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Screening for the sickle cell gene in Yavatmal District, Maharashtra, India: An approach to a major public health problem

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Abstract

Aim of the present study was to determine the feasibility of large-scale population screening for the sickle cell gene in different areas of Yavatmal District. A program designed to detect the sickle cell trait and sickle cell disease has screened 7568 subjects among several villages and in Yavatmal District of Maharashtra State. The subjects were screened by solubility test and positive samples were subjected to cellulose acetate membrane electrophoresis, used for the diagnosis of sickle cell disease under the sickle cell disease control program held on Jan 2010 to Dec 2014 by SVNGMC Yavatmal. Among total (7568) samples were screened, 1621 (21.41%) were detected with the sickle cell trait and 978 (12.92%) with heterozygous (Hb AS) and 593 (7.89%) with homozygous (Hb SS) sickle cell disease as well as 259 (3.4%) cases were found with other Hb. Out of total positive cases, 53.30% were female and 46.69% were male. The prevalence was found to be more in age group (1 month to 20 years) compared to other age groups. The caste wise distribution of solubility test confirmed samples were SC-52.06%, ST-20.17%, NT -16.34%, OBC-8.45%, SBC-1.97%, Open-0.98%. The study concludes that the prevalence of sickle cell disease among backward classes in Yavatmal district is more especially in schedule caste (SC) in comparison to general or open category with higher prevalence among female subjects.

Keywords: Population screening, Sickle cell trait, Sickle cell disease, Cellulose acetate membrane electrophoresis.

1. Introduction

Sickle cell disease (SCD) is an autosomal dominant haemoglobinopathy. Sickle haemoglobin (HbS) results from a substitution of one amino acid (Valine) for another amino acid (Glutamic acid) at position six of the β-globin polypeptide chain. This substitution is caused by a single-base mutation in codon 6 within the β-globin gene on chromosome 11, where the sequence GAG occurs instead of GTG [1]. Due to the abnormal amino acid in the β-globin chain, HbS forms long, insoluble polymers when deoxygenated, and the red blood cells (RBCs) containing HbS become less deformable and form a “sickle” shape [2]. SCD is essentially a multisystem disorder, affecting almost every organ system of the body. The clinical consequences can be divided into 4 groups: haemolysis and haematological complications, vasoocclusion, infection, and organ dysfunction [1].

The sickle cell gene is widespread in India, affecting predominantly the tribal peoples of central India (states of Gujarat, Maharastra, Madhya Pradesh, Chhattisgarh, and Orissa) with another focus of the gene in southern India in the north of Tamil Nadu and Kerala [3,4]. The tribal peoples in these areas are often relatively backward, living in rural areas with limited facilities, and generally marginalized from much of Indian society. Detection of the sickle cell trait and sickle cell disease among these peoples therefore poses particular challenges. There have been many programs designed to advance the tribal peoples in the Indian society, and sickle cell disease

has emerged as one of the important public health problems affecting these groups. Several states have initiated sickle cell control programs, Gujarat leading the way in early 2006, Chhattisgarh in 2008, and Maharashtra in 2010. These screening programs usually have two objectives: firstly, screening of the susceptible populations, seeking to identify carriers in order to conduct education and counseling to reduce affected births; and secondly, identifying patients with the disease in order to improve clinical management [5]. The present study has been envisaged with an intention to assess the prevalence of sickle cell disease among backward communities of Yavatmal District, Maharashtra. Sickle cell disorder is very common in tribal and backward caste, so a study of this kind will help the public health department to tailor-made the sickle cell disease control and prevention program in the district.

2. Experimental Methodology

The study was carried out in Department of Pathology, SVNGMC Yavatmal, Maharshtra, India over a period of Jan 2010 to Dec 2014. All participating individuals were 1 months - 56 years of age. The population was screened by solubility test. One ml of phosphate buffer reagent was taken in a glass tube and a small quantity of sodium dithionite was added to it and was mixed well to dissolve. A small drop of washed red cells was added and was mixed well to produce light pinkish violet colour. The test was read after 3 to 5 min. It was read as positive, if the turbidity impaired the visibility of dark, bold lines on a white paper held against bright source of light at one inch distance. Negative test was indicated by visible lines.

The sickle cell solubility test is a simple method that detects the presence of sickle haemoglobin, but does not distinguish between sickle cell trait and sickle cell disorders. The positive samples were subjected for cellulose acetate membrane electrophoresis at pH 8.8 to confirm the diagnosis and classify Hb SS and Hb AS pattern. Samples were collected into tubes containing dipotassium EDTA (vacutainers). The samples were run on cell counter to obtain hemoglobin value and red cell indices. The same sample was used for HPLC.

A total of 1621 samples were analyzed on HPLC. After collection, the samples were stored at 2-8^{0}c and tested within a week of collection. The samples were run on an instrument manufactured by BIO RAD laboratories. The instrument is known as BI0-RAD variant (Beta Thalassemia short program) utilizes the principle of high performance liquid chromatography. An HbA2F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run. The total area acceptable was between one to three million.

