CHAPTER 4

Discussion
Discussion

4.1 Haemoglobinopathy and thalassaemia

The situation of haemoglobinopathies and thalassaemia causing hereditary hemolytic anemia is very grim in India. Unfortunately, there is no attempt at the national level to enlarge the epidemiological database, establishing specific programmes for screening populations at risk, imparting genetic counseling and establishing special treatment centers to alleviate the sufferings of dwindling masses in India. Only piece meal and sporadic studies have been carried out in some communities in India. Hospital based case reports are available, but without having a central registry. The exact magnitude of the problem in India is still obscure. Only hospital-based data are available, which cannot be regarded as representative of the community or population. There is a genetic, ethnic and regional diversity of the hemoglobin variants as well as of the mutations in India which emphasizes to tackle the problem at a regional level.\textsuperscript{301,302,303} The generation of infra-structural diagnostic facilities at regional level, health education and prevention are needed for rehabilitation and amelioration of the affected masses in the country. Consanguineous marriages further compound the complexity by increasing the homozygosity in the community. Thus these genetic disorders of blood should be tackled at individual, family, community and national levels with full strength and sincerity.\textsuperscript{304,305,306}

Most of the patients of haemoglobinopathies have a high morbidity rate, intercurrent infections being unusually common, suffer from high economic burden, terminate fatally in childhood, and, have emotional and psychological trauma including the family members. The most effective approach to reduce the burden of the society is to reduce the incidence by implementation of a carrier-screening programme offering genetic counseling, prenatal diagnosis and selective termination of pregnancy of the affected foetuses in India.\textsuperscript{307,308,309}
4.2 Geographical Distribution.

The global distribution indicates a high prevalence in a belt around the earth, which is around the 40th parallel in the Mediterranean area but eastwards moves further south, reaching the equator in Indonesia. It is rare in people of Northern European origin, around 1: 1000 are carriers, while in the Northern Mediterranean countries (S. Europe) the carrier prevalence is 1-19%. In the Arab world the carriers are around 3% while in central Asia (Azerbaijan-Iran) 4-10%. From the Indian subcontinent to the S.E. Asia, α-thalassemia coexists with HbE in carrier rates which range from 1-40%.

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Italy represents a small group of high prevalence Mediterranean countries, which have developed control programs (treatment and prevention) within the context of a developed health provision system. Azerbaijan is a high prevalence country (carrier rate up to 15%) in which it is not possible to provide optimum treatment, resulting in early deaths, and prevention is still being considered.[314,315,316]

Changing Epidemiology.

This north-south divide has been gradually changing. The factors affecting epide-
miological change in genetic disease have been recognized as selection, genetic drift and migrations. Other interventions such as improved survival of patients, prevention programs, fall in total birth rate, improved education levels and cultural interactions are all contributing to changes in patient numbers, age distribution and needs for services^{317,318,319}. Selective advantage of the carrier state of the Thalassemias and haemoglobinopathies by increased resistance to malaria has been proposed as the reason for their high prevalence in these areas. Even though the relationship has not been proven conclusively for α-thalassemia, the geographical distribution coincides with historically malarial areas and there is more convincing evidence for α-thalassemia and sickle-cell disease (SCD). From these endemic areas however, there have been significant migrations during the 20th century towards N. Europe, the Americas, Australia and South Africa. In the earlier part of the century these migrations were mainly from the Mediterranean countries (Greece, Italy, Cyprus) bringing in the Thalassemia genes. The first generation of immigrants settled usually in ethnic communities (ghettos) and tended to marry within their ethnic groups. Later generations showed an increasing marriage-out rate as cultural barriers fell. The Americas already had sickle genes from the slave trade in previous countries^{320,321,322}. These migrations are continuing but now the Mediterranean countries have become recipients of emigrants rather than donors. Countries of sub-Saharan Africa are enriching Europe with SCD and the East is exporting α-thalassemia and HbE. HbE in particular is increasing in the West coast of N. America.
4.2.1 Analysis of relation of Consanguineous marriage with Thalassemia.

Our study confirms that in communities in which consanguineous marriage is common, an approach targeting the extended family is useful because it produces a high yield of information on carriers and couples at risk; family members often understand the condition because they have had contact with an affected child; and usually only one gene variant is present in a given family, simplifying and reducing the cost of DNA-based diagnosis. One of the outstanding features of the social relationships in Jordan is the existence of consanguineous marriages with considerable frequency. A consanguineous marriage is defined as marriage between individuals who are second cousins or more closely related. However, it is often possible to document lesser degrees of consanguinity quite relevant to pregnancy outcomes, particularly in highly inbred families.

