pISSN: 0976 3325 eISSN: 2229 6816

Original Article

PREVALENCE OF SICKLE CELL DISORDER IN RURAL PIPALWADA, GUJARAT

Doshi Neena[1], Chaudhari Sanjay[1], Shah Nigam[2], Joshi Trupti[1], Shah Menka[1], Uday Singh[3]

[1] MD Pathology, Department of Pathology, [2] DCP, Department of Pathology, [3] MD PSM, Department of PSM; Shree Krishna Hospital and Pramukh Swami Medical College, Karamsad, Anand-388 325, Gujarat, India.

Correspondence:
Dr. Neena Doshi,
47 Gulmohar Park, Near Akota Garden,
Akota, Vadodara-390 020, Gujarat, India
E mail: npd1997@yahoo.co.in

ABSTRACT

Sickle cell disorder has remained a neglected field of research in our country. The present study was started in rural population of Gujarat to find out the magnitude of sickle cell disorders. 395 subjects were studied. The prevalence of sickle cell trait was found to be 7.86 %. Through this study, we created awareness among people about the disorders. The villagers were explained about its' hereditary nature and that no curative treatment is available, although it can be prevented through marriage counseling. It is necessary to continue with such activities since prevention appears to be the only solution in present circumstances.

Keywords: sickle cell disorders, tribals, diothinite tube turbidity test, hemoglobin electrophoresis

INTRODUCTION

Sickle cell disorders are a group of autosomal recessive disorders, caused by point mutation at the sixth position in beta globin chain, valine substituting glutamic acid.[1] The resultant HbS has poor solubility in the deoxygenated state and can polymerize within the red cells. The red cell shows a characteristic shape change because of polymer formation and becomes distorted and rigid, the so-called sickle cell.

In India, sickle cell disorder is more common in central and southern parts of the country. It is the second most common haemoglobinopathy, next to thalassemia in India. In 1952, Lehman and Catbush reported the presence of this disease in India among the tribals of Nilgiri Hills for the first time. Almost at the same time, Dunlop and Muzumdar reported the presence of the disease in Assam.

In 1953, Buchi confirmed the presence of the disease in Veddoids of South India. In 1955, Sukumaran found it in Western-India. In Maharashtra, Bankar et al reported prevalence of disease from 1.9% to 33.5% in different communities. Shukla and Solanki were the first to report the disease in Vidarbha region of Maharashtra with prevalence from 9.4% to 22.2% in non-tribal population. Ankushe reported prevalence of 5.5% from few villages of Wardha.[1]

Sickle cell disorder has remained a neglected field of research in this country and magnitude of problem has never been appreciated in spite of the fact that the sickled RBCs were detected in the blood of Indian patients as early as 1952. This was largely because most of the subsequent reports spread a misconception that the sickle gene in India was confined to the tribal population or some scheduled castes only. The present study was started in rural population of Gujarat to find out the magnitude of sickle cell disorders.

It is well documented that the gene for sickle cell hemoglobin is located on the short arm of chromosome 11 and has an autosomal recessive

inheritance. Hence, it can manifest in two forms viz. heterozygous (carrier) and homozygous (sufferer). When two carriers marry, the chance of having a homozygous child is 25% with every pregnancy.[2]

In the homozygous state or sickle cell anemia, affected individuals characteristically are without symptoms until the 2nd half of the first year of life because of sufficient quantity of HbF ($\alpha_2\gamma_2$). Mild hemolytic anemia is apparent by 10-12 weeks of age, splenomegaly after 6 months of age, first vaso-occlusive episode between 6-12 months by approximately one-half of the subjects, before 6 years by the vast majority. Dactylitis and acute chest syndrome have the highest incidence during the first year of life. The clinical severity is extremely variable, partly due to the effects of inherited modifying factors, such as interaction with β-thalassaemia or increased synthesis of HbF and partly to socio-economic health.

Heterozygous state or sickle cell trait is 40-50 times more in number than sickle cell disease. Sickle cell trait is rarely associated with clinical or hematological manifestations of significance. Nevertheless, under unusual circumstances, serious morbidity or mortality can result from complications related to polymerization of deoxy-HbS. Such problems include increased urinary tract infection in women, gross hematuria, splenic infarct with altitude hypoxia or exercise and life threatening complications of exercise {exercise-related death (ERD)}, exertional heat illness (exertional rhabdomyolysis, heat stroke or renal failure) or idiopathic sudden death.[3]

Thus, there are two possible reasons to population screening for the presence of sickle cell:

1. To inform affected persons of health risk.
2. To provide information that might affect an individual's reproductive decisions.

