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Original Research Article

Screening extended families for identification of β-thalassemia carriers: an experience from north Maharashtra region

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ABSTRACT

Background: Beta thalassemias are group of autosomal recessive disorders of hemoglobin synthesis affecting many people worldwide. The study was undertaken to identify carriers of β-thalassemia through extended family screening in communities with high preference of consanguineous marriage.
Methods: Ninety families consisting of 130 index cases (β-thalassemia major) with 3466 living family members were approached for testing, of these 1702 were tested. All carriers and couple at risk received counseling and followed for three years.
Results: A total of 629 carriers were identified out of 1702 tested members. Of these 310 were married couples and 319 were unmarried. Of the 310 married couples 116 couples were at high risk for producing affected child as both the partners were carriers. There have been four new marriages and seven engagements. Of these no any new married couples were at risk but one engaged couple was at risk as both being a known carrier.
Conclusions: Following index cases is reliable and feasible way to study the inherited hemoglobin disorder in high-risk communities and in communities with high preference of consanguineous marriages. High numbers of carriers and couples at risk were identified by screening a very small number of populations.

Keywords: Beta thalassemia, Cascade screening, Consanguineous marriage, Index cases, Red cell indices

INTRODUCTION

Hemoglobinopathies are major public health problem in India. About 3-4% of the Indian population carries β-thalassemia, and around 8,000 to 10,000 β-thalassemia major patients are born each year.[1] The prevalence of β-thalassemia carriers varies between 8 to 10% or more in communities like Sindhis, Muslims, Cutchi Bhanushalis, and some tribal groups. Screening and genetic counseling by means of routine blood count, HbA2 estimation and DNA analysis to identify carriers can reduce the incidence of β-thalassemia major.[2] The epidemiology of thalassemia is changing globally during last few decades by means of successful implementation of preventive programs. The population of India is very diverse; more than 4000 ethnic groups with different religious beliefs and cultural backgrounds are present. According to 2011 census, the population of Dhule and Nandurbar districts of Maharashtra is more than 2 million and 1.6 million respectively.[3] Nandurbar district consisting of more than 60% tribal population and the frequency of sickle cell anemia carriers ranges from 15-25% which poses financial as well as mental burden on the affected families.[4,5]

Communities with strong preference of consanguineous marriages are at an increased risk for recessively inherited disorders.[6] The individuals from affected families and from communities who are at high risk have some knowledge of thalassemia as compared to the general

population where majority of the people are totally unaware of this disorder. Therefore, in such communities, screening extended families of index cases is more feasible than general population for identifying present and future couples at risk for producing affected children.

METHODS

Extended family screening for carrier identification was carried out from August 2013 to May 2015. In this cross-sectional study, 242 β-thalassemia major patients (receiving regular blood transfusion at Government Civil Hospital, Dhule) were followed.

Ethical approval was obtained from institutional review committee. Two inclusion and exclusion criterion was set; one was the thalassemia major taking monthly blood transfusion and second complete family information with address. Of these 242, only 181 β-thalassemia major patient's families were requested for testing to identify carriers, remaining patients and their families were not able to approach. Information regarding other family members and relatives were obtained. After getting consent from family members, a two or three-generation pedigree was drawn up and collection of blood samples was arranged at their homes. After testing, these families were approached again for explaining the results to the family members and make them aware of who is a carrier and who is at risk of producing affected child. A total of 1702 family members and close relatives were enrolled in this study and were undergone through different hematological examinations.Initial screening to identify carriers was performed by the strategy described earlier.[7] The samples undergone through initial screening like NESTROFT (Naked Eye Single Tube Osmotic Fragility Test) with different concentrations of hypotonic saline solutions and red cell morphology due to limited resources. The blood specimens who show abnormalities according to the criteria mentioned earlier were processed for further series of studies.[7] These include determination of red cell indices with an electronic cell counter (Coulter AcT 5diff, Beckman Coulter). Individuals who showed hypochromic microcytosis with mean corpuscular volume (MCV) value less than 80fl and mean corpuscular hemoglobin (MCH) value less than 27pg, further testing using cellulose acetate electrophoresis and/or automated HPLC system (Bio-Rad Variant-II β-thalassemia short program) for HbA2 estimation were performed.[8] Beta thalassemia trait was diagnosed when the percentage of HbA2 was 3.5% or higher.[9] The analysis of globin gene mutations was performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).[10] The data were fed in Microsoft Excel for statistical analysis; descriptive statistics like mean, percentage were applied.

