CHAPTER 6

CONCLUSION

Cost benefit analysis of thalassemia screening programs have shown that the single years’ treatment for a β-thalassemia major patient was much higher than a total cost per case prevented. Facilities for the optimum care for β-thalassemia major children are available in very few districts of Maharashtra while these services are better developed in Gujarat. However, the well-established services for prenatal diagnosis for both states are in Mumbai. Hence, few couples who avail of these services from different districts of Maharashtra and Gujarat often have to travel long distances. Thalassemic patients need lifelong blood transfusion for their survival, so it is very important that they should get blood from a reputed source because they are at high risk of acquiring several blood-borne diseases. Possible care is being provided to the β-thalassemia major patients by conventional treatment of optimal regular blood transfusion and iron chelation by Government civil hospital, Dhule and blood bank at civil hospital regularly provides blood to the patients.

The outcomes derived from the basic data collated in the present study would provide a sound platform on which future health care planning for the prevention and treatment of β-thalassemia in the present study area can be undertaken. The need for a paradigm shift in β-thalassemia-related research is however, indicated. While determination of the causative mutations has been an important initial step, there is a clear need for structured sampling programs to be planned and instituted to provide representative information on this region for which data are currently inadequate. Additionally, in a country with a population as large and ethnically and socially diverse as India, the further extension of sampling to facilitate state, district and village registers of persons with β-thalassemia major and carriers of the disorder is warranted. Indeed, given the continuing marked hereditary sub-divisions within Indian society that result from intra-caste and intra-community marriage, community-specific mutation testing would provide the basis for the optimum delivery of genetic education, screening and prevention programs.

Developing countries pose a major challenge to the health care services regarding the management of thalassemia major. Lack of facilities and coordination to this multi-disciplinary problem make the treatment difficult in a variety of ways. Awareness about the disease among the caretakers and the availability of antenatal
diagnosis are not available readily. On the other hand, in the developed countries much attention has been directed towards the prevention of disease by detection of thalassemia carriers and marriage counseling. By applying this prevention program in most of European countries, the incidence of thalassemia major patients has significantly dropped down.

There is diversity in the genotypic and phenotypic expressions of this syndrome that represents challenges in at-risk couple counseling and population screening. Presently, counseling and testing of at-risk populations is inadequate. More cases are being diagnosed unexpectedly in high prevalence areas. With this state of affairs, thalassemia is the best treated conservatively with all its inherent complications eventually resulting in death, unless a definitive treatment like bone marrow transplantation is carried out, which itself is beyond the resources of a large segment of the population. The only way to prevent the disease and reduce the morbidity and mortality is by educating the general population. For this reason in the present study, awareness among parents of thalassemic patients regarding the disease was evaluated.

In this study, very few cases had undergone antenatal screening and got their siblings screened for thalassemia. The reasons for this being the lack of diagnostic facilities, family's elders influences, religious beliefs, besides the universal factors of lack of knowledge and poverty.

The present study concluded that the overall awareness among parents regarding this chronic illness was inadequate and these patients will continue to suffer a slow and painful course ultimately leading to death. Hence, health education, carrier screening and premarital counseling remain the best ways to reduce disease incidence with potentially significant financial savings and social and health benefits.

6.1 Extended family screening

Extended family screening identified as many as 629 carriers by screening of 1702 family members of index cases and it would not be possible to identify such a high number of carriers by taking 1702 general population into screening program. On the basis of the prevalence of β-thalassemia carriers reported in the present study by screening of extended families, it would have had to screen about more than 20,000 school children to identify 629 carriers. Furthermore, for extended family screening the families are readily available for testing, counseling 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.

6.2 Screening of school children

This study comprises a large number of school children studied for frequency of β-thalassemia trait (βTT) and other hemoglobinopathies from five schools of Dhule city. Population groups with higher gene frequencies require screening programs and facilities for genetic counseling as well as increased awareness and educational programs to control the birth of homozygotes. The overall carrier frequency of β-thalassemia trait was found to be 0.9%, sickle cell disease 0.4%, sickle cell trait 1.1% and iron deficiency anemia 5.9% reinforces the differential frequencies of these hemoglobinopathies in school children from different areas as reported previously. The strategy adopted for the screening was found to be quite reliable and less time consuming. Therefore, screening of school children for detection of different hemoglobinopathies need to be undertaken urgently in all over the country to reduce the financial, social and health burden. This type of screening programs at schools and colleges level not only helps in reducing the burden of hereditary diseases but also helps in understanding the drawbacks of consanguineous marriages, selection and testing of marriage partner and the students can teach clinical aspects of these diseases to other family members.

6.3 Screening and diagnostic tests

6.3.1 Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT)

The single-tube osmotic fragility test (NESTROFT) is easy to perform, fast, cheap and does not require sophisticated equipment and potentially useful in under-resourced laboratories although it cannot replace automated red cell indices using electronic cell counters. There are certain limitations to this test as observed during the study, it gives false positive results in case of patients with iron deficiency anemia. This would affect the specificity of the test in a population with a high incidence of iron deficiency anemia. Therefore, subjects with positive NESTROFT need to undergo further investigations to confirm the diagnosis. However, the test was also positive in detecting other hemoglobinopathies like sickle/β-thalassemia, sickle cell trait and disease, HbE traits, HbE/β-thalassemia and α-thalassemia trait. The test
proved to be reliable and adaptable for mass screening coming close to an ideal screening test for β-thalassemia trait but also needs careful standardization.

