CHAPTER 3

Observations and Results
3.1 Hemoglobinopathies and Thalassemias

Most of the scientist expressed there views about hemoglobinopathy, but the most common definition of hemoglobinopathies is ‘A genetic defects that results in abnormal structure of one of the globin chains of the hemoglobin molecule. These condition comprise a very large number of genetic biochemical and physiological entities. The genetic defect was occurred due to the substitution of one amino acid for another, deletion of a portion of the amino acid sequence, abnormal hybridization between two chains or abnormal elongation of the globin chain.

The first description was written by Dr. Thomas Cooley in 1925. He was observed multiplicity of different β thalassemia genes. He was noted abundant nucleated red blood cells in the peripheral blood and initially thought that he was dealing with erythroblastic anemia, it was described earlier by Von Jaksh. Before long Cooley realized that erythroblastemia is neither specific nor essential in this disorder. Although Cooley was aware of the genetic nature of the disorder, but he failed to investigate the apparently healthy parents of the affected children.

Riette described Italian children with unexplained mild hypochromic and microcytic anemia, in almost the same year in which Cooley reported the severe form of anemia later named after him. Wintrobe and coworkers in the United States reported a mild anemia in both parents of a child with Cooley anemia. This anemia was similar to that described by Riette in Italy. Only then was Cooley severe anemia recognized as the homozygous form of the mild hypochromic and microcytic anemia described by Riette and Wintrobe. The severe form then was labeled as thalassemia major and the mild form as thalassemia minor. These initial patients now are recognized to have been afflicted with beta thalassemia.
Byrne was the first to perform experimental studies with thalassemia. How
conclude from his experiment, the thalassemia are inherited disorders of Hb syn-
tesis resulting from an alteration in the rate of globin chain production. He notice
that decrease in the rate of production of a certain globin chain impedes Hb syn-
tesis and creates an imbalance with the other normally produced globin chains. I
suggest that since two types of chains pair with each other to form normal Hbs,
excess of the normally produced type was present and accumulates in the cell as an
unstable product leading to the destruction of the cell. This imbalance was the
hallmark of all forms of thalassemia. According to Byrne, most thalassemias are not
considered hemoglobinopathies, because the globin chains are normal in structure
and the defect is limited to a decreased rate of production of these normal chain.
The type of thalassemia usually carries the name of the under produced chains. Th
reduction varies from a slight decrease to complete absence of production.

Guilliotis was the first confirm that when beta chains are produced at a lower
rate, the thalassemia is termed beta+, whereas beta-0 thalassemia indicates a com-
plete absence of production of beta chains.

Machado made the important discovery that in most common type of beta
thalassemia trait, the level of A2Hb usually was elevated due to the increased utili-
ization of delta chains by the excessive free alpha chains resulting from lack of ade-
quate beta chains with which to pair. The delta gene, unlike beta and alpha genes
was known to have a physiologic limitation in its ability to produce adequate delta
chains by pairing with the alpha chains delta chains produce HbA2. Machado was
noted that remaining alpha chains precipitate in the cells, reacting with cell mem-
branes, intervening with cell membranes, intervening with normal cell division, and
acting as foreign bodies, leading to destruction of RBCs.
3.2 Geographical Distribution of Hemoglobin Disorders including Thalassemia

3.2.1 World-wide frequency of hemoglobin disorders including thalassemia

It was a mistake to think of hemoglobin disorders as occurring only in the tropics or in black populations. In Britain and most other western European countries about two people in every thousand carry alpha or beta thalassemia trait or abnormal hemoglobin. Some of the genes responsible may have been brought to Western Europe in the distant past by Roman soldiers and oriental traders, but others appeared spontaneously in the areas where they are found today.

In 1995 estimates made for the World Health Organization indicated that about 6% of the world population carry a hemoglobin disorder, and 7% of children born today are carriers. This is because the fastest population growth is occurring in countries where hemoglobin disorders are most common. The global proportion of carriers will probably rise to over 8% by the year 2010.

Hemoglobin S

This is extremely common in Africa, particularly in countries south of the Sahara and in some Asian Indian tribes. It is also found in areas where beta thalassemia is common, such as the Middle East, Northern India, Pakistan, Greece, Sicily and Southern Italy, Albania, Southern Turkey and Southern Portugal.

Sickle cell was taken with African slaves to North and South America and the West Indies in the 17th to 19th centuries, and as many as 10% of all black people in these counties now carry it. In more recent years it has been brought to western Europe by migrants from the Caribbean and Africa, and is first becoming established in most industrial cities in the developed world. In Britain, about 10% of all Afro Caribbean and over 20% of all Africans carry it. It is also found in the Indian, Pakistani, Cypriot, Italian, Greek and Portuguese communities, and very
occasionally indeed in northern Europeans.

**Hemoglobin C**

This is African hemoglobin that originated in what is now northern Ghana. It is common in West and North Africa, but not in East Africa. It was taken to the Caribbean and to North and South America with slaves from West Africa as early as the 17th century. About 3% of black people in these countries now carry it, as do 3% of African Caribbean living in Europe.

**Hemoglobin D Punjab**

This occurs naturally but very rarely in all populations, but it is relatively common in northern India and in neighboring countries such as Iran and Central Asia. It has been taken to other parts of the world by migration from northern India, and is now also found in the Caribbean, South Africa and Britain.

**β-thalassemia**

Through over 100 different mutations can cause beta thalassemia. Only a few of them are common in any given area. Most mutations cause a severe thalassemia, but in some areas there are also mild mutations (mild thalassemia is the commonest type in Malta). Delta-beta thalassemia, hemoglobin Lepore thalassemia, normal A2 beta thalassemia and few other obscure forms are also found occasionally, in all areas where sickle cell or beta thalassemia are common.

Beta thalassemia is very common in the Mediterranean, the Middle East, Central Asia, the Indian subcontinent, Southeast Asia and North Africa. It is less frequent in the rest of Africa. It has been taken to northern Europe, North and South America, South Africa and Australia by people migrating from Italy, Greece, Cyprus, Turkey, India, Pakistan, Bangladesh, North Africa, the Middle East, Southern China and Vietnam. In North and South America and the Caribbean, about 1% of black people carry it.
Figure 3.1: Geographical distribution of haemoglobinopathies belt. 

**Observations and Results**
3.2.2 Forms of hemoglobinopathies in India.

The Indian peninsula is a vast reservoir of abnormal hemoglobin as well as thalassemias. Most of the abnormal hemoglobin either have first been detected in India or among the individuals of Indian origin abroad. The abnormal hemoglobin so far detected in India include Hb D, E, H, J, K, L, M, Q, S, Lepore, Norfolk, Koya Dora, Chandigarh and the hereditary persistence of HbF.

Several reviews are available in India on haemoglobinopathy and thalassemia\cite{12,13,14,15} sickle cell haemoglobin\cite{16,17} hemoglobin E85, haemoglobin D86, haemoglobin double heterozygosity and various mutations detected in India\cite{18,19,20,21,122}. A map depicting distribution of cases of major forms of hemoglobinopathies in India is given in Figure 3.2.

