Original Article

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Study of Sickle Cell Anemia in Tribal Area of Thane Region of Maharashtra

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Abstract

The cross sectional study was performed to find out the prevalannce of sickle cell anemia in tribal population of Thane district; Maharashtra from July 2012 to December 2013. The study was conducted at GMC & Sir JJ group of hospitals ; Mumbai. In this study we screened 1524 tribal subjects comprising adult men & women as well as children. Whole blood Samples were collected in EDTA bulb in different camps organized in PHC & RH centres located in tribal region of Thane as well as samples were also transported by health workers from these tribal areas health centres to Biochemistry special investigation laboratory. Samples were analysed by Automated High Perfomance Liquid Chromatography analyser. We found that 11% of tribal population was having sickle cell trait & 0.5% of were suffering from sickle cell disease.

Keywords: Sickle Cell Anemia; HPLC; Tribal Community; Chromatograph.

Introduction

Indian tribal populations constitute 8.2% of the total population [1]. In all, 461 scheduled tribes have been listed and they have their owncharacteristic cultural patterns, languages and social systems, by and large keeping to themselves [2]. However, Reich *et al* [7] concluded that "several thousand years ago, the entire subcontinent underwent a period of massive intermarriage, shuffling its population's genetic deck so thoroughly that it left clear traces even in the genomes of today's most isolated tribes [3]. Many population groups have been screened and the sickle cell gene has been shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes in India [4,5,6]. The prevalence of sickle cell carriers among different tribal groups varies from 1 to 40 per cent [7]. In Maharashtra, the sickle gene is widespread in all the eastern districts, also known as the Vidarbha region, in the Satpura ranges in the north and in some parts of Marathawada.

The prevalence of sickle cell carriers in different tribes varies from 0 to 35 per cent. The tribal groups with a high prevalence of HbS (20-35 %) include the *Bhils*, *Madias*, *Pawaras*, *Pardhans* and *Otkars* [8]. Sickle cell disease(SCD) is one of the most common monogenic disorders globally with an autosomal recessive inheritance [9]. Sickle cell anaemia is an autosomal genetic disorder caused by a defect in HBB gene (β 6Glu→Val). The beta (β) globins gene is located on short arm (i.e., P-arm) of chromosome 11 and there are over 475 allelic variants. HbS (Haemoglobin S) is responsible for sickle cell disease, one of the most prevalent genetic diseases, affecting millions of people in India. Individuals who are sickle cell carriers are referred as sickle cell trait and do not express symptoms of sickle cell disease. Either double

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copy of the HbS variant on both the chromosomes or one copy of HbS and one copy of HbC variant on different chromosomes results into disease manifestation. In addition SCD results in combination with mutation for beta thalassaemia on other chromosome [10].

The sickle cell gene in homozygous condition was found to be lethal in Africa [11]. The homozygous apparently suffer from severe anaemia and die before attaining reproductive age without contributing to the gene pool of the population. Majority of the children born with SCA die before the age of 5 years [12]. The incidences of SCT are higher among the tribal groups than other caste populations [13]. It was found that the trait was present to the extent of about 10% in African origins. The absence in other racial group led to the belief that sickling was an exclusively an African gene. Lehman and Cutbush reported the presence of the trait in considerable frequencies in some of the tribal populations in and around Nilgiri Hills in South India [14]. Buchi was of the opinion that "the sickle cell cannot be a character of Weddis as a whole". He further pointed out that "the possibility of direct contact with the African for the introduction of the trait in India than independent mutation". SCD is by no means an African characteristic alone [15]. Ingram obtained the molecular change in the haemoglobin molecule of SCD – Chemical structure Position of amino acid [16]. Normal HbA H3N – Val – His – Leu – Thr – Pro – **Glu** – Glu Sickle HbS H3N – Val – His – Leu – Thr – Pro – **Val** – Glu The primary pathophysiology is based on the polymerization of deoxyHbS with formation of long fibers within the RBCs causing a distorted sickle shape which eventually leads to increased haemolysis and vaso-occlusion of sickle red cells. However, the clinical presentation of SCD patients is extremely variable and there are several events that may trigger vasoocclusion. Recent work has shown the importance of red cell dehydration,abnormal adhesion of RBCs to the vascular endothelium, imflammatory events, and activation of all the cells in the vessel and abnormalities of nitric oxide metabolism in the pathophysiology of this multi-organ disease [17]. Most of the early studies on epidemiology of sickle haemoglobin in different parts of the country used the sickling or the solubility test and in many reports this was followed by Hb electrophoresis to determine the phenotypes. However, in recent years, high performance liquid chromatography (HPLC) analysis has been used in many large programmes to identify carriers of both sickle haemoglobin as well as â-thalassaemia. Capillary electrophoresis has also now been introduced at some centres Nonetheless, even the simple and cost-effective solubility test has been shown to have a sensitivity and specificity of 97.4 and 100 per cent, respectively in comparison to HPLC and could still serve as a good first line screen for sickle haemoglobin in remote areas where other facilities are not available [18].