The frequency of consanguineous marriages ranges from 50% to 66% in different parts of Jordan. First cousin marriage constitutes about one third of all marriages. Religion, culture, tradition, education, and major historic events affect the frequency of consanguineous marriages but the roles of tradition and historic events seem to dominate in the Jordanian culture. The frequency of consanguineous marriages correlates with an increase in recessively transmitted diseases, congenital malformations and infant mortality.

First cousin marriage in inbred families carries an even higher risk for autosomal recessive genetic diseases than first cousin marriage in non inbred families. In fact in Great Britain, this seemed to be the case, with a number of consanguineous marriages observed among Friedrich parents double the number expected. On the contrary our data did not support the hypothesis of genetic heterogeneity among Friedrich ataxia. This very approach was used to ask the same question regarding the possible genetic heterogeneity of cystic fibrosis.

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4.3 Analysis of Screening and Diagnostic Test

4.3.1 NESTROFT

The purpose of this study was to find the heterogeneity present in thalassemia affected population. The effectiveness of the NESTROFT is to used as a mass screening test. In present study NESTROFT was both sensitive (97.1%) and specific (100%) for identification of b-thalassemia trait.

Kattamis, raghawan, Gorashker, thomas and Mehta reported the sensitivity and specificity of NESTROFT in the range of 95 to 98.4 % and 66.6 to 91% respectively.

Gomber et. al reported that the sensitivity of ‘Nestroft’ was 95.59 per cent (grp I) & 85.71 per cent (grp II), specificity was 84.2 per cent (grp I) and 81.7 per cent (grp II) with a negative predictive value as high as 99.21 per cent in general population.

None of the normal subjects in present study showed positive NESTROFT test. Positive predictive value of NESTROFT in present study was 100% and negative predictive value was 98%. Kattamis in his study reported positive predictive value of 91.3 % and negative predictive value of 98.3% for NESTROFT.

Through NESTROFT was positive in 40% cases of sickle cell trait, 23.63 % cases of the sickle cell disease. In the study by Raghvan K, NESTROFT was positive in 29.46% and negative in 70.6% cases of sickle cell disease. Similar study by Thomas et al reported that NESTROFT was positive in 56.26% and negative in 43.75% cases of sickle cell disease.

Kattamis et al also found the test useful in picking up patients of sickle cell disease. When used as population screening, this will prove to be beneficial aspect of the test.
Though NESTROFT was positive in 100% cases of Thalassemia major, the detection is of major benefit as each of these conditions has its own health implications. NESTROFT does not miss out any β-thalassemia heterozygous and helps to pick up cases of sickle cell disease also.

4.4 Analysis of Hematopathology

4.4.1 Peripheral Blood Smear Examination

Peripheral smear examination can yield a wealth of information. Gross morphological abnormalities of red cells (hypochromia, microcytosis, anisocytosis, elliptocytosis, poikilocytosis, target cells, schistocytes) in a patient with Hb in range of 8-11 g% strongly suggests presence of beta thalassemia trait. Presence of microcytes indicates possibility of hereditary spherocytosis, auto-immune hemolytic anemia or hemolytic disease of newborn due to ABO incompatibility. Presence of schistocytes would indicate microangiopathic anemia. Leucoerythroblastic picture would indicate marrow infiltrative disorders (myeloma, metastasis, myelofibrosis, etc.). Presence of normoblasts in peripheral smear of patients with congestive cardiac failure signifies poor prognosis. Dimorphic anemia is best identified by peripheral smear examination showing microcytosis and macrocytosis, and hypochromia and normochromia. Presence of hypersegmented neutrophils (shift to right) indicates B12/folate deficiency.