Since the most commonly found abnormal haemoglobin in India, i.e. sickle cell haemoglobin (S), haemoglobin-E and haemoglobin-D have recently been extensively reviewed elsewhere, they have been excluded from the present consideration. A brief account of other important variants of haemoglobin is presented here.

The sickle cell haemoglobin is widely distributed all over India\cite{12,13,14}. Verma et al., after screening 3000 subjects belonging to various ethnic groups of Jammu region in the state of Jammu and Kashmir, detected 39 cases of HbAD trait and 3 cases of homozygous HbD disease. Agarwal et al. recorded 16 (1.5%) cases of haemoglobin-D trait in Lucknow, Uttar Pradesh, out of 1098 unrelated individuals who were tested. In their study, the prevalence of HbD trait in Khatris was 3.1%, compared to 0.5% in other Hindus. Balgir\cite{87} detected three families of haemoglobin-D in Orissa. Subbedar et al. reported one case of Hb-J in a scheduled caste family from Nagpur. Labie et al. recorded 3 cases of Hb-K among 114 Hindus of lower caste and another of unknown identity in Pondicherry. De Traverse et al. demonstrated 3 in-
stances of Hb-K among 10 South Indians in Chennai. Trincao et al. reported 2 instances of Hb-K in a survey of 1843 Indians in Goa. Verma et al. detected 18 cases of HbAK trait among the Hindus migrated from Poonch and Mussafrabad area of West Pakistan in Jammu. Sukumaran et al. demonstrated 8 instances of Hb-L in three Gujarati-speaking Lohana families in Mumbai. Only one family with haemoglobin-M has so far been detected in a Punjabi family from Amritsar. Three members of the family were found to have Hb-M levels of 7%, 33% and 50%, 138,139,140,141,147. In alpha-chain haemoglobin-M variants, the R-T equilibrium favours the T form. Oxygen affinity is reduced, and the Bohr effect is absent. Beta-chain haemoglobin-M variants exhibit R-T switching, and the Bohr effect is, therefore, present.3 In practice, these defects are known as heterozygotes. The blood is dark in colour, the affected individuals are cyanosed in appearance, but they survive into old age without difficulty. Trincao et al. detected 4 instances of Hb-Q in a survey of 1843 Indians in Goa. Sukumaran et al. recorded a new Hb-Qa (aspartic acid, histidine), or Hb-Q (India), in two Sindhi families in Mumbai. Recently, a new beta-chain variant, haemoglobin Chandigarh has been detected by Dash et al.

**Thalassemia and other haemoglobinopathies:**

Alpha-thalassemia is found in association with alpha-chain haemoglobin variants, e.g. Hb-Q and Hb-I; beta-chain variants, e.g. HbE, HbS; and with beta thalassemia.

**S-thalassemia:** Chatterjea reported 15 cases of Sthalassemia, 8 in Oriah Hindus, 1 each in Bengalee Hindus and Muslims, and 1 in South Indian Hindus and 2 in Tamil Muslims. Mital et al. recorded a high incidence of S-thalassemia among Sorathis in Palghar (3.7%). Lele et al. identified one family of S-thalassemia in a survey of 100 students belonging to scheduled caste in Aurangabad.

**E-thalassemia:** Chatterjea 67 detected 526 cases of Ethalassemia investigated in
Calcutta among Indian Hindus and the regional distribution was as follows: Bengalees (508), Oriahs (10), Biharis (4), Assamese (2), Punjabis (1), South Indians (1), and 48 cases among Bengalee Muslims and one in Bihari Muslims. Sarkar et al. detected 14 cases of E-thalassemia from Calcutta. Kochhar and Kathpalia and Praharaj et al. reported solitary instances of E-thalassemia in a Kannada and an Oriah family. Dash et al. demonstrated a case of E thalassemia in Punjab. Ghosh et al. described 7 cases of E-beta-thalassemia from Punjab and one case from Rajastan. High prevalence of haemoglobin-E in ten populations of Assam (20–60%) and in three populations of West Bengal (12–61%) has been studied by Deka et al. and Das et al. respectively, in North-Eastern India. DNA haplotypes analysis showed a common origin of haemoglobin E mutation in Assam and in South-East Asia.\textsuperscript{151,152,153,154}

**D-thalassemia:** Chatterjea recorded 9 cases of D thalassemia:
6 from Bengal, and one each from Bihar, Punjab and South India. Occasional cases of D-thalassemia have been reported in and around Delhi. Lele et al. detected one case in a Kunbi family from Aurangabad. Sukumaran et al. reported one case each in a Sindhi and Gujarati-Lohana family. One case of Hb-D trait with thalassemia was detected in a Muslim girl from Lucknow, Uttar Pradesh, by Agarwal et al.

**J-thalassemia:** Sanghvi et al. recorded one case of J-thalassemia in a Gujarati-speaking Lohana. Swarup et al. reported 4 cases of J-thalassemia in Bengalee Hindus.

**K-thalassemia:** Swarup et al. reported an interaction of Hb E and K with thalassemia in a Bengalee family of Calcutta.

**Q-thalassemia:** Sukumaran et al. recorded one case of Q-thalassemia major and 2 cases of Q-thalassemia minor in Sindhi families in Mumbai.

**Haemoglobin Lepore:** Chouhan et al.\textsuperscript{116} reported the only case of Hb Lepore in an Indian family from Koondapur of Karnataka.
Figure: 3.2 Geographical distribution of different forms of hemoglobinopathies in India
<table>
<thead>
<tr>
<th>States</th>
<th>b-Thalassemia trait frequency %,n/p</th>
<th>Sickle cell disease frequency %,n/p</th>
<th>HbE disease frequency %,n/p</th>
<th>HbD disease frequency %,n/p</th>
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</thead>
<tbody>
<tr>
<td>Western Maharashtra</td>
<td>7.04</td>
<td>2.3</td>
<td>0.61</td>
<td>0.15</td>
</tr>
<tr>
<td>N = 1291</td>
<td>n = 91</td>
<td>n = 30</td>
<td>n = 8</td>
<td>n = 2</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>–</td>
<td>7.2</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Jammu Kashmir</td>
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<td>–</td>
<td>–</td>
<td>3.3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>n = 15</td>
</tr>
<tr>
<td>Sindh</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Punjab</td>
<td>6.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>8.4</td>
<td>7.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Karnataka</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n = 110</td>
</tr>
<tr>
<td>South India</td>
<td>4.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gujarat</td>
<td>10-15</td>
<td>6.4</td>
<td>–</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 16</td>
<td></td>
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<tr>
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<td>–</td>
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<td>–</td>
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<td></td>
<td></td>
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</tr>
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<td>–</td>
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<td></td>
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<td>n = 1369</td>
</tr>
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<td>29.7</td>
<td>–</td>
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<td></td>
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<tr>
<td>Bengal</td>
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<td>–</td>
<td>7.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 19</td>
<td></td>
<td>P = 33</td>
</tr>
<tr>
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<td>7.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 60</td>
<td></td>
<td>n = 2916</td>
</tr>
<tr>
<td>Sikkim</td>
<td>–</td>
<td>–</td>
<td>0.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 13</td>
</tr>
<tr>
<td>India (Average frequency)</td>
<td>3.3</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 308</td>
<td></td>
<td>P = 93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 83</td>
</tr>
</tbody>
</table>

N = Total number of cases studied; n = Number of cases of a particular disease studied; P = Total number of population studied; p = Number of population of a particular disease studied.
3.2.3 Prevalence of thalassemia in different communities in Maharashtra

The communities which showed higher incidence of beta thalassemia were Navbudha, Maratha and Muslim. Amongst the total cases of beta thalassemia from Maharashtra studied, 33.5% were from Navbudha community, followed by Maratha 30.5%, Muslim 13.2%, Brahmin 4.8%, Gujarati 3.6%, Dhangar 3.0%, Koli and Patil 1.8%, Pardeshi, Banjara, Gargi, Sindhi and Lingayat 1.2% each and Ramoshi Kunbi and Mang 0.6% each.

