Disorder Scenario in the State of Maharashtra

Taking into our huge population size, more than 50 % of the world's sickle cell anaemia cases are in India. It is estimated that most of the cases are in the Central and South India. During last 50 years, because of simple, reliable and inexpensive laboratory methods are available[18], the large number of population genetic surveys conducted by different scientific groups and data on geographical distribution, clinical manifestation along with its variations, available from the state of Maharashtra [3-15.]

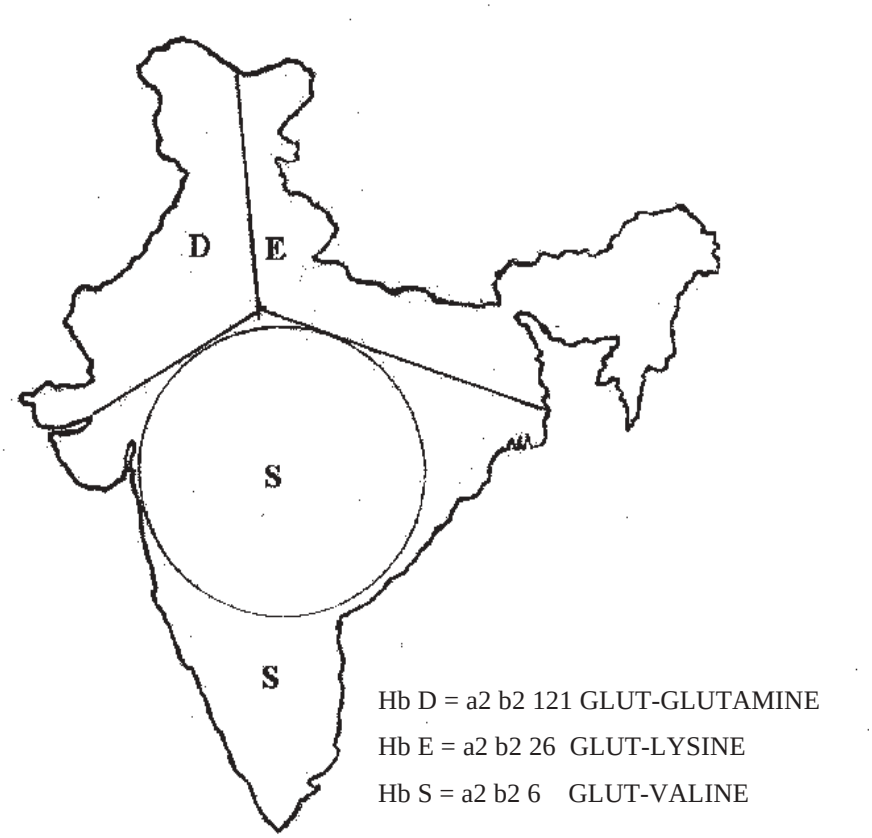

Fig. 1. Distribution of abnormal haemoglobin in India–DESH (D, E and S Haemoglobins)

METHODOLOGY

1. Solubility Test: Deoxygenated Sickle cell hemoglobin has an abnormally low solubility. A fibrous precipitate is formed when a concentrated solution of sickle cell hemoglobin is deoxygenated (This precipitate deforms red cells and gives them their sickle shape. The rate of fiber formation is proportional to about the tenth power of the effective concentration of deoxyhemoglobin S. Thus, fiber formation is a highly concerted reaction)[19]. HbS is deoxygenated form and is insoluble in phosphate buffer (giving turbidity to the solution) while other hemoglobins are completely soluble (giving clear solution).

2. Electrophoresis of Hemoglobin: Each of the major hemoglobin types has an electrical charge of a different degree, so the most useful method for separating and measuring normal and abnormal hemoglobins is electrophoresis. This process involves subjecting hemoglobin components from dissolved red blood cells to an electrical field. The components then move away from each other at different rates, and when separated, form a series of distinctly pigmented bands. The bands are then compared with the other samples on the same membrane strip called as control. Quantitation of different hemoglobins can also be made to indicate severity of any abnormality.