Tribal populations also have a high prevalence of β-thalassaemia. The role of these genetic modifiers in reducing the severity of the disease in tribal groups was first shown by studies done in Odisha [19]. Subsequently, studies have shown that tribal groups in Gujarat and Maharashtra have a milder presentation than non-tribal populations with the rates of painful crises, infections, acute chest syndrome and hospitalizations being fewer in them. This has partly been attributed to the very high prevalence of β- thalassaemia (90 to 97 %) in some tribes and/or much higher foetal haemoglobin (HbF) levels [20,21]. There are little data on the maternal and perinatal outcomes of women with sickle cell disease in India. A prospective study from Odisha showed that neonatal outcomes such as low birth weight, perinatal mortality rate, admissions to the neonatal care unit, intrauterine growth retardation and preterm births were significantly higher in sickle cell anemia mothers with successful pregnancies being achieved in 84.44 per cent of cases [22].

Maternal and perinatal outcomes were also evaluated retrospectively from patients' case files in women with sickle cell disease in a tribal population in Madhya Pradesh. There were 25 deliveries to women with sickle cell disease and preeclampsia and disseminated intravascular coagulation were common problems. There was no maternal mortality; however, there were five intrauterine foetal deaths and one early neonatal death [23].

Although there are significant advances in the management of sickle cell disease, yet increased morbidity and early death are not infrequent. Thus, prenatal diagnosis remains an important option for couples at risk of having a child with homozygous sickle cell anaemia, sickle-β-thalassaemia or HbSD disease despite the fact that it is impossible to predict the severity of the disease and many individuals may have a milder clinical presentation. With increasing awareness in the community more couples are opting for prenatal diagnosis [24,25]. Most of the tribal populations where sickle cell disease is common rely on the primary health care facilities in rural and often remote areas. Thus, the goals of medical genetic services should be to help these people with a genetic disadvantage and their families to have access to quality care as well as social and genetic counselling support to make informed choices for reproduction

to have healthy children with the availability of prevention programmes when needed. The Indian Council of Medical Research (ICMR) under its Tribal Health Research Forum (THRF) activities as well as other programmes under the National Rural Health Mission (NRHM) in different States have initiated programmes to enable advances in genetics to reach these communities.

Aims & Objectives

Identify high risk population and to create community awareness followed by counseling to the affected individuals/ families about sickle cell disease for its prevention and management.

Material and Methods

Study Design

The current cross sectional study was undertaken from Mumbai; Maharashtra.

Study Period

July 2012 to December 2013.

Ethical Approval

The study was approved by the GMC Mumbai & Sir JJ group of hospitals; Institutional Ethical Committee.

Inclusion

All males and females aging less than 40 years.

Site of Sample Collection

PHC and RH hospitals of Thane Vasai taluka. (Tribal Belt)

Site of Sample Study

Special Investigation Biochemistry laboratory, department of Biochemistry, J.J hospital, Mumbai.

Material

EDTA bulbs; 3 ml venous blood sample ; Sickle cell short program recorder pack,contains(whole blood primer, wash solution – deionised water, elution buffer 1, elution buffer 2, analytical cartridges, retention time marker set of retention time marker 1 (FAES) and of retention time marker 2 (FADC) contains lyophilised human red blood cell hemolysates with preservatives.

Method

Sample collection was done by venepunture withdrawing 5ml blood inn to EDTA bulbs.The sample was stored in refrigerator at 2-80 c till further assay.

Sample preparation – A dilution adjustment of the sample may be required due to variation in sample collection, transport and storage. 5 micro lit of umbilical cord sample is taken in a vial each time with the help of a pipette. A 0.5 micro lit deionised water is added to each sample vial which is allowed to stand for 30 min at room temperature. Each sample vial is mixed by inversion. The sample vial is placed into the sample tray.

Sample annalysis is based on the principle of cation exchange high performance liquid chromatography (HPLC - BIORAD VARIANT TM). All steps are automated.Diluted specimens are maintained at 12 +/- 20 c in the automatic sampler chamber. Each specimen is sequentially injected into the analysis stream and then separated by the analytical cartridge. Two dual piston pumps and a pre-programmed gradient control the elution buffer mixture flow through the analytical cartridge.