3.2.4 The effect of thalassemia traits on malaria.

Thalassemia trait protect people against Plasmodium falciparum in different ways. Thalassemia carriers are protected because their red cells are smaller and contain less haemoglobin than normal. Consequently, the parasite uses up all the haemoglobin before it has finished growing. So it cannot spread to neighboring cells. Carriers who migrate to non-malarial countries no longer need their natural protection against malaria, but the trait do not disappear. Even though they are redundant as protection against malaria, the thalassemia genes continue to be handed down in families for generations.

Hemoglobin disorders are common in parts of the world where malaria used to be rite, or where it is still a problem. This is because thalassemia give the carrier a strong natural protection against malaria. It does not stop carriers being infected with malaria; it only reduces the risk of dying from it. It is only sickle cell and thalassemia traits that help to protect people against malaria.
3.3 Risk of Consanguinity

3.3.1 Current prevalence of consanguineous unions

As a working definition, unions contracted between persons biologically related as second cousins ($F^3$ generation) are categorized as consanguineous. This arbitrary limit has been chosen because the genetic influence in marriages between couples related to a lesser degree would usually be expected to differ only slightly from that observed in the general population. Globally, the most common form of consanguineous union contracted is between first cousins, in which the spouses share 1/8 of their genes inherited from a common ancestor, and so their progeny are homozygous (or more correctly autozygous) at 1/16 of all loci. Conventionally this is expressed as the coefficient of inbreeding ($F$) and for first cousin offspring, $F = 0.0625$. That is, the progeny are predicted to have inherited identical gene copies from each parent at 6.25% of all gene loci, over and above the baseline level of homozygosity in the general population. In some large human populations genetically closer marriages also are favored, in particular uncle-niece and double first cousin unions where the level of homozygosity in the progeny is equivalent to $F = 0.125$. National populations can be approximately subdivided into four main categories: those in which consanguineous unions account for less than 1% of marriages, 1% to 10%, and 20% to over 50%, and populations where the level of consanguinity is unknown, either because it has not been reported or the data are of insufficient reliability and depth to make a prediction with any degree of confidence. Applying these definitions, the present numbers in each category are: less than 1% consanguinity, 1,061 million; 1% to 10% consanguinity, 2,811 million; 20% to 50% consanguinity, 991 million; and unknown, 1,064 million. As the data collection methods employed were con-
servative, these figures should be regarded as lower bound estimates. With the exception of Japan, which has undergone rapid industrialization and urbanization since World War II, past predictions of a rapid decline in the overall prevalence of consanguineous unions have proved to be largely incorrect. In fact, the recorded numbers of consanguineous unions appear to have grown at least in step with increasing national and regional populations, and in some economically less developed countries the proportion of marriages contracted between close biological kin has expanded. The simplest explanation for this observation is that as greater numbers of children survive to marriageable age, the traditional social preference for consanguineous unions can be more readily accommodated.\textsuperscript{156,157,158}

Migrant communities now permanently resident in Western countries may represent a special case, especially where they practice a religion not followed by the majority indigenous population. In such communities, the available evidence from Western Europe, North America and Australasia suggests that the prevalence of consanguineous unions is increasing, in many cases from an already high. Various reasons can be advanced for this finding, including the desire to find a marital partner from within the community, which itself may be numerically small and composed of a restricted number of kindreds, and the wish to maintain community traditions in a new and unfamiliar environment. However, explanations of this type underestimate the strong belief that marriage within the family, as opposed solely to community endogamy, is the most desirable and reliable marital.\textsuperscript{159,160,161}
Religious and legal regulation of consanguineous marriage

Religious proscription

There appears to be no particular rationale for the subdivision of human populations into opposing forms of marriage preference, and even within the major religions there are quite marked differences in attitude to close kin marriage. Thus in Christianity, the Orthodox churches prohibit consanguineous marriage, the Roman Catholic church currently requires Diocesan permission for marriages between first cousins, and the Protestant denominations permit marriages up to and including first cousin unions reported by Bittles et al. 2001.

A similar degree of non-uniformity exists in Hinduism. The Aryan Hindus of northern India prohibit marriage between biological kin for approximately seven generations on the male side and five generations on the female side reported by Kapadia 1958. By comparison, Dravidian Hindus of South India strongly favour marriage between first cousins of the type mother’s brother’s daughter (MBD) and, particularly in the states of Andhra Pradesh, Karnataka and Tamil Nadu, uncle-niece marriages also are widely contracted.

In general, Muslim regulations on marriage parallel the Judaic. However, uncle-niece unions are permitted in Judaism. Yet they are forbidden by the Koran, even though double first cousin marriages, which have the same coefficient of inbreeding \((F = 0.125)\), are recognized within Islam. In southern Asia, Buddhism sanctions marriage between first cousins, as does the Zoroastrian/Parsi tradition. The Sikh religion forbids consanguineous marriage, although some minority Sikh groups appear to exercise flexibility in the observance of this proscription162,163,164.
Legislation

A similar lack of coherence exists in legislation enacted in different countries to govern permitted types of consanguineous relationships in marriage. For example, first cousin marriages are legal in countries such as the U.K. and Australia, but they are criminal offences in eight of the states of the U.S.A. and illegal in a further 31 states. Yet exceptions can be incorporated into state laws. Legislation approved and adopted at the national level may also prove to be inoperable in practice, as exemplified by the Hindu Marriage Act of 1955 which includes a ban on uncle-niece marriage. Yet in a study conducted between 1980 and 1989 in Bangalore and Mysore, the two major cities of the state of Karnataka in southern India, 21.3% of Hindu marriages were uncle-niece unions\textsuperscript{159,160,161}.

