ORIGINAL ARTICLE

Prevalence of β thalassemia carrier state in Sindhi community of Wardha and evaluation of risk factors for β thalassemia trait

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Abstract

Objective: To determine the prevalence of β thalassemia in Sindhi community of Wardha and evaluation of risk factors. To give genetic counseling to those diagnosed as carriers.
Study Design: Cross-sectional (prevalence study).
Setting: Sindhi community residing in and around Wardha.
Study Period: 18 months.
Materials and Methods: A total of 578 individuals belonging to Sindhi community residing in and around Wardha in India were selected by systemic randomization. Those who fulfilled the inclusion criteria and voluntarily gave consent were subject to Naked Eye Single Tube Rapid Osmotic Fragility Test (Nestroft). Those found positive by Nestroft were labeled carriers and advised to undergo Hemoglobin A_2 (HbA_2) quantification for confirmation of carrier status. Carriers thus identified were given genetic counseling.
Result: The mean age of study population was 15.06 years with a range of 3-28 years. The largest group consisted of those between 12 and 18 years. The prevalence of β thalassemia carrier state as diagnosed by Nestroft is 36.36% (200/550) and incidence of carrier state by HbA_2 quantification in the study population was 17.2% (95/550).
Conclusion: Prevalence of β thalassemia in the Sindhi community of Wardha is higher than in other studies and equal to the highest prevalent communities in India. Thus, we recommend that urgent measures to increase awareness and control the disease be taken.

Key words: Hemoglobin A_2 quantification, naked eye single tube rapid osmotic fragility test, prevalence, Sindhi community, β thalassemia

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Introduction

Thalassemias are a heterogenous group of inherited hematological disorders characterized by defect in the rate of special globin chains. Thalassemia is considered the most common single gene disorder worldwide occurring with high frequency from Mediterranean basin through Middle East, Indian subcontinent, Burma and South East Asia, and Islands of Pacific.

Thalassemia is assigned α, β, γ, δ, and ε thalassemia depending on the chain whose synthesis is impaired. According to WHO data, there are 269 million carriers of thalassemia and 150 million of β thalassemia alone out of which 40 million are in South East Asia. In India, thalassemia is the most common single gene disorder with approximately 30 million carrying the defective gene, with carrier frequency ranging from 3% to 17%.[1-3] β thalassemia major is an inborn error of hemoglobin synthesis with clinical manifestations arising due

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to severe anemia, ineffective erythropoiesis and in transfused patients iron overload. Clinical result in untreated patients is a catastrophic failure of growth and development, inanition, high output cardiac failure, susceptibility to infections, bony deformities, and early death without treatment. The average life of untreated β thalassemia major was less than 4 years, <80% dying in the first 5 years. The main modality of treatment is regular blood transfusions every 2-4 weekly coupled with iron chelation. With this treatment, a few lucky ones have reached 3rd or 4th decade. The only curative treatment currently available, in the form of bone marrow transplantation, is beyond the reach of all but a lucky few.[4] Cost of ideal treatment for one thalassemic child is Rs. 1,25,000/annum hence for 50,000 children will be Rs. 620 crores. This staggering cost is beyond the reach of our country, India; moreover, this cost is expected to increase due to additional children being born. Transfusions alone cost 20-30% of the income of most families. The chronic nature of the disease and demanding treatment place significant financial, emotional, and social stress on the family.

In this scenario, prevention and control of thalassemia deserve a top priority. Experiences in Cyprus, Greece, and Italy show that this genetic disease can be efficiently controlled by public education, population screening, genetic counseling, and antenatal diagnosis. The incidence of thalassemia major by these simple measures has been reduced by 96% in Cyprus, 62% in Italy, and 52% in Greece.[5-7] Mass screening would involve huge outlay of resources and manpower; hence, targeted screening in the form of high-risk screening of individuals at risk is a good option. Naked Eye Single Tube Rapid Osmotic Fragility Test (Nestroft) is a highly sensitive screening test with negative predictive value of approx 99%, sensitivity ranging from 86% to 99%, and specificity ranging from 66% to 100% in various studies.[8-15]

Thalassemia is a widespread, highly prevalent disease not a disease of "*just a few people*." Worldwide improved medical and health care has resulted in increased life expectancy. Globally, this translates in increased life expectancy. It is hence warranted that more attention be paid to screening and prevention The Sindhi community residing in and around Wardha accounts for most of the β thalassemia patients in the region. As the first step in disease prevention is identification of carriers, hence this study aims to determine prevalence of β thalassemia in this community, increase awareness and impart genetic counseling to reduce incidence of the disease in the community, and as a small measure of disease reduction in the country.

