CHAPTER VI

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Even now, the knowledge of the wide spectrum of expression of many genes, exact mode of their inheritance and mechanism of their maintenance within a population or region is far from complete. Thalassaemia gene is one of those gene, whose mode of inheritance though known, its variable expression under different ecological conditions and fate in population groups are yet to be explained. The disease is a matter of great concern for many countries including India, which harbors a number of endogamous groups with wide range of variation in the incidence of the gene. Though reasons of the variation have been postulated, yet it remained to be explained in the context of specific population structure including demographic and social structure.

Among the Sindhis of India, the level of awareness about β-thalassaemia is relatively high in comparison to many other local populations, because of high incidence of the disease among them, and high educational and economic status of its average person. In spite of this fact, the frequency of thalassaemia gene is on a steady temporal rise (Jain et al 1984, Bandodkar 1996, Mulchandani 1997). A positive selection in favor of β-thalassaemia gene has been suggested from the pattern of its distribution outside India. But till date there is no evidence of this in the central Indian thalassaemia belt, which is stretching from Gujarat, the northwestern part of India to southern part of Orissa, on the east.

Against this backdrop, the present investigation was carried out in order to study the pattern of distribution of alleles of some common genetic markers including β-thalassaemia among the rehabilitated Sindhi immigrants from the Sind province of Pakistan, in Nagpur City, which is within the region highly endemic for malaria. The situation was expected to offer a good opportunity for the study of differential reproductive fitness between groups of families.
with and without thalassaemia gene under malarial environment. The study was carried out
with the following objectives:

1. Present a brief historical account of the migration of the Sindhi into India and their
   pattern of distribution in Nagpur City.
2. Explain the system of mating among the Sindhi, identify the subgroups, measure the
   index of endogamy for the subgroups and exogamy at the community level.
3. Show the distribution of allele frequency of the common marker genes such as
   A1A2BO, MN, and Rh blood groups, haemoglobin variants, G6PD and \( \beta \)-thalassaemia
   among the Sindhi. Compare the observed frequencies with those of local populations
   and populations with high incidence of \( \beta \)-thalassaemia trait.
4. Estimate the gene diversity and heterozygosity at locus and subgroup level.
5. Measure the extent of similarity between subgroups of Sindhi, and between Sindhi and
   non-Sindhi populations, which have relatively high incidence of \( \beta \)-thalassaemia by
   genetic distance analysis and predict the possible phylogenic relationship among the
   groups.
6. Observe some of the hematological parameters of the Sindhi with \( \beta \)-thalassaemia Trait
   and compare those with that of their normal counterpart, and
7. Measure the reproductive fitness of groups of families with and without thalassaemia
gene.

Material and Method

Collection of data for the present study was made by the author in the Nagpur City of
Maharashtra, in India during 1997-2001. The data include basic demographic information,
brief ethnography of the Sindhi, and gene and phenotype frequencies of common genetic
markers such as A1A2BO, Rh, MN, G6PD, Hemoglobin variants and \( \beta \)-thalassaemia. In total
blood samples of 1533 Sindhis belonging to different subgroups were tested for the genetic
markers and hematological parameters.

Collection of blood samples was made from the camps organised by the Anthropological
Survey of India, at various locations in the Nagpur City with the consent and help from
members of the local Sindhi Societies and Medical Officers. The number of randomly
selected Sindhi subjects from different camps varies from 50 to 80, which is less than the actual number of persons tested. The subjects tested positive for β-thalassaemia in the preliminary screening were later followed up by the author for detecting earlier evidence of β-thalassaemia gene in the family by adopting genealogical method. Inductive screening test was organized for other members of the family. Each of the targeted Sindhi settlements in Nagpur City was covered for inductive screening. The families were revisited by the author for the collection of ethnographic data and detail information on subgroup affiliation, history of migration, system of mating, and reproductive history of females.

Naked Eye Single Tube Red-cell Osmotic Fragility Test (NESTROFT) was performed after Kattamis (1981) and Dacie and Lewis (1991). Seven hematological parameters, such as WBC, RBC, HGB, HCT, MCV, MCH and MCHC were estimated by ERMA PC-607 (Japan) particle counter, within 6-10 hours of blood collection. AGD (Mumbai) make diluent and haemolyser were used for counting. The standard procedures as suggested in the instruction manual were followed.