3.3.4 Sociodemographic aspects of consanguinity

The specific types of consanguineous marriage that are favoured can vary quite widely between and within different countries, with religious, ethnic, and local or tribal traditions playing a major role at local and national levels. The reasons most commonly given for the popularity of consanguineous marriage can be summarized as: a strong family tradition of consanguineous unions; the maintenance of family structure and property, and the strengthening of family ties; financial advantages relating to dowry or bridewealth payments; the ease of marital arrangements and a closer relationship between the wife and her in-laws; and greater marriage stability and durability. The degree of social compatibility, and the close involvement of the entire family in consanguineous unions, may explain both the greater stability that has been claimed for consanguineous unions, which have lower divorce rates, and enhanced female autonomy.
Among the major populations so far studied, the highest rates of consanguineous marriage have been associated with low socioeconomic status, illiteracy, and rural residence. In some populations a high prevalence of marital unions between close relatives has however been reported among land-owning families, and in traditional ruling groups and the highest socioeconomic strata. Interactions between consanguinity and social variables can potentially complicate assessment of the genetic effects of human inbreeding, and failure to account for social variables when estimating the possible effects of inbreeding on mortality predictably would lead to biased results, with overestimation of the adverse biological effects ascribed to consanguinity. Conversely, where consanguinity has not been included as an explanatory variable, the influence of other more widely investigated demographic determinants, such as maternal age, maternal education, birth interval, and birth order, probably require significant downward revision.

3.3.5 Consanguinity, morbidity, and mortality

The detrimental health effects associated with consanguinity are caused by the expression of rare, recessive genes inherited from a common ancestor(s). In populations where inbred unions are common, increased levels of morbidity and mortality caused by the action of detrimental recessive genes can be predicted. Generally, inbreeding is associated with loss of biological fitness. It is however appropriate to note that, even in the absence of preferential consanguinity, alleles which are rare in large populations can rapidly increase to high frequency in a breeding pool of restricted size, because of factors such as founder effect and random genetic drift.

Empirical studies on the progeny of first cousins indicate morbidity levels to be some 1% to 4% higher than in the offspring of unrelated couples (reviewed in Observations and Results 47
Bittles and Makov 1988). The less common a disorder, the greater the influence of consanguinity on its prevalence, a generalization that applies to recessive multigene disorders as well as to single gene conditions. For this reason, many previously unrecognized genetic diseases have first been diagnosed in highly endogamous communities, and in a significant proportion of cases the underlying mutation may be unique to the community. At a practical level, this community-specific pattern of disease leads to major problems when attempting to estimate the burden imposed by consanguinity-associated morbidity at national or even at regional and local levels.

In a study based on combined data from 38 populations in eastern and southern Asia, the Middle East, Africa, Europe, and South America, with average coefficient of inbreeding (φ) values ranging from 0.0005 to 0.0370, mean excess mortality at the first cousin level was 4.4%. This estimate appears to be valid for all of the large
Figure 3.5 Geographical comparison of distribution of蚕毛รอย and haemoglobinopathies
human populations so far examined. However, consanguinity interacts with a range of sociodemographic variables in determining rates of mortality during infancy and early childhood, including some common cancers and cardiovascular disease.

3.4 Screening and Diagnostic tests.

3.4.1 Results of NESTROFT

A total of 1154 peoples were screened, NESTROFT was positive in 56 cases of β-thalassemia trait (True Positive, TP). There were no False positive. It was negative in 1 cases. (false negative, FN) and other cases did not have β-thalassemia trait (True Negative, TN). Sensitivity of NESTROFT was 96.1% and specificity was 100%. Positive predictive value was 100% and negative predictive values was 98%.

NESTROFT was also positive in 29 cases of sickle cell trait and 13 cases of sickle cell disease and 21 cases of β-thalassemia major. However, none of the normal subjects showed positive NESTROFT test.

Figure 3.6: Showing NESTROFT instrument for rapid screening.
Table- 3.4 : Results of Nestroft in β-thalassemia trait.

<table>
<thead>
<tr>
<th>NESTROFT</th>
<th>Normal Subject n= 500</th>
<th>β-Thalassemia trait n= 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>00</td>
<td>55</td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>01</td>
</tr>
</tbody>
</table>

3.4.2 Peripheral Blood Smear Examination

The examination starts with a macroscopic view to evaluate the quality of the smear based on overall appearance. The microscopic analysis begins on lower power (10x), primarily to assess cellular distribution, staining quality, and to select an area where the RBCs are barely touching each other. This area is used to conduct a complete assessment of the cellular elements on higher magnification. All of the detailed analysis of the cellular elements is performed using oil immersion. This final microscopic examination was perform at 50x and 100 x oil immersion and includes:

- A WBC differential
- The identification of abnormal and peculiar leukocytes
- Assessment of RBC morphology
- The number and morphology of the platelets
- The identification of intra- and extra-cellular elements.
- Assessment of any organisms present.

Following criteria was used to examine the peripheral blood smear of thalassemic patients.

- Size
- Shape
Sample 1

Peripheral blood smear shows that fragmented red blood cell. Fragmented cells are cells that are broken up or otherwise misshapen. Specific terms, depending on the shape, include schistocyte, acanthocyte, spur cells, and burl cells.

Figure 3.8: Microphotograph showing abnormality in shape of RBCs of thalassemic patients.
Sample 2

This microphotograph depicts polychromasia. Referring to the blue-gray color of the red cell. Peripheral blood smear also showing microcytic, poikilocytosis including elliptical and elongated RBCs.

Figure 3.9: Microphotograph showing abnormality in shape of RBCs of thalassemic patients.
Sample 3

The arrowed cells are anisocytes, target cell and tear drop cell also clearly seen. Microcytosis also depicts. A microcyte is a small red blood cell, having a diameter of less than 7 um.

Figure 3.10: Microphotograph showing abnormality in shape of RBCs of thalassemic patients.
Sample 4

Peripheral blood smear shows an platelet aggregation, vacuoles in RBCs. Microcytic, hypochromic, anisocytosis and poikilocytosis also observed.

Figure 3.11: Microphotograph showing abnormality in shape of RBCs of thalassemic patients.
Sample 5
Peripheral blood smear shows an platelet aggregation, vacuoles in RBCs. Microcytic, hypochromic, anisocytosis and poikilocytosis also observed.

Figure 3.12: Microphotograph showing abnormality in shape of RBCs of thalassemic patients.
3.5 Biochemical analysis

3.5.1 Estimation of serum bilirubin

Bilirubin is one of the products of haemolysis of erythrocytes by hepatic macrophages (Kupffer cells) in the liver and by other macrophages in the spleen and bone marrow.

As the life span of red blood cells is approximately four months or 120 days, the destruction or haemolysis is carried out by phagocytic reticulo-endothelial cells. These cells are found in many tissues but the main sites of haemolysis are the spleen, bone marrow and liver. When erythrocytes age, changes in their cell membranes make them more susceptible to haemolysis. During this destruction, the amino acids from globulin chains and iron from the heme units are salvaged and rived. The bulk of heme unit is converted to bilirubin. In its original form bilirubin is insoluble in water and is carried in the blood bound to albumin. In hepatocytes it is conjugated with glucuronic acid and becomes water soluble before being excreted in bile. Bacteria in the intestine change the form of bilirubin and most is excreted as sterobilinogen in the faeces and a small amount is reabsorbed and excreted in urine as urobilinogen.