6.3.2 Red blood cell morphology

Initial diagnosis for screening of hemoglobinopathies based on morphological characteristics has an excellent diagnostic reliability and it can be used as a screening tool. The present study results lend quantitative support to target cells, tear drop cells and pencil cells as morphologic features favoring the diagnosis of β-thalassemia trait but fail to support the diagnostic usefulness of prekeratocyte and basophilic stippling in discriminating β-thalassemia trait and IDA. The average numbers of target cells tear drop cells and pencil cells reported in β-thalassemia trait were higher as compared to IDA. The level of microcytosis and hypochromasias was found to be higher in RBCs of IDA and these features can be used as diagnostic tool. As the examination of red cell morphology was found to be fast and effective in the present study in the diagnosis of different hemoglobinopathies, it is recommended to be used as a diagnostic tool in areas with high prevalence and poor resourced laboratories like present study area.

6.3.3 Red cell indices

The measurements of RBCs, Hb, Hct, MCV, MCH, MCHC and RDW are now fully automatic, fast and not as expensive as another screening tests. The automated cell counter based parameters provide an excellent hematological data continue to play a crucial role for screening and differentiation of all forms of thalassemias like α-thalassemia traits, homozygous α-thalassemia, β-thalassemia major and minor etc. The key characteristic features for initial diagnosis of β-thalassemia trait were found to be MCV < 80fl and MCH < 27pg. It has also been concluded that the RBC count, MCV and MCH are suitable for epidemic screening in a large population and are the best discriminant functions among all red cell indices and continue to provide an essential support to the diagnosis and monitoring of hematological diseases. Automated cell counters with differential counts are now readily available in almost all primary health care clinics of Maharashtra state, where expensive laboratory equipments for screening and diagnosis of different hemoglobinopathies are not adoptable.
6.3.4 Cellulose acetate electrophoresis

Cellulose acetate electrophoresis is reliable for measurement of Hb fractions and allows the detection and the separation of the most common Hb variants. It also provides a precise, quick and very easy quantification of HbF and HbA2, even in the presence of HbS. It is very suitable for routine investigation and screening of hemoglobinopathies in many clinical laboratories. This accurate and fast system gives appropriate characteristics for use as a routine method for the diagnosis of thalassemias and hemoglobinopathies in high prevalence areas. Intra- and inter-laboratory variations in HbA2 determination may cause difficulties in evaluating this measurement in screening programs for hemoglobinopathies. Therefore, knowledge of genetic factors both related and unrelated to the globin gene clusters, iron metabolism, endocrinological disorders and some types of anemia, is important for reducing or eliminating the risk of mistakes in screening programs for hemoglobinopathies. For qualitative analysis, it is the best system for large number of samples analysis. It is easy to carry, easy to handle, does not require any high-class instruments and separate A.C. lab, harmful and costly chemicals etc. and the system is not so costly therefore, it should be made available in high prevalence areas.

6.3.5 HPLC studies

The HPLC method performed satisfactorily throughout the evaluation and the results compared well with existing electrophoretic methods for all common and clinically significant Hb abnormalities. The increase in HbA2 level is the most significant parameter in the identification of β-thalassemia carriers. However, in some cases, the level of HbA2 is not typically elevated and some difficulties may arise in making the diagnosis. For these reasons, the quantification of HbA2 has to be performed with great accuracy and the results must be interpreted together with other hematological and biochemical evidences. The HPLC system has been developed to provide excellent resolution of frequently encountered human hemoglobins and thus lead to an accurate diagnosis of hemoglobinopathies in the clinical laboratory. The method has the sensitivity to detect total hemoglobin in minute quantity of whole blood, thus making it an excellent method for the screening of high-risk populations for hemoglobinopathies. The turnaround time for detection of each specimen is approximately 6.0 min per sample with superior resolution. The simplicity of sample preparation and accurate quantitation of hemoglobin concentration, combined with
complete automation, makes this an ideal methodology for the diagnosis of hemoglobin disorders in a routine clinical laboratory. The use of an automated HPLC system will produce substantial time saving for large population screening in high prevalence areas.

6.3.6 ARMS-PCR

The application of simple and rapid PCR-based methods has major implications for screening and molecular diagnosis in populations where β-thalassemia is common. The ARMS-PCR based method represents an important step toward the use as a diagnostic tool for molecular screening of common β-thalassemia mutations based on the principle of sequence-specific PCR primers that allow amplification to the test DNA only when the target allele is contained within the sample. It is a simple method for detecting any mutation involving single base changes or small deletions and provides a quick screening assay that is cheap, fast and reliable and does not require high technology or dedicated instruments. Screening for β-thalassemia mutations using ARMS-PCR for the five most frequent alleles in India succeeded in determining the β-globin genotype in 86.4% of patients and it could be quite useful for the implementation of a strategy for carrier detection, genetic counseling and prenatal diagnosis in high-risk communities. The technique has also been used for prenatal diagnosis in countries with limited resources, large population and high affection towards consanguineous mating like ours because of its rapid and inexpensive properties for screening and prenatal diagnosis and the primers can be multiplexed to screen for multiple mutations in a single PCR assay.

6.3.7 Discriminant function analysis

None of the 12 DFs selected for discrimination purposes was found to be 100% sensitive and specific. The RBC count and E&F Index showed reliable results with greater AUC (area under the curve) and can be used for differential diagnosis between these two entities in areas with high prevalence of hemoglobinopathies in the tribal districts like Dhule and Nandurbar. Similarly, the EI and SI were effective for differentiating βTT from IDA but the specificity for detection of IDA of these two indices were unsatisfactory and gave significant false positive rate. But in these situations further confirmatory tests like determination of HbA₂, serum iron studies should be recommended because these DFs did not show reliable sensitivities and specificities.