Laboratory screening and confirmation of β-thalassaemia trait and other haemoglobin variants involve the following steps:

1. Cello-gel wet-strip alkaline-buffer electrophoresis for screening
2. Cellulose acetate membrane electrophoresis in alkaline buffer medium for confirmation
3. Quantitation of HbA2 by elution spectrophotometry method (Dacie and Lewis 1991) and Quantitation of other abnormal haemoglobins (ibid)
4. Quantitation of HbF by alkaline denaturation (ibid)

G6PD deficiency spot test: Fluorescent spot test (Beutler and Mitchell 1968) was performed on male samples only for identification of G6PD deficiency. Microtube technique (Race and Sanger 1958, Dunsford and Bowley 1955) was adopted for A1 A2 BO, Rh and MN blood grouping.
Summary of Observation

Sindhis of Nagpur City of India constitute people belonging to a number of its subgroups, which were named after the ancestral place of origin. These subgroups are well differentiated from each other both culturally and linguistically and are largely endogamous in nature. Their distribution in Nagpur City was not even. Members of a particular subgroup could be found concentrated in a limited number of settlements, while in other settlements they were very thinly distributed. Four numerically large subgroups and a number of comparatively smaller territorial groups could be identified during the present study.

Surnames among the Sindhis are after the names of ancestors. Surname and the Nukh (synonymous to clan or Gotra) were exogamous. Few of these are specific to certain subgroups, whereas others are common across the subgroups. Sindhis of Nagpur city are mostly Hindu. Many of them also visit Gurdwara on a regular basis. 'Jhulelal' is being worshipped in almost every Sindhi family.

Study of population structure and system of mating have revealed that though subgroup endogamy was still in practice, there has been temporal change in the rates of exogamy i.e. after migration, the Sindhi were increasingly opting for subgroup exogamy and also selecting mates from a longer distance.

As stated above, subgroups of Sindhi were not strictly endogamous in Nagpur City, yet in most of them the allele frequencies for marker genes were found in equilibrium. Every subgroup and the Sindhi community in general were marked by the presence of extreme low frequency of A2 allele of the A1A2BO locus. The frequency varies from zero to 0.00986 with an average frequency of 0.00471 among the subgroups. However, the order of prevalence of alleles of this locus is the same for all subgroups (O>B>A1>A2). Frequency of Rh negative persons among the subgroups ranges from 0.8% to 12.5% with an average of 4.6%. ‘R1R1’ was the most common phenotype of the complex Rh locus, with a frequency variation of 38.53% to 50.72% among the subgroups. It is followed by ‘R1r’ phenotype. The order of prevalence of four of the alleles at the Rh locus found to be R1>R2>r /Rz. At the MN blood group locus M allele frequency was greater than N in all the subgroups. The range
Frequency of heterozygote AD for HbD β-chain variant varies from 0.79% to 3.83% among the subgroups except Shikarpuri, in which the variant was not found. Only one case of homozygote DD for this variant was found among the Sakhrui. Five of the six heterozygote for alpha-chain variant HbQ belong to Ladkana subgroup and the remaining one belongs to the Sahiti subgroup. HbQ was not found in other subgroups and its average frequency in the Sindhi was 0.40%. Prevalence of β-thalassaemia trait was considerably high (14.49%) among the Sindhi. Its subgroupwise distribution showed a variation of 9.25% (among Sahiti) to 20.47% (among Sakhrui). Not a single case of homozygote or β-thalassaemia major case was found among the 1498 adult Sindhi tested during the present study. Only two cases of double heterozygotes were detected in the sample. One subject of Sakhrui subgroup was found heterozygote for β-thalassaemia and HbD (D-β-thalassaemia) and the other from Ladkana subgroup was heterozygote for β-thalassaemia and HbQ. Test for G6PD deficiency was performed on only 266 male Sindhis. Three of them (one from Sahiti and the other two from Ladkana subgroup) were found deficient in this enzyme. Like HbD and HbQ, G6PD gene has a very low prevalence among the Sindhi.

Wide variation of locuswise average heterozygosity was found over all subgroups studied, where as subgroupwise average heterozygosity over all loci show little variation. A1A2BO blood group locus has the highest average heterozygosity among all the genetic loci studied. The range of average heterozygosity was 24.8% to 29.9%. The average heterozygosity for all the loci was 28.32%. The observed range was within the range reported for different populations groups in India (Malhotra et al 1978, Chakraborty and Roychoudhury 1978, Papiha et al 1984).

The genetic diversity within the subgroup was found greater than that between subgroups. The FST values with a mean of 1.84 ± 0.020 for the alleles, were at a lower limit compared to the range (0.09 – 5.86) reported for various population groups of India (Roychoudhury 1977, 1978, Das et al 1978). The test of the ratio of genetic diversity to the intra-subpopulation
diversity (k) showed that though the distribution indicated panmixia (also the computation of ‘d’ i.e., the deviation from hypothetical panmixia showed a high value of about 53%) among the subgroups, but the scattered shape of the plot also suggests an effect of preferential mating on the genetic differentiation among the Sindhi subgroups of Nagpur City. The distribution of $F_{ST}$ values also has taken a ‘L’ shape suggesting a recent stage of divergence and genetic differentiation among the subgroups with respect to the selected genetic markers.