The plasma-insoluble form of bilirubin is referred to as unconjugated bilirubin; the water soluble form is referred to as conjugated bilirubin. Serum level of conjugated and unconjugated bilirubin can be measured in the laboratory and are reported as direct and indirect, respectively. If red cell destruction and consequent bilirubin production are excessive, unconjugated bilirubin accumulates in the blood. This results in a yellow discolouration of the skin, called jaundice. When red blood cell destruction takes place in the circulation, as in haemolytic anaemia, the Hb remains in the plasma. The plasma contains a Hb-binding protein called haptoglobin.
Other plasma proteins, such as albumin, can also bind Hb. When extensive intravascular distruption of red blood cells, Hb levels may exceed the Hb-binding capacity of haptoglobin. When this happens, free Hb appears in blood (i.e. hemoglobinemia) and is excreted in the urine\textsuperscript{255,256} (i.e. hemoglobinuria)

**Normal values**

In adult the conjugated (direct) bilirubin value is 0.0 to 0.2 mg/dl while unconjugated (indirect) bilirubin value is 0.2 to 0.8 mg/dl

In infant premature : > 10 mg/dl
And full term : > 04 mg/dl

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
<th>Standard Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Bilirubin</td>
<td>1.10 - 14.65</td>
<td>4.761334</td>
<td>3.65</td>
<td>±1.0891</td>
<td>7.90012</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>14.93 - 31.55</td>
<td>23.09812</td>
<td>22.34</td>
<td>±1.8111</td>
<td>23.609</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>0.23 - 0.93</td>
<td>0.124</td>
<td>0.93</td>
<td>±0.13108</td>
<td>0.0312</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>15.7 - 398.8</td>
<td>135.9356</td>
<td>95.44</td>
<td>±1.481</td>
<td>8120.26</td>
</tr>
<tr>
<td>Serum electrolytes i) Na\textsuperscript{+}</td>
<td>3.4-371</td>
<td>282.1281</td>
<td>218</td>
<td>±3.181</td>
<td>1451.65</td>
</tr>
<tr>
<td>ii) K\textsuperscript{+}</td>
<td>2.3-6.8</td>
<td>5.012</td>
<td>4.3</td>
<td>±0.109</td>
<td>1.3121</td>
</tr>
</tbody>
</table>

Table 3.5: Showing the biochemical estimations of thalassemia diseased patients.
Fig. 3.13: Showing bilirubin level in thalassemic patients
3.5.2 Estimation of Serum creatinine

Creatinine is a breakdown product of creatine, which is an important constituent of muscles. Creatinine are the waste products of protein metabolism. They formed in the liver and conveyed in blood to the kidneys for excretion. It is removed from plasma by glomerular filtration is then excreted in the urine without being reabsorbed by the tubules to any significant extent.

The test of creatinine is performed to see the kidney function. Because, if the kidney function is abnormal, creatinine level will increase in the blood as the excretion of creatinine in urine get decreases. Creatinine can be convert to the ATP molecule, which is a high energy source. The daily production of creatine and subsequently creatine, depends on muscle mass, which filtrates very little. The serum creatine generally decreases in pregnancy and in conditions characterized by muscle wasting\textsuperscript{268,269,270}.

Creatinine determinations have one advantage over urea determinations, they are not affected by a high protein diet as in the case for urea levels. The normal values of it in serum is 0.9 to 1.5 mg/dl and in urine 90 to 150 mg/dl.
Fig. 3.14: Showing Serum creatinine level in thalassemic patients
3.5.3 Estimation of serum alkaline phosphatase

Alkaline phosphatases are a group of enzymes found primarily the liver and bones. Small amount of it also produced by intestinal cell lining, the placenta and the kidney in the proximal convulated tubules. This enzyme works best at an alkaline pH 10 and thus the enzyme itself is inactive in the blood. Alkaline phosphatases act by splitting of phosphorus (an acidic mineral) creating an alkaline pH.

The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. When the liver, bile ducts or gall bladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatases is released into the blood stream. Thus the serum alkaline phosphatases is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.296,297

Very high alkaline phosphatase activity is seen in those patients having bone cancer, in obstructive jaundice and biliary cirrhosis.

Moderate elevation have been observed in congestive heart failure, infective hepatitis, and abdominal problems.

3.5.4 Estimation of serum sodium and potassium

The major abnormality within red blood cell (RBC) results from the precipitation of unstable hemoglobin chain. The RBC membrane is consist of lipid protein, sialoglycoproteins and glycolipids, all the components are altered. The membrane alteration affect the transport of sodium and potassium.296,297

Sodium, the major extracellular cation, plays a role in fluid distribution among body compartments. The ingested sodium is filtered in the renal glomerulus and approximately 70% is reabsorbed in the proximal tubule. Further reabsorption oc-
Fig. 3.15: Showing Alkaline phosphatase level in thalassemic patients
curs in the loop of Henle and <5% is reabsorbed distally under the influence of aldosterone. About 65-70% of the total body sodium is in its exchangeable form. The exchangeable sodium is made up of extracellular and intracellular sodium. The intracellular sodium concentration is about 10 mmol/L and the extracellular, i.e. the plasma sodium concentration, is about 140 mmol/L. Sodium maintains the osmotic pressure of the extracellular fluid and helps in retaining water in the extracellular compartment. Along with other cations it is also involved in neuromuscular irritability, acid base balance, maintenance of blood viscosity and resting membrane potential\textsuperscript{301,302}.

A high plasma sodium concentration of more than 145 mmol/L is referred to as hypernatremia. This can occur due to simple dehydration, excess sodium intake, steroid therapy as well as in diabetic insipidus. Hyponatremia, with plasma sodium concentration less than 130 mmol/L, can occur due to diuretic medication, kidney disease, excessive sweating, congestive heart failure or gastrointestinal disorder.

Potassium is the major intracellular cation. It is widely distributed in the body in muscle tissue, nerve tissue, blood cells and plasma. It is filtered in the glomerulus, absorbed in the proximal tubule and finally excreted by exchange for sodium in the distal tubule. Potassium influences muscular activity, cardiac function and nerve conduction process. In hyperkalemia the plasma potassium concentration exceeds 5.5 mmol/L. Acute hyperkalemia is a medical emergency. In hypokalemia the plasma potassium level will be less than 3.5 mmol/L. This can occur due to excessive loss in gastrointestinal secretions and urine, and also in renal tubular acidosis.
SODIUM

Increase in serum sodium is seen in conditions with water loss in excess of salt loss, as in profuse sweating, severe diarrhea or vomiting, polyuria (as in diabetes mellitus or insipidus), hypergluco- or mineralocorticoidism, and inadequate water intake.

Drugs causing elevated sodium include steroids with mineralocorticoid activity, carbenoxolone, diazoxide, guanethidine, licorice, methyldopa, oxyphenbutazone, sodium bicarbonate, methoxyflurane, and reserpine.