Social classification--The nomenclature was derived from Articles 340(1) and 340(2) of the Constitution of India. General castes include the four categories of the Indian caste system (Brahmin, Kshatriyas, Vaishas and Shudras). Scheduled castes (SC) traditionally occupy the lowest status in Indian society, were previously known as the 'untouchables' and now officially referred to as 'Harijans' or 'Dalits'. Scheduled tribes (ST) are tribal communities which have been declared as such by the President through public notification. They are widespread but occur mainly in forest and hilly regions, are often geographically isolated, shy of other human contact, economically backward and outside the Indian caste system. Other backward classes (OBC) are defined in the Constitution of India as 'socially and educationally backward classes' and falling outside the definitions of scheduled castes and tribes, these are also targeted for special programmes of social and educational advancement.

3. Results

Out of 7568 screened population prevalence of sickle cell affected person was 21.41% (n=1621). Electrophoresis pattern revealed that out of all 1621 subjects, 978 (60.33%) were found heterozygous state (Hb AS) and 593 (36.58%) had homozygous state (Hb SS) as well as 50 (3.08%) cases was found with other Hb. Out of total positive cases majority of subjects (53.30%) were female and 46.69% were male. Male: Female ratio was 0.72: 1 (411males:567females) in Hb AS and 0.52: 1 (203 males: 390 females) in Hb SS cases, (Figure 1).

The prevalence of sickle cell anemia was higher in 1 month to 20 years age group (54.59%) followed by 21 to 40 years age group (40.34%) and then 41 and above year age group (5.05%). Similarly out of total positive cases scheduled castes (SC) were 52.06%, scheduled tribes (ST) - 20.17%, NT -16.34%, OBC-8.45%, SBC-1.97% and Open-0.98% (Figure 2).

This study clearly shows that the backward communities of Yavatmal district are seriously affected by sickle cell disease. Out of six different castes, the scheduled castes was found to be most affected group in the community and next to it were ST, NT, OBC, SBC and Open in a descending order.

Figure 1: Shows gender wise distribution of sickle cell patients

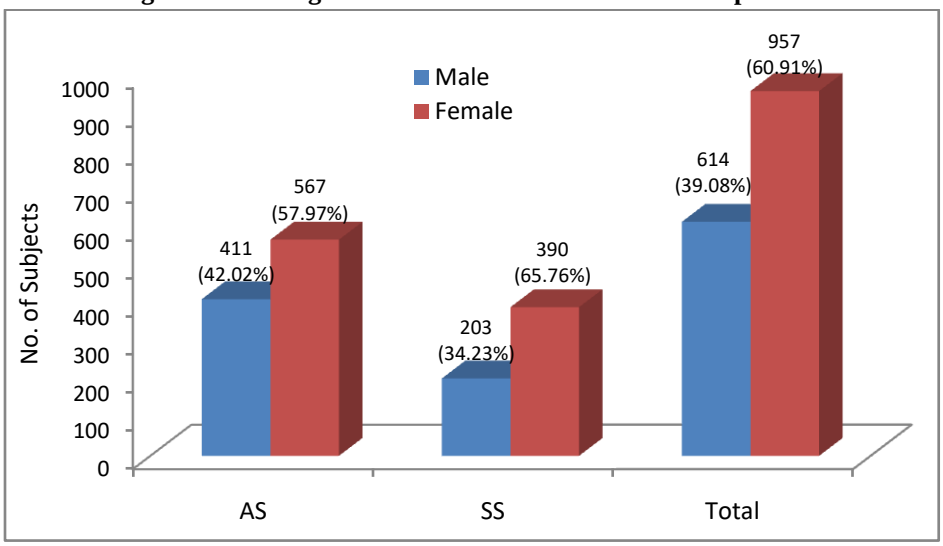
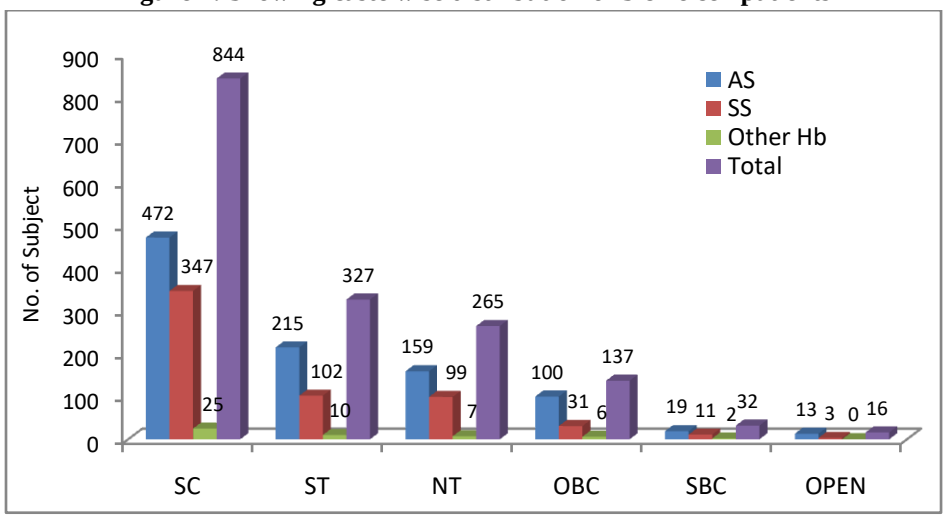