4.4.2 Foetal hemoglobin

After the first year of life, Hb-F usually does not account for more than of the total haemoglobin. Levels higher than this are commonly seen in haemoglobinopathies (thalassemia, HPHF or abnormal haemoglobins). Investigation of foetal haemoglobi-
bin in other haematological disorders have shown it to be significantly raised in a number of benign or malignant acquired haematological disorders. In the present study, over one third of the cases of haematological malignancies were shown to have an acquired rise in foetal hemoglobin. The incidence and degree of rise in foetal Hb were variable in different disorders. Maximum rise was seen in juvenile chronic myeloid leukemia, where both cases had very high level of foetal Hb (28.2% and 32.6%) together with practically insignificant amount of HbA2 (i.e. 0.1 and 0.3%). Parents of both these cases showed no evidence of haemoglobinopathies. In the present series, cases of erythroleukemia did not show markedly raised HbF level (HbF: 5.4 and 8.2%). This was rather unusual as majority of the cases of erythroleukemia reported in the literature have shown HbF above 10%. Plat et al. (1994) examined predictive factors for life expectancy and risk factors for early death (among black Americans). In their study, a high level of foetal haemoglobin augured improved survival. Koshy et al. (1989) reported that foetal haemoglobin levels above 10% were associated with fewer chronic leg ulcers in American children with sickle cell disease.

An interesting feature was the occurrence of raised foetal Hb in more than half of the cases of haematological malignancies in children under the age of 10 years, while this was seen in only 35.4% of the cases over 10 years. The difference was statistically significant (p <0.05). In half of the cases where HbF was repeated after induction of remission in acute leukemia or after controlling the total leukocyte count in CML, the amount of HbF had increased. In one third of cases, the HbF levels had decreased while in the remaining it had remained constant. There was no obvious clinical or laboratory feature to predict alteration in HbF level and its clinical significance remains obscure.

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Production of foetal proteins by malignant tissue is a recognised feature seen in many neoplastic disorders. It is difficult to arrive at any significant conclusion regarding the need and the mechanism for the switch over from adult to foetal haemoglobin production in these different disorders. That this is not secondary to hypoxic stress on the marrow is clear as foetal Hb has not been shown to rise in congenital cyanotic heart diseases. In JCML and EL, Weatheral has shown a genuine reversion to foetal erythropoiesis. The fact that this reversion occurs more commonly in children may suggest that it may be affecting the basic mechanism by which the gamma chain synthesis is replaced by beta chain synthesis after birth. It has been stated that some of the cases may show raised foetal Hb as an effect of rapid regenerative process occurring after the aplasia due to the treatment used in leukemia. However, this does not appear to explain the whole problem as not all cases show rising Hb-F during the recovery phase. Interestingly, in a similar study, it was concluded that only myelogenous leukemias are characterised by acquired rise in foetal haemoglobin. However, from the present study this definitely appears to be disproven. Overall, the present study shows that foetal Hb rises in a significant number of cases of haematological malignancies. There appears to be no consistent relationship between the rise of foetal Hb and the nature or stage of malignancy and its response to treatment. Children with haematological disorders do show raised foetal Hb more often.

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4.4.3 Analysis of Complete Blood Count

Microcytosis and hypochromia are frequently observed findings in a hematology laboratory of which b-thalassemia trait and other hemoglobinopathy are common causes. Differentiation between other hemoglobinopathy and β-thalassemia can be reasonably made using red cell counts. MCV and MCH based on an elevated RBC count. The used of red cell indices in the form of discriminant functions further helps in differentiating the hemoglobinopathies.

It was observed in this study that elevated red cell count specially in the presence of mild anaemia was a reliable indicator of the presence of β-thalassemia. One hundred and fifty nine (85.2%) traits had an elevated RBC count. β-thalassemia traits generally have mild anaemia. The mean haemoglobin value is reported to be 12-13 g/dl in males and 9-10 g/dl in females.

An elevation in erythrocyte count despite low haemoglobin concentration in b-thalassemia has been reported earlier. RBC count was most reliable primary measurement for differentiating b-thalassemia and other hemoglobinopathy. However, about 10% of patients were misclassified based on an elevation of RBC count in their study.

Of one hundred and fifteen heterozygous b-thalassemia with iron deficiency, 96 (82.5%) had an elevated RBC count. Mean Hb concentration in this group was 10.7 (+-1.5) g/dl. It is evident from this study that elevation in red cell count remains an important screening test for b-thalassemia even in presence of iron deficiency.