Decrease in sodium is seen in states characterized by intake of free water or hypotonic solutions, as may occur in fluid replacement following sweating, diarrhea, vomiting, and diuretic abuse. Dilutional hyponatremia may occur in cardiac failure, liver failure, nephrotic syndrome, malnutrition, and SIADH. There are many other causes of hyponatremia, mostly related to corticosteroid metabolic defects or renal tubular abnormalities. Drugs other than diuretics may cause hyponatremia, including ammonium chloride, chlorpropamide, heparin, aminoglutethimide, vasopressin, cyclophosphamide, and vincristine.

POTASSIUM

Increase in serum potassium is seen in states characterized by excess destruction of cells, with redistribution of K⁺ from the intra- to the extracellular compartment, as in massive hemolysis, crush injuries, hyperkinetic activity, and malignant hyperpyrexia. Decreased renal K⁺ excretion is seen in acute renal failure, some cases of chronic renal failure, Addison's disease, and other sodium-depleted states. Hyperkalemia due to pure excess of K⁺ intake is usually iatrogenic.

Drugs causing hyperkalemia include amiloride, aminocaproic acid, antineoplastic agents, epinephrine, heparin, histamine, indomethacin, isoniazid, lithium, mannitol, methicillin, potassium salts of penicillin, phenformin, propranolol, salt
substitutes, spironolactone, succinylcholine, tetracycline, triamterene, and tromethamine. Spurious hyperkalemia can be seen when a patient exercises his/her arm with the tourniquet in place prior to venipuncture. Hemolysis and marked thrombocytosis may cause false elevations of serum K+ as well. Failure to promptly separate serum from cells in a clot tube is a notorious source of falsely elevated potassium. Decrease in serum potassium is seen usually in states characterized by excess K+ loss, such as in vomiting, diarrhea, villous adenoma of the colorectum, certain renal tubular defects, hypercorticotidism, etc. Redistribution hypokalemia is seen in glucose/insulin therapy, alkalosis (where serum K+ is lost into cells and into urine), and familial periodic paralysis. Drugs causing hypokalemia include amphotericin, carbenicillin, carbenoxolone, corticosteroids, diuretics, licorice, salicylates, and ticarcillin.

In the present study, when the blood samples of thalassemic patients were analysed for serum Na+ and K+ concentrations. It was found that serum Na was increased significantly. The mean value of serum Na concentration increased significantly (P < 0.05) in male patients whereas it was decreased significantly (P < 0.01) in female patients.

In both male and female patients of Thalassemia the K+ was non-significantly increased, did not show any variation as compared to control subject.

3.5.5 Estimation of serum urea

Urea contributes most of the body’s non-protein nitrogen, accounting for about 45% of the total. It is the major end-product of protein catabolism in humans. It is synthesized in the liver, released into blood circulation and excreted by the kidneys. Measurement of urea in blood is a useful indicator of renal and hepatic integrity.
Fig. 3.16: Showing the serum sodium level in thalassemic patients
Fig. 3.17: Showing the serum potassium level in thalassemic patients
Fig. 3.18: Showing the serum urea level in thalassemic patients
3.6 Hematopathology

3.6.1 CBC Interpretation

Red cells develop from stem cells in the bone marrow and are released as reticulocytes into the blood. The primary function of the red blood cells, or erythrocytes, is to carry oxygen from the lungs to body tissues and to transfer carbon dioxide from the tissues to the lungs. Oxygen transfer is accomplished via the hemoglobin contained in red blood cells. Hemoglobin combines readily with oxygen and carbon dioxide.

Red cell in thalassemic patients are microcytic hypochromic usually with mild degree of anaemia. With the availability of electronic particle counters, red cell indices have become more attractive and are combined or used singly to identify possible heterozygotes\textsuperscript{278,279}.

In the present study the 380 patients of beta thalassemia major and trait, iron deficient subjects and 920 normal subjects were investigated from different hospitals and thalassemia camps held in Amravati region.

3.6.1 a Erythrocyte count

The RBC is a count of the number of red blood cells per cubic millimeter of blood. Red blood cell count showing lower values than normal. Normal red blood cell values at various ages are:

Adults (males) : 4.6 – 5.9 million

(females) : 4.2 – 5.4 million

newborns : 5.5 – 6 million

children : 4.6 – 4.8 million
3.6.1 b HGB Count

Referred to simply as “hemoglobin,” this test involves lysing the erythrocytes, thus producing an evenly distributed solution of hemoglobin in the sample. The hemoglobin is chemically converted mole-for-mole to the more stable and easily measured cyanmethemoglobin, which is a colored compound that can be measured colorimetrically. Normal hemoglobin values are:

Adult (males): 13 – 18 gm

(females): 12 – 16 gm

Pregnancy: 11 – 12 gm

Newborn: 17-19 gm. 77% of this value is fetal hemoglobin, which drops to approximately 23% of the total at 4 months of age.

Children: 14 – 17 gm

3.6.1 c Mean corpuscular volume (MCV) count

Mean corpuscular volume measures the mean or average size of individual red blood cells. To obtain the MCV, the hematocrit is divided by the total RBC count. The MCV is an indicator of the size of red blood cells.

Normal values for MCV

Male: 80 – 90 cubic microns

Female: 82 – 98 cubic microns
3.6.1 d Hematocrit (PCV)

The hematocrit, also known as the “Hct”, “crit” or PCV (packed cell volume) determines the percentage of red blood cells in the plasma. The term hematocrit means “to separate blood.” When the patient’s blood sample is spun in a centrifuge, the white blood cells and platelets rise to the top in what is known as the “buffy coat”. The heavier red blood cells sink to the bottom, where they can be calculated as a percentage of the total blood sample.

Normal hematocrit values are:

Adults: (males) : 45–52 %, (females) : 37 – 48 %

Pregnancy: decreased hematocrit, especially in the last trimester as plasma volume increases

Newborn: up to 60%

Children: varies with age

3.6.1 e Mean corpuscular hemoglobin (MCH)

MCH measures the amount of hemoglobin present in one RBC. The weight of hemoglobin in an average cell is obtained by dividing the hemoglobin by the total RBC count.

3.6.1 f Mean corpuscular hemoglobin concentration (MCHC)

MCHC measures the proportion of each cell taken up by hemoglobin.