The ionic strength of the elution buffer mixture is increased by raising the percentage of elution buffer 2. As the ionic strength of the mixture increases, more strongly retained haemoglobin elute from the analytical cartridge. A dual wavelength filter photometer (415 and 690 nm) monitors the elution from the cartridge. As the haemoglobin elute from the cartridge and pass through the photometer flow cell, changes in the absorbance at 415 nm are detected. The secondary filter at 690 nm corrects the baseline for changes caused by the buffer gradient. Changes in absorbance are monitored versus time, producing a chromatogram (graph of absorbance versus time).

Each haemoglobin has a characteristic retention time. Retention time is measured from the time of sample injection to the maximum point of each peak. Identification of unknown haemoglobin is accomplished through the comparison of the unknown haemoglobin's retention time with the retention time of known haemoglobin, analyzed on the same system. A built in integrator performs reduction of the raw data collected from each

analysis. At the end of each sample analysis, a copy of the chromatogram and report data is automatically printed [26].

Results

The screening was completed over a period of 18 months during which venous blood samples 1524 subject was collected, analyzed by HPLC and finally results were obtained as chromatograms.

Out of total sample screened; total number male tribal subjects screened was 990 while total number of female tribal subjects screened was 534. More delineation was made by separating total number of tribal children screened which was equal to 476.Out of this 330 were boys and 176 were girls.

Table 1:

Type of Sickel Cell Anemia	Male tribal subjects affected	Female tribal subjects affected	Tribal Boys affected	Tribal Girls affected
Sickel Cell Trait(Heterozygous)	87	58	22	8
Sickel Cell Disease (Homozygous)	0	1	3	4

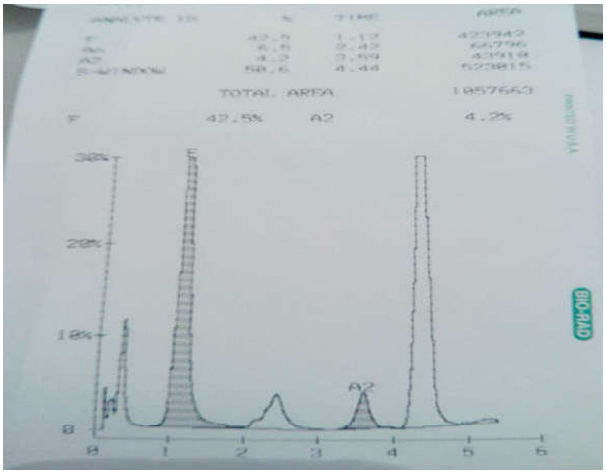

Fig. 1: Chromatogram of Sickle cell Trait sample. (Heterozygous)

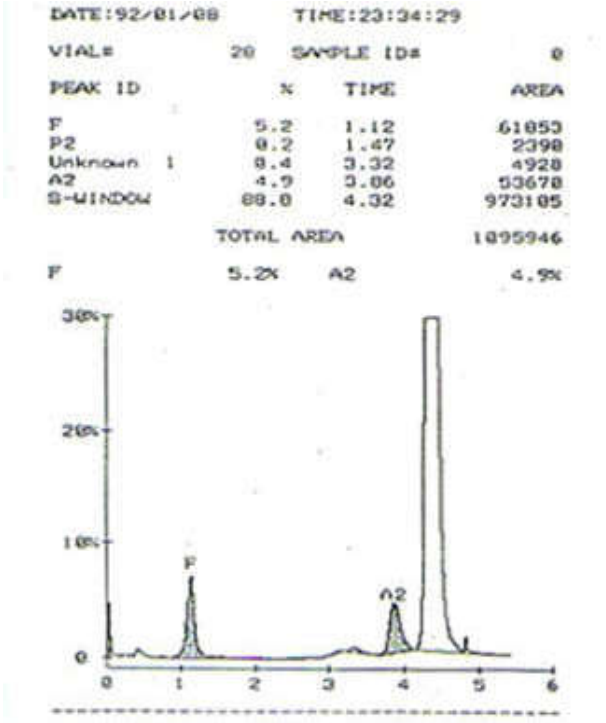

Fig. 2: Chromatogram of Sickle Cell Disease. (Homozygous)