Figure 2: Showing caste wise distribution of Sickle cell patients



4. Discussion

In present study, solubility (DTT) test was used as a screening test, as it is a rapid method and easy to be carried out in the field setting. Bankar *et al* [6] had used it and ICMR network on Sickle Cell Disorders coordinated by institute of Immunohaematology, Mumbai, have also recommended the solubility test as a screening test. Total 7568 subjects were screened detecting 1621 (21.41%) with the sickle cell trait. Capillary Electrophoresis results distinguished the individuals into AS, SS and other Hb individuals. In each caste group the number of AS individuals were more than SS individuals. Overall number of AS individuals was 978 (60.33%), SS individuals were 593 (36.58%) and other Hb found in 50 (3.03%) subjects. These results were supported by another study wherein they screened a total of 3479 individuals, amongst them, 172 (4.94%) individuals were found to be positive for sickle cell disorder, of which 135/172 (3.88%) with AS and 37/172(1.06%) with SS from the urban parts of east Maharashtra [7]. In each age group, the number of AS individuals was quite higher than the SS individuals studied. This is because the number of SCD heterozygote is increasing at alarming rate. Based on 1981 census figures of population in India, it was estimated that there were 24, 34,170 carriers and 1, 21,375 sickle cell homozygotes among the tribes of India [8].

Age–wise data of SCD prevalence showed that the youngest age group (1 months-20 years) had 28.10% of SS and 26.49% of AS individuals. However, the next two age groups, 21-40 years had 19.95% of SS and 20.39% of AS individual as well as 40 and above age group had 2.5% of SS and 2.5% of AS. These two age groups showed a less frequency of SCD than the 1 months-20 year's age group. In the study conducted in a population of eastern part (Vidarbha) of Maharashtra also showed similar percentage i.e. high prevalence between the age 0 and 30 years and its severity declined with increasing age [7]. The reason for the low prevalence in higher age groups may be attributed to

very small sample size; secondly most of the sickle cell patients could have succumbed to the disease in early age. The number of female individuals was found to be more than the male individuals in all age groups. Similarly study conducted at Rural Wardha found that the prevalence of the disorder was 2.8% in males and 3.0% in females. As regards to sex distribution of the disorder, Wintrobe states that sickle cell trait is more common in females [9]. Younger age group (1 month -20 years and 21-40 years) showed higher percentage of parental consanguinity than the members of elder age groups indicating that the number of consanguineous marriages is increasing day by day. Children of such a marriage, therefore, are at a greater risk of being homozygous for the harmful gene and consequently suffer autosomal recessive genetic disorders [10]. The sickle cell trait is most serious problem which acts as a carrier for propagation of anemia among the society through consanguineous marriages [11].

Sickle cell disease is a major genetic disorder amongst Scheduled Caste (SC), Scheduled Tribe (ST), and Other Backward Communities (OBC) population groups of Maharashtra. Caste wise, the prevalence of sickle cell trait in our study was found to be highest in Mahar (SC) i.e. 52.06% followed by ST - 20.17%, NT -16.34%, OBC-8.45%, SBC-1.97% and Open-0.98%. These findings were consistent with the findings of other investigators. The study carried out by Bhobate SK *et al* [12] in Chandrapur district of Maharashtra in their study found sickle cell trait, 9.42% i.e. maximum in Mahar caste. Kar BC *et al* [13] also reported high prevalence of sickle cell trait in Mahar (19.8%). Another study carried out by Shukla RN *et al* [14] among 1010 subjects in Nagpur found the prevalence of sickle cell trait about 22.2% in Mahar. These observations support the hypothesis that the sickle cell disorders are present in scheduled castes, scheduled tribals, NT and few communities of OBCs and not found in so called higher castes; though the review of literature says it is present invariably in all castes [15].

5. Conclusion

The present study concluded that the prevalence of sickle cell disease among backward classes in Yavatmal district is more in comparison to general or open category with higher prevalence among female subjects and preponderance in schedule caste (Mahar). The study also shows that most of the subjects are in the age group of 1 month to 20 years.

The district is rich in tribal and other related backward communities, thus the sickle cell disease has been widely spread in the tribal population of Yavatmal, which needs to be deeply investigated with proper implementation of control programs.

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