Genetic distance analysis based on 16 of the alleles of the six loci studied suggests that there was apparently no significant genetic differentiation among the four major subgroups of Sindhi such as Sahiti, Ghotki, Ladkana and Sakhru. Sahiti and Ladkana were found forming a single cluster predicting their apparent closeness and genetic similarity. and they Sindhi-O. Similarly Ghotki and Sakhru have close similarity. Sindhi-G was found having greater genetic distance from rest of the subgroups.

The reproductive performance of average normal woman was compared with that of $\beta$-thalassaemia carrier woman (trait). The mean live birth per $\beta$-thalassae mia trait woman was higher (4.42) and the variance in the number of live birth (2.97) lower than the values 3.85 and 4.74 respectively obtained for normal woman in the completed families. But the proportion of births surviving to reproductive age was greater for normal women. Index of the opportunity for natural selection was found greater among normal women, and 74.89% of it was due differential fertility and the rest due to differential mortality. Among the $\beta$-thalassae mia trait women 46.98% of the index was due to differential mortality.
CONCLUSION

From the present investigation on "A genetic study of the Sindhis of Nagpur City, with special reference to β-thalassaemia trait", the following conclusions have been derived:

- The Sindhi community of Nagpur City though endogamous, is not panmictic to be considered as a mendelian population.
- The community constitutes groups of people belonging to different populations, which used to practice strict endogamy in their homeland, the Sind Province of Pakistan. These groups or subgroups of Sindhi in Nagpur City are no more closed populations. Since the time of their migration there has been temporal change of the index of endogamy, as each of the groups practice exogamy with one or more groups of their community.
- Increasing preference for selection of mate from a longer distance and from other group, if continue would lead to loss of differences between groups.
- Allele frequencies of common genetic markers among the subgroups were not significantly different, and those were within the range observed among other population groups of the region.
- The genetic diversity within the subgroup was greater than that between subgroups.
- From the dendrogram drawn on the genetic distance values, it could be concluded that genetic relationships among the Sindhi subgroups are weak.
- The high frequency of β-thalassaemia gene among the subgroups is not due to selective advantage in favour of heterozygote under the new environment in Nagpur. Its preexistence in the original population was evident.
- The hematological features of β-thalassaemia trait persons from the Sindhi community were not different from those of other communities.
• Some of the hematological parameters like NESTROFT status, MCH and HbA2 quantity could be indicative of β-thalassaemia trait in a person.

The recent advancement in the clinical management facilities for β-thalassaemia major patients over the past decade has dramatically improved the life expectancy and quality of life in the developed regions in India. The prenatal diagnostic techniques are now available with comparatively less associated risk. Peoples' changing attitude towards access to the available management facilities and preventive measures has been able to prevent pregnancies with β-thalassaemia major fetus to a considerable extent. However, in the City of Nagpur, though the Sindhi are having growing awareness about β-thalassaemia and its consequences, still due to many reasons β-thalassaemia major children are being born and they are succumbing to the dire consequences of the disease. No β-thalassaemia major child has been reported to have survived beyond puberty in the Sindhi of Nagpur. There are cases reported from different parts of the country, where β-thalassaemia major patients have survived to a much later age maintaining near-normal health with the management (Eleftheriou 2003).

Among the Sindhi, the frequency of thalassaemia carrier is high. The carriers are without any clinical symptom of the disease and they are often left undetected as carriers. The identification of the gene in a carrier requires special tests, which are generally not done unless the person comes for consultation in connection with similar medical problem. Even in many cases, after the gene is detected, the information is either suppressed, especially by the marriageable group, because of the social stigma attached with the disease or not paid serious attention due to ignorance or insufficient knowledge about the inheritance of the gene and consequences of the disease. The situation is suspected to be one of the reasons, which has led subliminally to enhance the probability of high-risk marriages and carrier frequencies in the Sindhi and resulting in the appearance of more number of births of β-thalassaemia major child. A structured counseling protocol with proper follow-up measures is of utmost necessity to a country like India in general and to a high-risk community like the Sindhi of Nagpur in particular. However, the issues related to the human right and socio-cultural aspects of the communities in concern are also to be considered as far as arresting the β-thalassaemia major births is concerned.