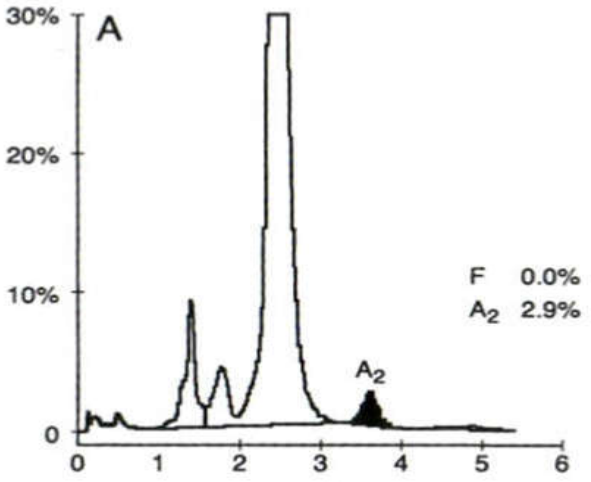

Fig. 3: Normal Chromatogram

On analysis of these chromatograms following observation were made.

Conclusion

We concluded that in our study in which total 1524 of tribal population was screened for sickle cell anemia by HPLC; 11% of tribal population was found to be affected from sickle cell trait & 0.5% of were suffering from sickle cell disease.

Discusion

When the samples of tribal population were analysed by using HPLC we found that about 11% of tribal population shared the burden of sickle cell trait while about 0.5% were suffering from sickle cell disease. Prepondrence of sickle cell gene in tribal

population was founded in number of studies which justified our selection of subjects in this study.Saha and Banerjee [27], Goud and Rao [28] while reviewing the incidence of sickle cell trait in Indian populations concluded that the HbS gene is mostly present in scheduled tribes and scheduled caste and very rarely in caste groups. The frequency distribution of HbS allele among various Indian populations groups has been reviewed and summarized by Bhasin *et al* [29]. One of the reasons for occurring in such a high incidences could be attributed to the practice of consanguinity among most of them and the similar findings were reported by Mukherjee and Das [30]. But it is surprising to note that why the other neighboring populations who live in the similar ecological niche do not exhibit similar trend for the SCT? In some of the tribal groups the HbS gene is completely absent [31].

Mukherjee and Das [30] are of the view that the highest gene frequency of HbS occur in region where malaria is highly endemic. HbS was largely absent in the areas of Horn of Africa and south of the Zambezi. Piel et al [32] shown a similar pattern of distribution of HbS frequency in malaria-free, hypoendemic ares of Africa.Some of the studies conducted on tribal population of Maharashtra vidharb region also found prephderance of sickle cell gene in their tribal subjects but prevalence was much higher than what we concluded in our study. The tribal groups with a high prevalence of HbS (20-35 %) include the *Bhils*, *Madias*, *Pawaras*, *Pardhans* and *Otkars*. It has also been estimated that Gadchiroli, Chandrapur, Nagpur, Bhandara, Yoetmal and Nandurbar districts would have more than 5000 cases of sickle cell anaemia [33]. Similar findings were shared by other studies on tribal population of states other than Maharashtra. The entire tribal population of 1,25,000 individuals in the Wayanad district of

Kerala was screened, followed by genetic counselling where carriers of HbS were advised not to marry carriers [34]. A very high prevalence of HbS is seen in these tribes (18.2 to 34.1 %) [35].

In Gujarat, the *Dhodia*, *Dubla*, *Gamit*, and *Naika* tribes have a high prevalence of HbS (13-31 %)10. More recently very extensive population surveys have been done by the Indian Red Cross Society, Gujarat State Branch where 1,68,498 tribals from 22 districts were screened and the overall prevalence of sickle cell carriers was 11.37 per cent.

Referrences

1. http://censusindia.gov.in/2011-common/censusdataonline.html.
2. Singh KS. Calcutta, India: Anthropological Survey of India; 1992. People of India: An introduction.
3. Reich D, Thangaraj K, Patterson N, Prince AL, Singh L. Restructuring Indian population history. Nature. 2009;461:489–94.
4. Bhatia HM, Rao VR. Bombay: Institute of Immunohaematology (ICMR); 1987. Genetic atlas of Indian Tribes.
5. Rao VR. Genetics and epidemiology of sickle cell anemia in India. Indian J Med Sci. 1988;42:218–22.
6. Kaur M, Das GP, Verma IC. Sickle cell trait and disease among tribal communities in Orissa, Madhya Pradesh and Kerala. Indian J Med Res.1997;55:104–9.
7. Reich D, Thangaraj K, Patterson N, Prince AL, Singh L. Restructuring Indian population history. Nature. 2009;461:489–94.
8. Kate SL, Lingojwar DP. Epidemiology of sickle cell disorder in the state of Maharashtra. Indian J Hum Genet. 2002;3:161–7.
9. Serjeant GR, Serjeant BE, editors. Sickle cell disease. 3rd ed. Oxford: Oxford Univ Press; 2001.
10. BP Urade ; Sickle Cell Gene (HbS) Scenario in Tribal India ; Urade, J Health Med Inform 2012, 3:3.
11. Allison AC (1954) Protection afforded by sickle cell trait against subtertian malarial infection. Br Med J 1: 290-294.
12. BP Urade ; Sickle Cell Gene (HbS) Scenario in Tribal India ; Urade, J Health Med Inform 2012, 3:3
13. Urade BP (2008) Haemoglobinopathies in Vidarbha region of Maharashtra. Presented a Paper in a National Conference (March, 7-9, 2008) on Prevention of Beta-thalassaemia in India, at Anthropological Survey of India, Head Office, Kolkata.
14. Lehman H, Cutbush M. Sickle cell cell trait in Southern India. Br Med J 1952;1:404-405.
15. Buchi EC. Is sickling a Weddid trait? The Anthropologist 1955;2:25-29.
16. Ingram VM. Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin. Nature 1957;180:326-328.