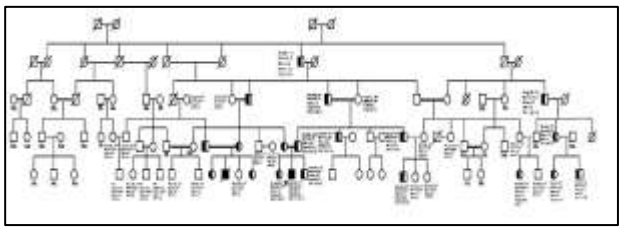
RESULTS

The present study was conducted on 242 clinically proved β-thalassemia major patients and their family members. The ages of thalassemia major patients were between 6 months to 22 years, 136 being males and 106 females. A total of 181 β-thalassemia major patients and their families were approached and requested testing to identify carriers. Of these, 90 families with 130 index cases (β-thalassemia major patients) were agreed to undergo testing and remaining 51 β-thalassemia major patient's families declined for various reasons including desire to avoid testing, elder influence, religious faith etc. Out of 242, remaining 69 β-thalassemia major patients and their families were not able to approach; might be their information was incorrect.

Table 1: Characteristics of families involved in screening.

Caste	Total No. of families	Total family members	Total marr-ied (%)	Total un-married (%)	Total No. of couples	No. (%) consanguineous	No. (%) from same caste	No. (%) un-related	Total No. of tested family mem-ber (%)	Total No. of carriers identified (% of tested famil members)	Total No. of affected children
Hindu	33	1034	466 (45)	568 (55)	233	19 (8)	201 (86)	13 (6)	469 (45)	189 (40)	41
Muslim	11	754	354 (47)	400 (53)	177	66 (37)	107 (61)	4 (2)	417 (55)	129 (31)	23
Adivasi	26	903	414 (46)	489 (54)	207	40 (19)	154 (74)	13 (6)	393 (44)	161 (41)	38
Sindhi	11	454	242 (53)	212 (47)	121	16 (13)	103 (85)	2 (2)	262 (58)	84 (32)	16
Budhist	6	181	80 (44)	101 (56)	40	8 (20)	29 (72)	3 (8)	96 (53)	45 (47)	9
Sikh	2	109	58 (53)	51 (47)	29	3 (10)	24 (83)	2 (7)	45 (41)	15 (33)	2
Jain	1	31	18 (58)	13 (42)	9	0	9 (100)	0	20 (65)	6 (30)	1
Total	90	3466	1632 (47)	1834 (53)	816	152 (19)	627 (77)	37 (4)	1702	629 (37)	130

The 90 study families included a total of 3466 members (Table 1). There were 816 married couples, of which 152 (18.62%) were consanguineous (second cousins or close relatives), 627 (76.8%) were from the same caste and sub-caste (tribe or sub division of a tribe) and only 37 (4.5%) were completely unrelated. A typical pedigree of a family with an index case was drawn and is shown in Figure 1. There was considerable inter family and inter caste variation in the proportion of marriages that were consanguineous, especially between Muslims and others



	Normal male and female		**Marriage**
	Family members who are homozygous for β-thalassemia		Consanguineous Marriage
	Family members who are heterozygous for β-thalassemia	NA	Not available for testing
	Deceased family members	DL	Testing declined
	Index cases		

Figure 1: A typical pedigree showing results of testing for β-thalassemia in index family.