Electrophoresis of Hemoglobin at Alkaline pH (pH 8.6) Using Cellulose Acetate Membrane as Supporting Medium: Hb A has faster mobility than Hb A which is slower. Hb D and Hb S have similar mobility in between Hb A and Hb A_2. In case of Hb S solubility test is positive.

Criteria used is as follows:

Combination of electrophoretic technique with solubility test is a golden standard for detecting sickle cell hemoglobin in carrier and sufferer state. It is very cost effective (about

Rs.10/- per hemoglobin blood samples) hence screening on large scale can be undertaken by different institutions[18].

From the available data [5-10, 14.] it is found that Sickle cell gene is widely spread in all districts of Eastern Maharashtra (known as Vidarbha region), North Maharashtra (Satpuda ranges) and some parts of Marathwada region [16, 17.]

Table 2: Methods used for identification of haemoglobins

Solubility test	*Electrophoretic mobility (major bands)*	*Genotype*
+ ve	A+S	Heterozygote (Carrier)
+ ve	S+S (One single band at S position)	Homozygote (Sufferer)
-ve	A+A (One single band at A position)	Normal

Table 3: Prevalence for sickle cell disorder (carrier) amongst Scheduled Tribe population groups (State of Maharashtra).

Tribe	*District*	*Prevalence %*
Otkar	Gadchiroli	35
Pardhan	Gadchiroli, Chandrapur, Yewatmal	32
Pawara	Nandurbar, Jalgaon	25
Madia	Gadchirli	20
Bhill	Nandurbar	20
Halbi	Gadchiroli	13
Rajgond	Gadchiroli	11
Korku	Amravati	10
Kolam	Yewatmal	09
Warli	Thane	09
Katkari	Pune, Raigad	07
Kokana	Nashik	04
Andha	Nanded	02
Mahadeo Koli	Pune, Nashik	01
Thakar	Pune, Raigad	01
Paradhi	Solapur	00

EPIDEMIOLOGICAL SUMMARY OF SICKLE CELL DISORDER IN THE STATE OF MAHARASHTRA

Sickle Cell Anemia is a single point mutation red cell hereditary disorder. It is an autosomal recessive disorder and hence occur in two forms i.e. Carriers (AS type) and Sufferers (SS type). Haploype studies suggest that in majority of cases it is Arab-Indian haplotype. This disorder is mostly confined to economically and socially backward commu-

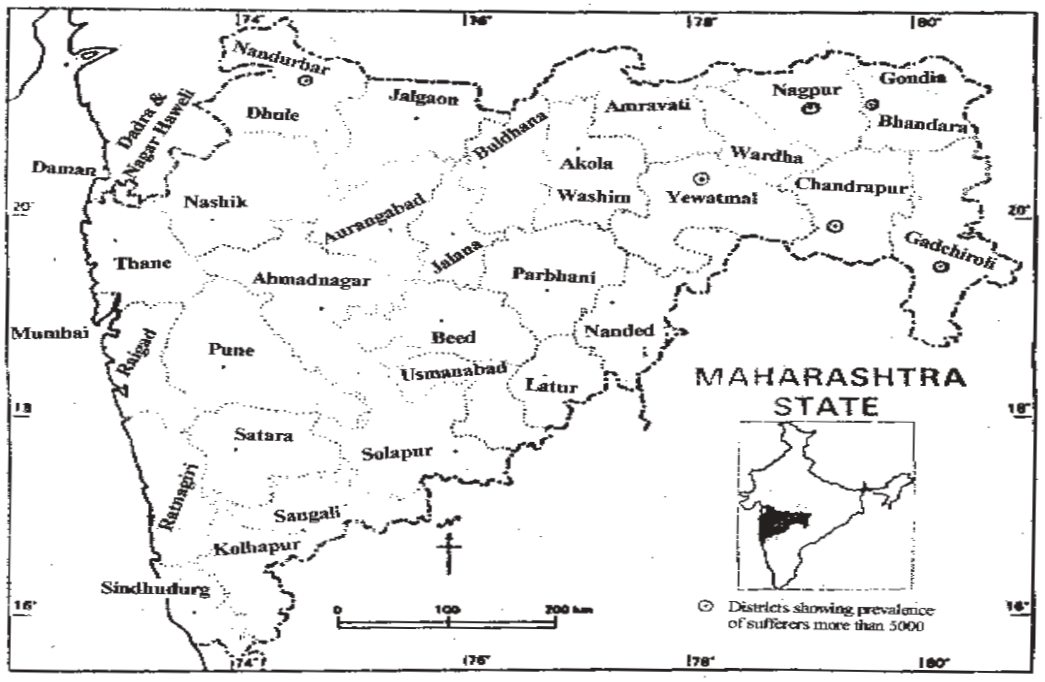

Fig. 2. Maharashtra state:⊙ Districts showing prevalence of sufferers more than 5000