MATERIAL AND METHODS

The study was conducted in the tribal population of Pipalwada village, taluka Thasara, district Kheda of Gujarat state, India; from May 22, 2003 to March 17, 2005. The chosen target population was for a purposeful screening.

The population was screened after taking in confidence the Anganwadi workers and the Medical Officer at the Primary Health Center. Villagers were educated in groups, during day time, with the help of booklets and posters. A performa and a consent form were duly filled; which was signed (or thumb impression) by the individual. 2 ml EDTA anticoagulated blood was collected from each.

The samples were run on automated blood cell counters in the laboratory in the same evening. Diothinite tube turbidity test (DTT or solubility test), the screening test for Sickle cell disorder, recommended by ICMR network on Sickle Cell Disorders coordinated by Institute of Immunohematology, Mumbai;[1] was performed the next morning. The test works on the principle that HbS is less soluble than other hemoglobin in presence of reducing agent, dithionite, in concentrated organic buffer. The polymers of reduced HbS obstruct light rays from passing through the solution. The reagents required are:

1. Saponin solution (pH : 7) :

   KH_2PO_4 : 14.35 gm

   K_2HPO_4 : 25.00 gm

   Purified Saponin (S.D.): 250.00 mg

   Distilled water : 100.00 ml

Saponin is dissolved in 5 ml distilled water. The salts are dissolved in 95 ml distilled water. The two solutions are then mixed.

2. Sodium dithionite powder
3. Normal saline

Cell wash, in excess of normal saline, were given to the test whole blood to remove plasma proteins, which may give false positive results. Two test tubes were taken, one labeled as C (control) and other T (test). 1 ml Saponin solution was added to each of the test tubes. A very small quantity (about 10 mg) of sodium dithionite powder was added only to the T test tube. The tube was shaken gently to dissolve the powder. 1-2 drops of washed test cells were added to both the test tubes and mixed. The results are read after 3-5 minutes. The reaction mixture in T test tube produces light pinkish violet color. A test is read positive, if the turbidity impairs the visibility of dark bold lines on a white paper kept at a distance of 1 inch and held against a bright source of light. A test is read negative, if the solution is clear pinkish violet, through which the dark lines are easily

seen. The solution in the control test tube will be clear with an orange tint.

Positive samples were subjected to hemoglobin electrophoresis on cellulose acetate membrane, at pH 8.5.[4] Hemoglobin electrophoresis is done based on the principle that when electric field is applied through supporting medium, hemoglobin proteins migrate according to their net charge. Negatively charged proteins move towards anode and positively charged ones towards the cathode. The reagents required are

1. Tris - EDTA - Boric (TEB) buffer (pH: 8.5)
   Tris (Hydroxy methyl) amino methane :10.2 gm
   E.D.T.A. (disodium salt) :0.6 gm
   Boric acid :3.2 gm
   Distilled water up to :1000.0 ml
2. 0.5% solution of Ponceau S in 5% Trichloroacetic acid solution
3. 5% acetic acid in distilled water
4. Normal saline.

Known sickle cell trait (HbA & HbS) or infant (HbF) and normal adult (HbA) blood is taken as control. Two test tubes are taken and labeled as C (control) & T (test). 2-3 drops of washed red cells are added to the respective test tubes. 3 cell washes are given to whole blood to remove plasma. Hemolysate is prepared by adding 2-3 drops of 5% Saponin solution to the washed cell button and mixed. Cellulose acetate strip is placed gently in a beaker containing TEB buffer for few minutes. The strip is blotted between two sheets of Whatman filter paper No. 3. 20 µl of hemolysate was added to the applicator groove or on clean glass slide. Applicator or a small piece of coverslip or a blade was dipped in the hemolysate. The strip was charged with the hemolysate on it's rough side, by gently touching the applicator/coverslip/blade to the strip for a few seconds. The two side chambers of electrophoresis tank was filled with equal quantity of TEB buffer, such that the electrodes dip in the buffer. Appropriately cut Whatman filter papers were placed on the two bridges that divide the tank into three compartments. The charged cellulose acetate strip was then placed on the bridges covered with filter paper, in such a manner that the charged rough surface faced downwards and smooth surface faced upwards and the charged end was near the cathode (negative electrode). The power of the electrophoresis tank was switched on and the electrophoresis was run at 200 volts for 15-20 minutes or till a good separation of hemoglobin bands was achieved. The instrument was then switched off. The strip was then carefully removed from the tank and put in the 0.5% Ponceau S solution for few minutes. The strip was held with forceps and placed in a Petri dish containing 5% acetic acid solution. 2-3 washes were given with 5% acetic acid, with gentle shaking till the background became clear.