Testing was greatly facilitated in rural areas than urban. Most of family members living in rural areas were readily available including men and school-age children, whereas in cities it was difficult. However, most of family members declined carrier testing in spite of being parents or siblings or close relatives of affected children and there is no indication that they are at lower risk than the rest of their family.Of the 130 index cases (β-thalassemia major child), 114 were alive and 16 of whom had died. Of 3466 living family members, 1702 (49 %) were tested and 629 (37%) were found to be carriers. Among the 699 married individual tested, 310 were found to be carriers, 232 of whom were married to another carrier. One hundred ten of the 116 couples were the parents of the child with the index cases (already had one or more affected children) and who were at risk to produce another affected child; another 6 more couples consisting of two carriers were identified by testing. of 1702 tested family members, 1003 were unmarried and of which 319 carriers were identified. Further history of unmarried carriers revealed that there was one engagement between two carriers in a Muslim family. The unmarried carriers were informed about their genetic risk, premarital testing of their partner and the availability of prenatal diagnosis.

Regular follow-up was conducted during period of study in most of the family members. It was possible to ask various questions during visits like engagements, marriages and birth. The index families and tested family members taking screening results into consideration when they are going to arrange engagements. To date, there have been four new marriages and seven engagements. There were no any new married couples at risk. Of these four, one marriage was consanguineous and one partner was a known carrier, so another partner was tested and found to be non-carrier.

Remaining three married couples were from same caste; in these, one partner of each three was a known non-carrier, that's why another partner was not tested. Of the seven engagements, two engagements were consanguineous; of this one engagement couple was at risk both being tested as carriers. The family was advised first to break the engagement and second option for prenatal diagnosis before going to plan for a child. Of remaining five engagements, three were from same caste and two from unrelated or other caste and these engaged couples were found not to be at risk; in four, one partner of each four couple was a known non-carrier and remaining one was a known carrier so the family was advised to test the other partner before going to marriage.

The selection of marriage partner was majorly carried out under the influence of elder person or head of the family. It was also noted that the preference of marriages in all communities was chosen from same caste and sub-caste only. Consanguineous mating was preferably adopted by Muslim community and the marriages between same caste and sub-caste (called biradri).

The lowest or no consanguinity was reported from Jain community, as well as the marriage partner from other caste was also strictly avoided. Hindu community from the study area also practicing consanguinity at some extent and the marriages from same caste and sub-caste were preferably accepted. Marriages among Adivasi community were carried out by their traditional way, consanguinity and the marriage from other communities was also reported. In Sindhi community the preference of marriages from same caste and at some extent consanguineous marriages were seen with acceptance of marriage partner from other community. Community wise marriage pattern like consanguineous marriage, marriage from same caste and marriage from unrelated caste are shown in (Figure 2).

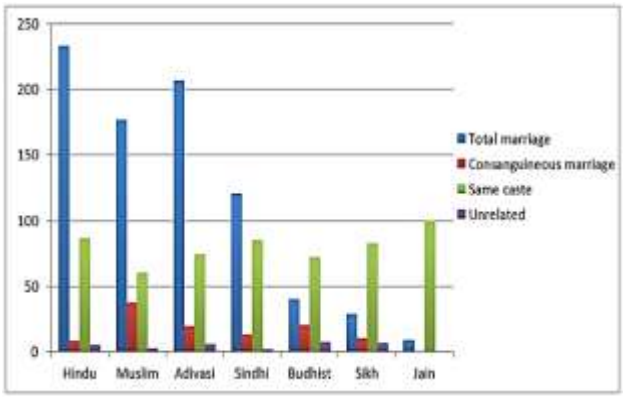


Figure 2: Caste wise preference of marriage.

DISCUSSION

Several modern screening approaches and protocols are being used for identification of carriers in many countries.[2,11] The present study tested the feasibility and efficiency of modern screening protocols for extended family screening and is widely used for carrier identification. The percentage of carriers identified in cascade screening was 5-6 times more than the carriers identified by other approaches.[12] The carriers of hemoglobinopathies are asymptomatic and affected genes ran through generation to generation; therefore, studies of the extended family may identify many carriers and couples at high risk. The best example of extended family screening for carrier identification was reported earlier in Sardinia and in Pakistan.[13,14]

The index families included 816 couples, 350 of whom were tested. One hundred sixteen couples were found to be at risk; 33% of those tested or at least 14 percent of all couples. Thus, in these families, majority of couples are at high risk for entering into pregnancy for producing affected children, and 20-30% unmarried carriers are at risk for entering a marriage to a carrier partner. Identification of unmarried carriers among these families has an important alternative as it gives them the option of selecting a partner who is a non-carrier. If they are engaged with a carrier or the partner is not tested, they have another option for prenatal diagnosis before going to plan for a child.