17. Odièvre MH, Verger E, Silva-Pinto AC, Elion J. Pathophysiological insights in sickle cell disease. Indian J Med Res. 2011;134:532–7.
18. Surve RR, Mukherjee MB, Kate SL, Nagtilak SB, Wadia M, Tamankar AA, et al. Detection of the beta S gene: an evaluation of the solubility test against automated chromatography and haemoglobin electrophoresis. Br J Biomed Sci. 2000;57:292–4.
19. Kulozik AE, Kar BC, Serjeant GR, Serjeant BE, Weatherall DJ. The molecular basis of alpha thalassemia in India. Its interaction with the sickle cell gene. Blood. 1988;71:467–72.
20. Mukherjee MB, Lu CY, Ducrocq R, Gangakhedkar RR, Colah RB, Kadam MD, et al. The effect of alpha thalassemia on sickle cell anemia linked to the Arab-Indian haplotype among a tribal and non-tribal population in India. Am J Hematol. 1997;55:104–9
21. Mukherjee MB, Surve RR, Ghosh K, Colah RB, Mohanty D. Clinical diversity of sickle cell disease in western india - influence of genetic factors.Acta Haematol. 2000;103:122–3.
22. Daigavane MM, Jena RK, Kar TJ. Perinatal outcome in sickle cell anemia: A prospective study from India. Hemoglobin. 2013;37:507–15.
23. Natu N, Khandelwal S, Kumar R, Dave A. Maternal and perinataloutcome of women with sickle cell disease of a tribal population in central India. Hemoglobin. 2014;38:91–4.
24. Colah R, Surve R, Nadkarni A, Gorakshakar A, Phanasgaonkar S, Satoskar P, et al. Prenatal diagnosis of sickle syndromes in India: Dilemmasin counseling. Prenat Diagn. 2005;25:345–9.
25. Colah RB, Gorakshakar AC, Nadkarni AH. Invasive and non-invasive approaches for prenatal diagnosis of hemoglobinopathies: Experiences fromIndia. Indian J Med Res. 2011;134:552–60.
26. Sonone.K , Abichandani.L ; IJAPB: April 2015; 2(4).
27. Saha, N and Banerjee, B. 1973. Haemoglobinopathies in the Indian Sub-continent. Acta Genet.Med.Genet., 25:117-138.
28. Rao,P.R. and Goud, J.D. Sickle cell haemoglobin and glucose-6-phosphate dehydrogenase deficiency in tribal population of Andhra Pradesh. Ind.J.Med.Res., 1979;70:807-813.
29. Bhasin MK and Walter H. Genetics of castes and tribes of India. Kamala-Raj Enterprises, Delhi. 2001;26-78.
30. Mukherjee BN, Das MK. Spatial distribution of two predominant abnormal haemoglobins – HbE and HbS in Indian subcontinent. J Indian Anthrop Soc 1990;25:39-59.
31. Urade BP. Sickle Cell Gene (HbS) Scenario in Tribal India. J Health Med Inform 2012;3:114.
32. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. Nat Commun 2010;1:104.
33. Kate SL, Lingojwar DP. Epidemiology of sickle cell disorder in the state of Maharashtra. Indian J Hum Genet. 2002;3:161–7.
34. Verma IC. Kochi: Proc. Indo-French Symposium on Recent Trends in Clinical, Diagnostic and Reserch Aspects of hemoglobinopathies; 2004. Nov 21-24, Hemoglobinopathies in India-An overview; pp.2–4.
35. Feroze M, Aravindan K. Sickle cell disease in Wayanad, Kerala: Gene frequencies and disease characteristics. Natl Med J India. 2001;14:267–70.