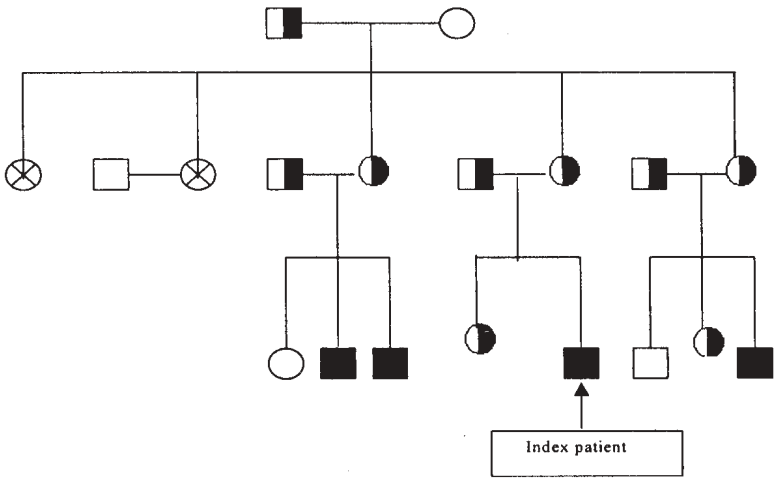

Fig. 3a. Typical pedigree from Vidarbha region

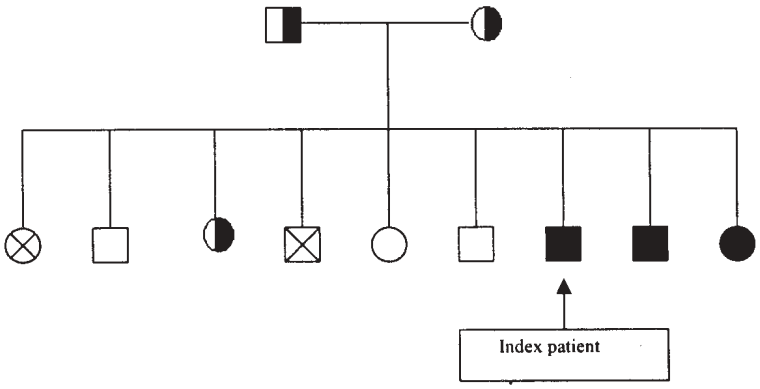

Fig. 3b. Typical pedigree from Nandurbar district

Table 4: Prevalence of sickle cell disorder (carrier) amongst scheduled caste population (State of Maharashtra)

District	*Prevalence*
Chandrapur	24
Gadchiroli	23
Nagpur	22
Bhandara	18
Gondia	15
Thane	12
Wardha	07
Washim	07
Aurangabad	07
Nandurbar	05
Pune	04

nities known as Scheduled Caste (SC), Scheduled Tribe (ST) and Other Backward Communities (OBC) groups. It is rare in other communities.

Table 5: Prevalence of sickle cell disorder (carrier) amongst OBC population groups (State of Maharashtra)

Groups	*District*	*Prevalence%*
Teli	Gadchiroli	12
Teli	Nagpur	10
Kunbi	Gadchiroli	10
Kunbi	Nagpur	04
Banjara	Nanded	05
	Yewantmal	
	Osmanabad	

Expected Carriers and Sufferers in the State are as Follows

Total population of Maharashtra (Census 2001)	100 millions
Total S.C., S.T., and O.B.C. population	25 millions

Expected carriers (10%) 2.5 millions
Expected sufferer (0.5 %) 0.125 millions
(Rough estimates as per available record from 2001 census).