Materials and Methods

Study design

This study was a cross-sectional study (prevalence study).

Setting

This study was conducted in Sindhi population residing in and around Wardha, a high-risk community for thalassemia major. Sindhis in Wardha are primarily a trading community comprising 1,004 heholds and a population of 5,500.

Inclusion criteria

Belonging to Sindhi community, residing in and around Wardha. Children, adolescents, unmarried adults, and married adults desiring more children.

Exclusion criteria

Community other than Sindhi, not residing in or around Wardha. Married individuals having desired number of children and thus not planning to have children in future (unlikely to benefit from screening) and known cases of thalassemia major.

Individuals were screened in three camps conducted during a period of 18 months.

Study population

Out of 3,500 individuals who fulfilled the inclusion criteria, every 6th individual was selected by systematic randomization. Out of the 578 individuals thus selected, Nestroft was performed on all. Those found positive by Nestroft were advised to undergo HbA_2 quantification to confirm their carrier status. However, 28 individuals refused to undergo tests and hence have been excluded from the study.

Selection

Individuals to be screened were selected by systemic randomization among those who fulfilled the inclusion criteria and registered voluntarily for the camp and were enquired about marital status, sex, consanguinity, and family history. Consent was taken from parents of children and adolescents and individuals before inducting in the study.

Sensitizing the community

Attempts were made to sensitize the community regarding severity of the disease, nature of inheritance; how a carrier may be apparently normal. This was done by distributing information brochures in Hindi. Informative talks by eminent personalities who were masters on the subject were arranged before the camps. Posters were put up at camp sites and individual visits to household with the help of volunteers.

Screening

Screened individuals were subjected to Nestroft.

Methodology of naked eye single tube rapid osmotic fragility test

Stock solution in the form of 10% buffered saline with pH 7.4 was prepared with NaCl 90 g, anhydrous Na_2HPO_4 13.65 g,

and NaH_2PO_4 2.43 g (can be stored in well-stoppered bottle in refrigerator for 6 months). Working buffer was prepared fresh by putting 3.6 ml of stock solution for 100 ml buffer (by adding distilled water).

Collection of blood sample

Finger prick for Nestroft and 2 ml of venous sample for HbA_2 quantification. Blood collected for HbA_2 quantification can be stored at 2-6°C in dipotassium ethylenediaminetetraacetic acid (EDTA) bulb for up to 1 week; however, sample was sent to the laboratory the very same day and temperature of 2-6°C maintained during transport.

Procedure and interpretation of naked eye single tube rapid osmotic fragility test

To 2 ml of working solution, 1 drop of blood was added and kept aside in room temperature for half an hour. It was observed half an hour later against a 1 mm line drawn on a white sheet. Control with distilled water was done. If clear line is visible, test is positive. Test was observed independently by three observers and even one positive was considered positive.[4,8,16,17]

Hemoglobin A_2 quantification

Nestroft positive patients were advised for HbA_2 quantification. This was done by separating bands by cellulose acetate membrane electrophoresis. These bands were then eluted and HbA_2 quantification done by spectrophotometric analysis of solution (optical density).[5]

Interpretation

Normal: 1.7-3.5%, Minor: 3.5-10%, Major: >10% (not of diagnostic significance).

Genetic counseling

Parents of thalassemia carriers were reassured regarding the innocuous nature of disease and explained about the need of avoiding marriage of their child to another thalassemia carrier. Married couples who were both carriers were told that in each pregnancy, they have 25% chance of having a normal child, 25% of thalassemia major, and 50% chance of thalassemia carrier. They were counseled regarding the option of prenatal diagnosis and of selective termination of affected fetuses if they so desire. All individuals attending camps were counseled regarding available treatment facilities and facilities for prenatal diagnosis of thalassemia major.

Methodology of statistical analysis

Data thus collected were used for estimating the prevalence of thalassemia in Sindhi community residing in and around Wardha. Fischer's exact test was done to evaluate the risk factor using graphpad. The data were analyzed using EpiInfo6 software (developed by CDC, Atlanta USA courtesy Wikipedia) and Microsoft Excel and Stata program release 8.0.

Results

The study was a cross-sectional study conducted in the Sindhi community residing in and around Wardha which is a high-risk community for thalassemia. 550 individuals were screened in three camps.