3.6.1 g PLT Count

Platelets are cell fragments formed in the bone marrow that circulate throughout the
Table 3.6 Showing the hematological characteristics of the thalassemic patients under study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
<th>Variables</th>
<th>Standard Deviation</th>
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</thead>
<tbody>
<tr>
<td>RBC</td>
<td>1.14-5.45 million</td>
<td>3.325</td>
<td>4.589</td>
<td>1.254</td>
<td>±0.958741</td>
</tr>
<tr>
<td>HGB</td>
<td>2.2-10 gm</td>
<td>6.587</td>
<td>6.985</td>
<td>3.568</td>
<td>±0.25489</td>
</tr>
<tr>
<td>MCV</td>
<td>52.3-84.3 fL</td>
<td>68.325</td>
<td>62.324</td>
<td>95.325</td>
<td>±1.3589</td>
</tr>
<tr>
<td>PCV (HCT)</td>
<td>6-35 (%)</td>
<td>20.5</td>
<td>21.658</td>
<td>15.3756</td>
<td>±0.25678</td>
</tr>
<tr>
<td>MCH</td>
<td>19.6-31.5 pg</td>
<td>25.55</td>
<td>24.254</td>
<td>19.356</td>
<td>±1.2587</td>
</tr>
<tr>
<td>MCHC</td>
<td>25.1-37.6 g/dL</td>
<td>31.45</td>
<td>30.2</td>
<td>15.3654</td>
<td>±0.2547</td>
</tr>
<tr>
<td>PLT</td>
<td>1.5-3.4 lac/cu. M.M</td>
<td>2.45</td>
<td>2.36</td>
<td>1.278</td>
<td>±0.6547</td>
</tr>
<tr>
<td>WBC</td>
<td>3100-9000 /C.M.M</td>
<td>6050</td>
<td>5365</td>
<td>25.3689</td>
<td>±0.2478</td>
</tr>
<tr>
<td>NEUTROPHIL</td>
<td>1-5 (%)</td>
<td>2.5</td>
<td>1.987</td>
<td>1.254</td>
<td>±0.26378</td>
</tr>
<tr>
<td>MONOCYTES</td>
<td>1-6 (%)</td>
<td>3</td>
<td>2.345</td>
<td>1.278</td>
<td>±0.8647</td>
</tr>
<tr>
<td>EOSINOPHILS</td>
<td>1-5 (%)</td>
<td>2.5</td>
<td>2.987</td>
<td>1.254</td>
<td>±0.25378</td>
</tr>
<tr>
<td>BASOPHILS</td>
<td>0-3 (%)</td>
<td>1.5</td>
<td>1.025</td>
<td>0.598</td>
<td>±0.5478</td>
</tr>
<tr>
<td>LYMPHOCYTES</td>
<td>24-48 (%)</td>
<td>36</td>
<td>33.65</td>
<td>5.986</td>
<td>±0.2314</td>
</tr>
</tbody>
</table>
blood stream. A normal platelet count ranges between 150,000 and 450,000.

3.6.1 h WBC counting

The total WBC count was invariably done using an automated method.

3.6.2 Estimation of Foetal haemoglobin

The overall incidence and the degree of raised haemoglobin-F values in thalassemia are shown in Table 3.6. No inclusion bodies were seen in the red cells in any case. Hb-F showed heterogenous intraerythocytic distribution in all the cases. The effect of age at onset of the disease on the rise of Hb-F is shown in (Table 3.6) There were no significant differences between the regularly transfused and untransfused groups.

Table 3.6 Showing Foetal hemoglobin concentration in minor and major thalassemic patient.

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Age / Sex</th>
<th>Thalassemia</th>
<th>% of Hb F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/F</td>
<td>Major</td>
<td>4.35</td>
</tr>
<tr>
<td>2</td>
<td>3/M</td>
<td>Major</td>
<td>7.59</td>
</tr>
<tr>
<td>3</td>
<td>9/M</td>
<td>Major</td>
<td>6.87</td>
</tr>
<tr>
<td>4</td>
<td>11/F</td>
<td>Minor</td>
<td>5.36</td>
</tr>
<tr>
<td>5</td>
<td>14/M</td>
<td>Minor</td>
<td>4.25</td>
</tr>
<tr>
<td>6</td>
<td>5/M</td>
<td>Major</td>
<td>4.29</td>
</tr>
<tr>
<td>7</td>
<td>10/M</td>
<td>Major</td>
<td>2.65</td>
</tr>
<tr>
<td>8</td>
<td>5/F</td>
<td>Major</td>
<td>5.97</td>
</tr>
<tr>
<td>9</td>
<td>3/F</td>
<td>Minor</td>
<td>3.36</td>
</tr>
<tr>
<td>10</td>
<td>8/F</td>
<td>Minor</td>
<td>2.98</td>
</tr>
<tr>
<td>11</td>
<td>2/M</td>
<td>Major</td>
<td>8.34</td>
</tr>
<tr>
<td>12</td>
<td>4/M</td>
<td>Major</td>
<td>10.25</td>
</tr>
<tr>
<td>13</td>
<td>3/F</td>
<td>Major</td>
<td>8.78</td>
</tr>
<tr>
<td>14</td>
<td>12/F</td>
<td>Minor</td>
<td>2.14</td>
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<tr>
<td>15</td>
<td>6/M</td>
<td>Major</td>
<td>6.78</td>
</tr>
<tr>
<td>16</td>
<td>3/F</td>
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<td>2.48</td>
</tr>
<tr>
<td>17</td>
<td>4/M</td>
<td>Major</td>
<td>6.89</td>
</tr>
<tr>
<td>18</td>
<td>7/M</td>
<td>Minor</td>
<td>4.10</td>
</tr>
<tr>
<td>19</td>
<td>9/F</td>
<td>Major</td>
<td>5.26</td>
</tr>
<tr>
<td>20</td>
<td>12/F</td>
<td>Minor</td>
<td>2.98</td>
</tr>
</tbody>
</table>
Fig. 3.19: Showing RBC count in thalassemic patients
Fig. 3.20: Showing percent of haemoglobin in thalassemic patients.
Fig. 3.21: Showing Mean Cell Volumes in thalassemic patients
Fig. 3.22: Showing haematocrit value in thalassemic patients
Fig. 3.2: Showing mean corpuscular haemoglobin in thalassaemic patients.
Fig. 3.24: Showing mean corpuscular haemoglobin concentration in thalassaemic patients
Fig. 3.25: Showing platelet count in thalassemic patients
Fig. 3.26: Showing lymphocyte count in thalassemic patients
Fig. 3.27: Showing leukocyte count in thalassemic patients
Fig. 3.28: Showing eosinophils count in thalassemic patients
Fig. 3.30: Showing basophils count in thalassemic patients
Fig. 3.31: Showing monocytes count in thalassemic patients.
Fig. 3.32: Showing the HbF level in thalassemic patients
3.6.3 ABO blood group frequency in thalassemia affected population under study.

The ABO blood group is based on two glycolipid isoantigens called A and B. People whose RBCs display only antigen A have type A blood. Those who have only antigen B are type B. Individuals who have both A and B antigens are type AB, whereas those who have neither antigen A nor B are type O. In addition to isoantigens on RBCs, blood plasma usually contains isoantibodies or agglutinins than react with the A or B antigens if the two are mixed. These are anti-A antibody, which reacts with antigen A, and anti-B antibody, which reacts with antigen B.

From this observation it was cleared that the frequency of blood types O and A is more as compared to blood types B and AB in thalassemic population of the region.

3.6.4 Rh Blood Group

The Rh blood group is so named because the antigen was discovered in the blood of the Rhesus monkey. The alleles of three genes may code for the Rh antigen. People whose RBCs have Rh antigens are designated Rh+ (Rh positive); those who lack Rh antigens are designated Rh− in various populations.

In the present study, the blood types and Rh blood types of thalassemic population under study showing following frequency.