It is also estimated that the districts with more than 5000 cases of sickle cell anaemia are Gadchiroli, Chandrapur, Nagpur, Bhandara, Yewatmal and Nandurbar districts. (Fig. 2).

The common clinical features observed among sickle cell anaemia cases are Anaemia (Moderate type), Intermittent jaundice (yellow sclera) figure 4, Joint pains (severe),Vaso-occlusive crisis (painful) and Spleenomegaly. Intermittent jaundice and joint pains are characteristic of the disease. These symptoms usually visible at the age of 3 to 4 years and

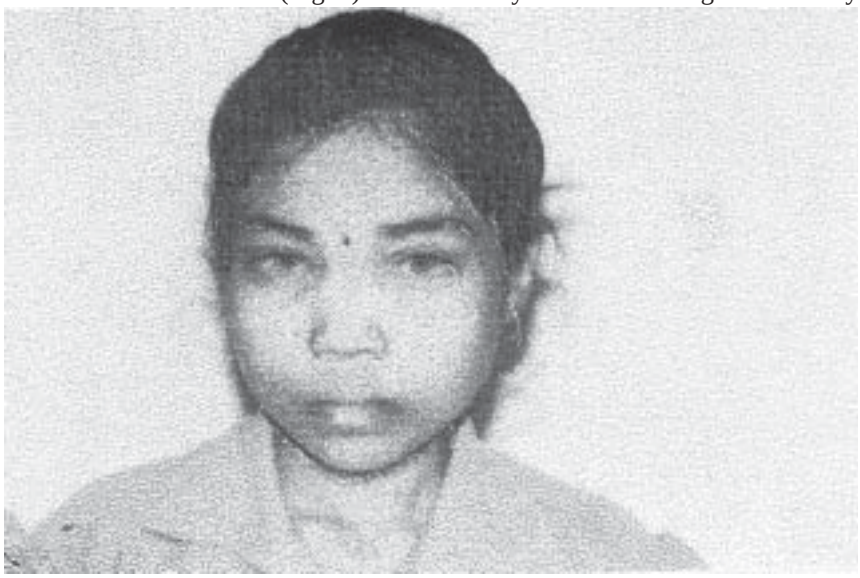

Fig. 4. Sickle cell anaemia patient (HbSS) showing intermittent jaundice (yellow sclera)

Fig. 5. A family with three sickle cell Diseased patients (Hb SS) from hill tribal community, Dhadgaon

severity increases with the age. Extreme hot and cold and unexpected changes in the atmosphere aggravate the symptoms. Though rare, most of the complication recorded in the world literature are also found in few cases. Cholelithosis and Hand and foot syndrome observed in few cases. There is a large variation in clinical presentation. Some are mild some are severe type. Clinically severe cases are found in Eastern Maharashtra than other parts of the state.

Avascular necrosis of bones and grade I early proliferative changes involving periphery of the retina are observed in elderly patients. Foot ulcers and priapism not observed. Pregnancy lost i.e. repeated abortion in family where both parents are carriers are recorded. Carriers are usually asymptomatic except few cases of painless hematuria. High prevalence observed in malaria endemic areas.

Compound heterozygotes are seen frequently i.e. S-β thalassaemia. It is common amongst non-tribal and non-scheduled caste groups.

The disease is incurable and hence patients are not only physically affected but mentally too. Due to presence of sickle cell anaemic patient whole family is disturbed. (Fig.3 and 5).

High prevalence is observed in the rural area from Eastern part of Maharashtra and hence population is at high risk in this area. In this rural area general practitioners have very little knowledge about this disease. Moreover, diagnostic and treatment facilities are not available. Modern interventions like Bone Marrow Transplantation (BMT), Gene Therapy (GT), Preimplantation Genetic Diagnosis (PGD) and Prenatal diagnosis is beyond their capacity (the population with sickle cell anaemia inheritance). Lack of knowledge and awareness enhances superstitions about the disease.

It is necessary to establish community control programme involving people, doctors, social workers, and sympathizers. This programme will undertake diagnosis, treatment, management and counselling. Government of Maharashtra is aware of these facts but unable to undertake major projects because of financial constraint. Similarly there is need to have Central Institute to study epidemiology and clinical course aspects in detail. It needs support from Central agencies.

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