Prevalence of β thalassemia carrier state in children and young adults of Sindhi community of Wardha

Total individuals selected were 578 out of which 28 individuals refused to undergo tests and hence have been excluded from further calculations of prevalence. Prevalence based on (screening test) nestroft is 36.36%(200/550). Total individuals found to be β thalassemia trait by taking HbA_2 3.5-10 were 97/550. Hence, 17.2% of the population has thalassemia trait.

Table 1 shows the baseline characteristics of study population and profile of carriers.

The mean age of the study population was 15.06 years with a range of 3-28 years. They were arbitrarily divided into three groups depending on difference in post-test counseling. Among the carriers, 22.9% were < 12 years, 47.3% were 12-18 years, and 29.8% were > 18 years (13.1% married and 16.7% unmarried). Among the carriers, 51.5% were males and 48.4% females. 11.8% of the population had a family history of thalassemia major. Family history of consanguineous marriage was found in 5.1% of the population. Various risk factors for thalassemia are evaluated in Table 2. Thalassemia carrier state was similar in married versus unmarried adults; hence, marital status is not a risk factor. Prevalence of thalassemia was similar in both sexes as inheritance is not sex linked. The prevalence of thalassemia in those with a family history of thalassemia (64.6%) was significantly higher than those without family history. It shows that the proportion of thalassemia carrier in those with and without a family history of consanguinity was 60% and 15.3%, respectively. Hence, consanguineous marriage

Table 1: Baseline characteristics of study population

	Total cases (%)
Age group	
<12 years (126)	(22.9)
12-18 years (260)	(47.3)
>18 years (164)	(13.1)
Sex	
Male (285)	(51.8)
Female (265)	(48.2)
Consanguinity	
Present (28)	28 (5.1)
Absent (522)	522 (94.9)
Family h/o thalass major	
Present (65)	11.8
Absent (485)	88.2

among high-risk communities is a risk factor for thalassemia trait. The study shows that thalassemia carrier is an innocuous disease, usually asymptomatic; hence, screening is essential for diagnosis. Pallor visible on examination was also seen in just 2% of carriers.

Discussion

Birth of a thalassemic child places immense stress on the family as it is associated with regular hospital visits, expensive treatment, and painful procedures. Only 10-15% of all thalassemic children born per year get optimal therapy in the form of regular blood transfusions and chelation. The only curative treatment currently relevant in India in the form of bone marrow transplant is beyond the reach of all but a lucky few. In this scenario, prevention and control of thalassemia deserve a top priority. This can be made possible by increasing awareness and screening individuals so that prevention is possible [Figure 1]. Mass screening would involve a huge outlay of resources and manpower; hence, targeted screening in the form of high-risk screening of communities at risk, extended family screening, routine antenatal ANC screening, and screening as part of school health program are options available to us. High-risk screening is a good option as it targets a population most likely to benefit from screening and also easy to motivate.