Of the 116 at risk couples, 66 were consanguineous and remaining 50 were related through the same caste or sub-caste. This is due to the affection towards the consanguineous marriage, religious faith and elder influence at some extent. This finding suggests that affected genes are not only present in extended families but also in the same caste and sub-caste. The marriage trend in India usually in all communities is endogamous and traditionally parents found the match for their children and the marriage was formalized strictly as per the rituals and it is ethically unacceptable to discourage for consanguineous marriage in certain communities. However, the consanguineous marriage and marriages from same caste should logically be discouraged to avoid genetically fatal outcomes especially the families with variant genes. The present study shows an option for providing genetic counseling in such a way that it is compatible with the social way of life and family structure of the affected population involves accurate identification of carriers and the supplement of precise information regarding presence of risk.

Several studies have been postulated for determining the outcomes of carrier identification after few years of testing and the authors have found unfavorable result.[15-17] This shows that there has been a change in the attitude of parents and relatives of index cases in the understanding and management of thalassemia. This may be due to the social stigmatization related to carrier status and/or awareness generated in the population over the years.

The persons who are at risk are generally avoiding marriage to another carrier or using prenatal diagnosis for pregnancy planning. However, the information of carrier status has seemed to be little different for the choice of partner in Mediterranean countries.[18] The selections of marriage partner especially in Muslim community are under the influence of parents or elder person of the family. Our findings suggest that close relatives agreed marriage more easily to a known carrier with in the family than unrelated person. This is because in latter situation; the parents of a carrier (particularly women) generally hesitate to ask whether the partner is tested or not and finally they arrange marriage by thinking that testing could be carried out after marriage or if required will prefer prenatal diagnosis.

Extended family studies provide a highly effective approach for prediction of risk in such a diverse and multi ethnic society. Regular follow-up of carriers can identify increased risk before the birth of any affected child and hence, the ideal policy is to provide both family studies and premarital or antenatal screening for the relatives of affected children. This approach would become a long-term strategy and its effects are likely to increase with time.

In our study most affected couples who had healthy children generally avoid further pregnancy, but in couples who had already one or two affected children requested prenatal diagnosis for healthy children, as it has been reported earlier.[18,19] In some families (two Muslim and one Buddhist) who had already one or two affected children and two or more healthy children avoided any intervention in further pregnancy for religious reasons as well as elder influence. These observations do not permit us to come to any firm conclusion that many families use information on risk to avoid fatal outcomes as far as possible.

This study also confirms that for the communities practicing consanguinity an approach targeting the extended family is useful because it produces a high yield

of information on carriers and couples at risk; second, family members often understand the fatal outcomes and conditions because they have had an affected child and third one is only one gene variant is usually present in a given family which simplify and reduces the cost of DNA-based diagnosis. These approaches are also useful to overcome from problems such as weak health care system and lack of information in the affected families because this type of study can be undertaken at the center where the index case is diagnosed and treated and information regarding carrier testing is communicated directly to the parents of that index cases. The major limitation of the present study was the lack of molecular diagnostics and genetic screening to establish the genetic profile of individuals to identify carriers. Furthermore, premarital and prenatal screening for thalassemia is not widely practised in the present study area as well as at country level.

CONCLUSION

In the present study 629 carriers were identified by extended family screening within 1702 family members of index cases. Such a huge number of carriers were identified by taking only few hundred family members into consideration and it would not be possible to identify such a high number of carriers by taking 1702 general population into screening program. Furthermore, for extended family screening the families are readily available for testing, counselling and minimal efforts are required to create awareness. Therefore, in areas with high incidence of autosomal recessive disorders of hemoglobin and in communities where consanguineous marriage is common, the protocol is most applicable and it seems to be one of the most cost-effective and practical approach to identify carriers.

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