The reduction in MCV and MCH was significantly (p < 0.0001) greater in heterozygous b-thalassemics as compared other hemoglobinopathy. Beutler and Klee have been reported similar results earlier. In present study MCV was less than 78 fl...
in majority of traits (97.3%). MCV is observed to be almost always reduced in β-thalassemia with values of 60-70 fl being the rule. Values this low are seen in only severe iron deficiency anemia. Jimenez and Minchinela has been proposed that the electronic measurement of MCV should be used as a screening test for b-thalassemia and patients with MCV < 80 fl should have quantitative estimation of HbA2 done.

MCH < 26 pg was observed in 94.5%, 97.3% and 83.4% patients of β-thalassemia with iron deficiency, β-thalassemia and iron deficiency anemia respectively. MCH has been observed to be sensitive and specific marker of b-thalassemia.

Four discriminant functions were calculated in each patient and comparative analysis made. The most sensitive discriminant functions was MCHC which identified 96.2% traits. However, the specificity of MCHC was lower than the other discriminant functions.

In a study on β-thalassemia patients in Orissa state, India, Andreas E. kulozik et. Al. reported that the total haemoglobin levels were highly variable (3.6 – 11.5 g/dl) although same pattern emerged.

Present study confirm most of the haematologic data reported by other investigators. The mean haemoglobin value was found to be 8.145. The mean values for MCHC, MCV and MCH were found to be 29.5 g/dl, 85.3 mm, and 27.14 pg, respectively. The red cell counts showed a low value with a mean of 1.356 x 10^6 mm^-3.

4.5 Biochemical Analysis

Gillis et. al. reported that thalassemia is associated with partial or complete deficiency of α or β globin chain synthesis, which leads to denaturation and degradation of the remaining globin chains. This process is associated with loss of the normal
asymmetrical distribution of the RBC membrane phospholipids and translocation of phosphatidylserine to the external membrane leaflet (flip-flop). The membrane damage may be related to lipid peroxidation mediated by free iron and increased amounts of membrane-bound hemichromes and immunoglobulins and modifications in the membrane band 3 protein and spectrin. The membrane changes may partly explain the enhanced aggregation of phosphatidylserine exposing RBCs, their increased adherence to endothelial cells, and their capacity to enhance thrombin generation via the assembly of the prothrombinase complex. The enhanced thrombin generation leads to activation of platelets, monocytes, granulocytes, and endothelial cells and expression of tissue factor, which further enhances the thrombotic process. The low levels of the coagulation inhibitors, protein C and protein S, further facilitate the resultant hypercoagulable state.

Sonakul D and P Pacharee reported that hypercoagulation was responsible for excessively permeable to cations such as sodium and potassium.

In the present study, the serum Na+ levels of the thalassemia diseased patients exhibited quite high values with a mean value of 287.52 mEq/L. However, the serum K+ levels showed a considerable variation with values ranging from 3.4 to 8.3 mEq/L. These results indicate that there may be a state of electrolyte imbalance present in the patients suffering from the thalassemia.

Christakis et. al. (1990) did investigations in Greek patients and reported the median serum bilirubin in 15 females as 32 mmol/l (range 12-55 mmol/l) and in 13 males was 41 m (15-128 mmol/l).

In the present study, the mean bilirubin level was found to be 4.56 mg/dl. This high bilirubin value indicates that the population under study might be suffering from haemolytic blood disease.
However the serum urea and seru creatinine levels analysis of the thalassemia diseased patients showed a normal range thus indicating that renal failure was a rare occurrence in the population studied.

The serum alkaline phosphatase activity measured in thalassemia diseased patients showed considerable high levels with a mean value of 125.38 IU/L. This indicate that the population under study may be suffering from hepatobiliary disorder.

4.6 Ethnobiology
Performing genetic counseling by Ambekar and Phadke et. al were the patients and their families referred to the Genetic Clinic of Sassoon General Hospital, Pune for the diagnosis of thalassemia. A total of 1291 subjects were studied. These included 891 cases from the pediatric age group and 400 cases from the adult age group. Of the total 891 cases from pediatric age group, 273 (21.1%) cases were from 0-2 year age group, 381 (29.5%) cases were from 2-5 year age group and 237 (18.45%) were from 5-10 year age group. From the pediatric age group, 591 (66.38%) males and 300 (33.77%) females were studied.

Present study also depict that wherein the male : female ratio was found to be 71% : 29%.

The communities which showed higher incidence of beta thalassemia were Navbudha, Maratha and Muslim. Present study also demonstrate that, in Amravati region sindhi community is on high risk.