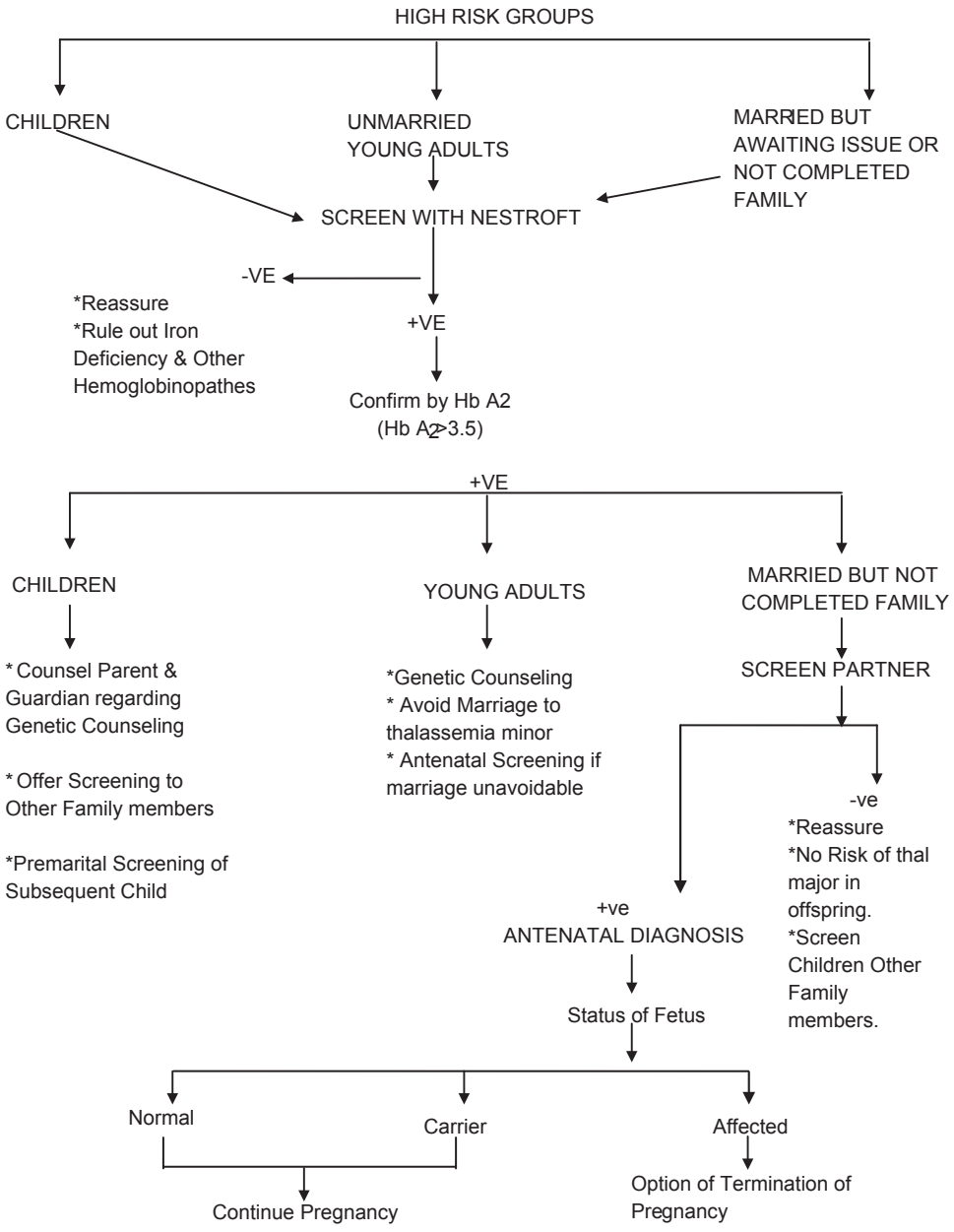

Figure 1: Approach to high risk screening for thalassemia

It is estimated that about 1,00,000 children with transfusion-dependent thalassemia are born worldwide annually, out of which 8,000-10,000 are born in India. Cost of optimal treatment for 50,000 children will be Rs. 6.25-10 billion and expected to rise by about Rs. 100 crore every year due to additional children born every year. 40% of 8,000 blood units collected in Red Cross Blood banks are taken up for transfusion of these children.[18] Carrier frequency ranges from 3% to 17%.[4,19] Sindhi community residing in and around Wardha is a high-risk community for thalassemia and accounts for most of the thalassemic patients in the region. Prevalence of thalassemia in the Sindhi community residing in and around Wardha in this study ,using Nestroft is 36.36%. Gene frequency using HbA_2 quantification as the gold standard is 17.2%.

Result of studies on prevalence of β thalassemia trait in Sindhi communities are given in Table 3. Thalassemia, as was initially believed is clearly not restricted to the Mediterranean regions but also prevalent in Indian Subcontinent in different castes and communities like Gujaratis, Khoja, Bora, Sindhis, Punjabis, Baniyas and different religions such as Muslims, Jains, etc.[1] Large-scale population surveys undertaken in India showed incidence of β thalassemia in Bengalis to be 3.7%. Chaterjee *et al.*[20] and Sen *et al.*[21] found the incidence of β-globin chain mutations up to 20% in tribals of Eastern India. Mathur *et al.*[22] found incidence to be 1.37% in Uttar Pradesh (immigrant population). Balgir *et al.*[23] found the prevalence of β thalassemia trait to be 18.2%, β thalassemia major 5.3%, and thalassemia intermedia 0.5% in Orissa. Ramot *et al.*[24] found an incidence of 16.6% in Kerala Jews. Verma[25] found thalassemia to contribute 8.8% of entire burden of genetic disorder in India and found that β thalassemia has a frequency of 1 in 2,700 births. In another study, incidence in Halai Lohanas was found to be 17.24%.[26]

The prevalence in this study is higher probably as there have been no attempts to screen individuals or prevent marriage between carriers, and marriage in the same closely knit community over the years have resulted in higher prevalence. All the previous studies have been conducted in urban areas with a greater degree of awareness, underlying the importance of urgent intervention in rural areas. Family history of thalassemia in 12% and history of consanguinity in 5% of screened population may have been responsible for higher prevalence.