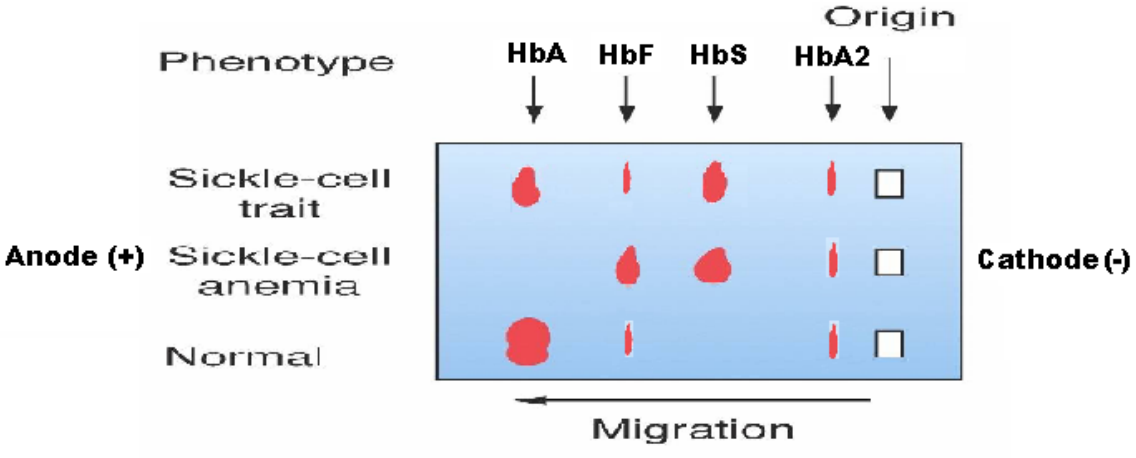

Fig. 1: Schematic diagram of hemoglobin electrophoretic pattern of sickle cell disorder

The villagers were explained about the hereditary nature of sickle cell disease and that no curative treatment is available although it can be prevented. Yellow (signifying anemia) cards were printed for sickle cell disease, half white and half yellow colored cards were distributed to sickle cell trait individuals and white cards to individuals not harboring sickle gene. The concept of marriage counseling to prevent the

disease was explained while distributing identity cards.

RESULTS

395 cases were studied. 28 cases were positive for diothinite tube turbidity test; of which 27 showed electrophoretic pattern of sickle cell trait and one was labelled sickle β-thalassemia.

Table 1: Caste distribution of sickle cell traits

Caste	Frequency	Percentage
Chavda	01	03.6
Rathod	20	71.4
Solanki	01	03.6
Vasava	06	21.4

In all seventeen castes were encountered. The prevalence of sickle cell disorder was found in Rathod (71.4%), Vasava (21.4%), Chavda (3.6%), and Solanki (3.6%) community, (Table 1). The disorder was not found in Chauhan, Christi, Gohel, Joshi, Parmar, Patel, Prajapati, Raval, Rohit, Vaghela, Vankar, Visani and Zala communities.

Out of the 28 positive cases, 14 were males and 14 females. The mean age amongst the positive cases was 32.8 years. Parameters of histogram of sickle cell trait showing logistic regression (significance) were: low HCT (mean : 35.6 %), low MCV (mean : 73.2 fl) & low MCHC (mean : 30.2 gm/l) with normal RBC & platelets.