Regarding risk factors, sex and marital status are not related to the inheritance of thalassemia as is well known and corroborated from the study. Consanguineous marriage among high-risk groups is a significant risk factor understating the autosomal recessive nature of inheritance. Family history of thalassemia is also a significant risk factor for thalassemia due to genetic nature of the disease.

The following strategies are recommended for control of thalassemia in India

- Sensitizing the community using newspapers, advertisements, TV, and mass media

Table 2: Parameters used for predicting thalassemia carrier state

Parameters	Nestroft positive	Significance	HbA_2>3.5%	Significance
Marital status (>18 years)		*P* value 0.34 not significant		*P* value 0.55 not significant
Married (72)	36		13	
Unmarried (92)	42		17	
Sex		*P* value is 0.5948, not significant	HbA_2>3.5	*P* value is 1.000 not significant
Male (%)	107 (37.5)		49 (17.19)	
Female (%)	93 (35.1)		46 (17.3)	
Consanguinity	Nestroft positive	*P* value is 0.0001, extremely significant	HbA_2>3.5	*P* value 0.0001
Present (%)	20 (71.4)		17 (60)	
Absent (%)	179 (34.3)		80 (15.3)	
Family h/o thalass major	Nestroft positive	*P* value is less than 0.0001, extremely significant	HbA_2>3.5	*P*<0.0001
Present (%)	49 (75.3)		42 (64.6)	
Absent (%)	151 (31.1)		53 (10.9)	

HbA=Hemoglobin A_2, Nestroft=Naked eye single tube rapid osmotic fragility test

Table 3: Result of Prevalence studies in Sindhi Community

Community	Region	No tested	Method	Prevalence of gene %	Conducted by
Sindhi lohanas	Bombay	134	HbA_2 quantification	6-8	Bhatia H.M. *et al.*[18]
Sindhis	Ulhasnagar	82	HbA_2 quantification	12.4	Sukumaran P.K.[2]
Sindhis	Gujarat and maharashtra	2525	HbA_2 quantification	7.7	Manglani M. *et al.*[9]
Sindhis	Wardha	550	HbA_2 quantification	17.2	Present study

HbA_2=Hemoglobin A_2

- Screening high-risk groups in the form of voluntary screening of high-risk communities and extended family screening
- Genetic counseling to those diagnosed as carriers: For parents of children <12 years counseling is aimed at first reassurance regarding the innocuous nature of the disease and prevention of marriage of their children to thalassemia traits to prevent birth of thalassemia major children. 12-18 years children and those >18 years but married along with parents are given information regarding inheritance of thalassemia and the need to prevent marriage to traits. For carriers aged >18 years and married to a thalassemic carrier, the need to screen their unborn child is explained. Options available (for diagnosing thalassemia) widely by amniocentesis, cordocentesis, and isolation of fetal cells from maternal blood should be explained with associated adverse effects. If a fetus is thus diagnosed to be thalassemia major, option of termination of pregnancy is given. For those couples for whom termination of pregnancy is not acceptable, pre-implantation diagnosis following *In vitro* Fertilization (IVF) is made using Polymerase Chain Reaction, Fluorescent (PCR), Fluorescent *in situ* Hybridization (FISH).

Limitations

Individuals who were married and had completed their family and older individuals were excluded from the study. This could affect the prevalence; however, this was done as these people were unlikely to benefit from the screening. 28 individuals screened by Nestroft were lost to follow-up due to economic reasons.

Conclusion

More efforts are needed to increase awareness of high-risk communities like Sindhis regarding β thalassemia before dreams of disease control can be fulfilled.

One of the most cost-effective and simple methods of decreasing thalassemia is by high-risk community screening and genetic counseling. Prevalence of β thalassemia in Sindhis residing in and around Wardha is 36.6% by Nestroft and prevalence of the defective gene by HbA_2 quantification is 17.2% which is higher than Sindhis in Ulhasnagar[2] and Sindhi Lohanas[18] and equal to the highest prevalence in communities like Cutchi Lohanas, Cutchi Bhanusalis, and Halai Lohanas.[5]

Thus, we recommend that urgent measures be taken to increase the awareness of high-risk communities about the disease, inheritance, and prevention. When one considers the repeated yearly expenses of bringing up a child with thalassemia, preventing thalassemic births by diagnosing and counseling of β thalassemia traits seems to be the more easier and effective solution.

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