DISCUSSION

As per the 1991 census, the total population of Gujarat state is 50, 671, 017. Gujarat ranks 10th amongst the most populated states of India and is the 7th largest state, area-wise. Scheduled Tribes, comprise of about 10%-15% of India's population.[5] The scheduled tribe population of Gujarat is estimated to 7,481,160; accounting to 14.8 % of the total population. Gujarat is the 4th most schedule tribe populated state of India after Madhya Pradesh, Maharashtra and Orissa. The tribal community of Gujarat inhabitates in the geographically difficult terrains of the Eastern belt, extending from Ambaji in the North to Dang in the South. Sickle cell disorder is known to be prevalent in the descendents of Bhil community, namely Damor, Bhabhor, Hathila, Ninama, Nayala, Bariya, Bhuriya, Rathva, Vasava, Tadvi, Chaudhary, Gamit, Ghodiya, Kukna, Dubada, Varali, Kodcha, Dangi and others.

With a large population, burgeoning birth rate, and consanguineous marriage practices, there is a dangerously high prevalence of genetic disorders among tribal populations. There remains a conspicuous lack of maternal and child health services among the hilly tribal areas and consequently, the tribal demographic scenario is one of high fertility, high maternal and infant mortality rates. Epidemiological studies confirmed that sickle cell anemia is rampant in the tribal populace.[5] Genetic diseases have traditionally received little attention from urban health services in India, and even less so in tribal areas. As a result, virtually all studies carried out regarding tribal populations and sickle cell disease have strongly recommended that genetic health services be integrated into existing primary health care and medical services to combat the epidemic.

The higher prevalence of the sickle cell trait may be a result of a higher frequency of consanguineous marriages within the relatively small community. Association for Health Welfare in the Nilgiris (ASHWINI), Tamil Nadu also reported prevalence of sickle cell trait in non-tribal Chetti community to be as high as 30%.[5] Studies by Dr. S. L. Kate, at B.J. Medical College, Pune and other scientists from different institutions indicate that the overall prevalence of sickle cell disorder in different tribal populations is 10% for carrier state and 0.5% for the sufferer.[2]

In the present study, the prevalence of sickle cell trait was found to be 7.86 %. The prevalence amongst the different communities in the decreasing order of frequency was Rathod (71.4 %), Vasava (21.4 %), Chavda (3.6 %) and Solanki (3.6 %). These observations support the hypothesis that the sickle cell disorders are present in scheduled castes, tribals and few communities of other backward classes (OBC), and not found in so called higher castes; though the review of literature says it is present invariably in all castes.[1]

The prevalence of the disorder was 50% in males and 50% in females. As regards to sex distribution of the disorder, Wintrobe states that sickle cell trait is more common in females. But Samal et al, P. Deshmukh et al did not find any such correlation. In the present study also, such a correlation could not be found.[1]

Although we carried out these activities for a limited period, our experience suggests that we have definitely created awareness among people about sickle cell disorders in the areas where the disease is prevalent. However, it is necessary to continue with such activities and with our experience we are hopeful that with devotion from medical scientists from multi-disciplinary fields, people will accept marriage-counseling program.

With advances of molecular genetics, it is possible to detect this defect at early stage (10 to 15 weeks) of pregnancy.[6] The management cost of these patients shall be exorbitant and the resources with the Government are limited. Hence, prevention appears to be the only solution in present circumstances.

Source(s) of support: Charutar Arogya Mandal and Medical Research society, Gokalnagar, Karamsad

REFERENCES

1. Deshmukh P. et al. Prevalence of Sickle Cell Disorders in Rural Wardha. IJCM 2006; 31(1).
2. Kate S.L. Health Problems of Tribal Population Groups from the State of Maharashtra. Imuunohaematology Bulletin Oct. 23, 2000.
3. John P. Greer et al. Sickle cell anemia and other sickling syndromes. Wintrobe's Clinical Hematology, 12th Edn 2008;Chp37:1039-71.
4. Dacie and Lewis. Investigation of abnormal hemoglobin and thalassemia. Practical Hematology, 10th Edn 2006;Chp12:272-307.
5. Association for Health Welfare in the Nilgiris & Tribal India Health Foundation. Sickle Cell Disease Center, Operations report version 1.4. www.tihf.org/scd.htm.
6. Gupta R.B. Sickle Cell Disease in Central India - Need for Micro Level Planning. Proceeding of National Symposium on Tribal Health; 110-15.