3.6.5 Sex ratio of people suffering from thalassemia.

In the present study the total number of males and females suffering from thalassemia were investigated. It was found that the percentage of males suffering from
thalassemia is more as compared to female.

The male:female ratio was found out to be 71%:29% in thalassemia affected population.

3.6.6 Blood Transfusion.

Despite the differences in RBC antigens reflected in the blood group systems, Blood is the most easily shared of human tissues, saving many thousands of lives every year through transfusions. A transfusion is the transfer of whole blood or blood components (red blood cells only or plasma only) into the bloodstream. A transfusion is most often given to alleviate thalassemia. Regular red cell transfusions to maintain hemoglobin above 10gm% is the mainstay of treatment. The current trend is to maintain hemoglobin to near normal levels. This leads to normal growth and development. Since the deficiency in thalassemia is that of red cells only packed red cells and not whole blood should be transfused and that too using a leucocyte filter to avoid any allergic reactions or antibody formation which may create problems during future transfusions. Tranfusions should be given in an out patient setting and in a thalassemia care center run by Dr. Punjabrao Deshmukh Memorial Medical College, Amravati which has medical and para medical staff trained to care for these patients. This is beneficial to the patients as they meet other patients with similar illness, leading to better psychological acceptance of the disease and its treatment. Also, they learn about the disease and outcome of treatment from one another and share experiences which lead to better compliance. Decisions as to volume replacement and transfusion of blood components must be based on an assessment of the patients cardiovascular status. Measurement of hematocrit or hemoglobin will not accurately reflect the loss of blood in the acute situation.
In the present study the frequency of blood transfusion along with their Hb level were monitored of thalasemic patients.

3.7 Inheritance of β-thalassemia in different family

The mutation causing thalassemia in an individual is in one of their hemoglobin chain genes. These are located on chromosomes number 11 and 16, which are called autosomes. The effect of the mutation in these genes is "recessive" or hidden by the presence of the correct copy of the gene. The pattern of inheritance in families of the faulty gene causing thalassemia is thus described as autosomal recessive inheritance. The mutations could either be in either the alpha or beta haemoglobin genes; the inheritance pattern is the same for both types of thalassemia. As babies are mixtures of the genetic information passed from their parents, there are four possibilities in every pregnancy for the combinations of genes inherited. A couple who are both carriers of the same type of thalassemia mutation they will both have alpha thalassemia minor or both have beta thalassemia minor, can each either pass on their faulty gene copy or their correct gene copy to their children. Their children have a 1 in 4 chance of having alpha or beta thalassemia major respectively. To have a child with thalassemia major, both parents must be genetic carriers of the same type of thalassemia. However, if one partner has beta thalassemia minor and the other has alpha thalassemia minor, they cannot have a baby with thalassemia major.

Table 3.7: Parents showing homozygosity. All offspring suffered with thalassemia.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha^+\beta^-$</th>
<th>$\alpha^+\beta^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha^+\beta^-$</td>
<td>$\alpha^+\alpha^+\beta^-\beta^-$</td>
<td>$\alpha^+\alpha^+\beta^-\beta^-$</td>
</tr>
<tr>
<td>$\alpha^+\beta^-$</td>
<td>$\alpha^+\alpha^-\beta^-\beta^-$</td>
<td>$\alpha^+\alpha^-\beta^-\beta^-$</td>
</tr>
</tbody>
</table>
The tabular forms show the combinations of parents with the possible genotypes of their offspring’s.

Both parents in this family being homozygous, have two copies genes which is responsible for β-thalassemic diseases. Every gametes carries defective gene, that’s why each offspring produced by parents have a possibility of thalassemia.

**Table 3.8 : Both parents showing heterozygosity.**

<table>
<thead>
<tr>
<th>σ</th>
<th>α⁺/β⁻</th>
<th>α⁺/β⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>α⁺/β⁺</td>
<td>α⁺⁺β⁺ β⁺⁺</td>
<td>α⁺⁺β⁺ β⁻⁻</td>
</tr>
<tr>
<td>α⁺/β⁻</td>
<td>α⁺⁺β⁻ β⁺⁻</td>
<td>α⁺⁺β⁻ β⁻⁻</td>
</tr>
</tbody>
</table>

In the above table, both parents has heterozygous for thalassemic gene in this family, means one gamete carries normal copy of gene and one gamete carries a defective copy of gene from both parents. The result of such type of mating is 25% offspring suffered with β-thalassemia major. 50% offspring will be carrier for the disease. 25% offspring will be normal.

**Table 3.9 : One parent homozygous and one parent heterozygous.**

<table>
<thead>
<tr>
<th>σ</th>
<th>α⁺/β⁻</th>
<th>α⁺/β⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>α⁺/β⁺</td>
<td>α⁺⁺β⁻ β⁺⁻</td>
<td>α⁺⁺β⁻ β⁻⁻</td>
</tr>
<tr>
<td>α⁺/β⁻</td>
<td>α⁺⁺β⁻ β⁺⁻</td>
<td>α⁺⁺β⁻ β⁻⁻</td>
</tr>
</tbody>
</table>
In the above table, one parent has showing homozygous recessive condition for the disease means parent carries both defective copies of genes. And one parent has showing heterozygous condition. Result of such a type of mating is 50% offspring suffer with β-thalassemia major and 50% offspring has act as a carrier for that and showing the symptoms of thalassemia minor.

Table 3.10: one parent normal and one is heterozygous.

<table>
<thead>
<tr>
<th></th>
<th>α⁺/β⁺</th>
<th>α⁺/β⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>α⁺/β⁺</td>
<td>α⁺⁺⁺⁺/β⁺⁺⁺⁺</td>
<td>α⁺⁺⁺⁺/β⁺⁺⁻⁻</td>
</tr>
<tr>
<td>α⁺/β⁻</td>
<td>α⁺⁺⁺⁻/β⁻⁺⁻⁻</td>
<td>α⁺⁺⁻⁻/β⁻⁻⁻⁻</td>
</tr>
</tbody>
</table>

In another family from Amravati region, one parent has show heterozygous condition for disease and another parent having normal. In such a condition 50% off spring will act as a carrier and 50% offspring will be normal.

Table 3.11: One parent is normal and one is homozygous recessive.

<table>
<thead>
<tr>
<th></th>
<th>α⁺/β⁻</th>
<th>α⁺/β⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>α⁺/β⁺</td>
<td>α⁺⁺⁻⁻/β⁻⁺⁻⁻</td>
<td>α⁺⁺⁻⁻/β⁻⁻⁻⁻</td>
</tr>
<tr>
<td>α⁺/β⁻</td>
<td>α⁺⁺⁻⁻/β⁻⁻⁻⁻</td>
<td>α⁺⁺⁻⁻/β⁻⁻⁻⁻</td>
</tr>
</tbody>
</table>

If one parent has homozygous for disease and one parent carries normal copies of gene, in such a condition there is a possibility of all their offspring showing heterozygous condition for the disease.
Electrophoretic pattern of family I showing thalassemia major.

Electrophoretic pattern of family II showing thalassemia major and symptoms of thalassemia major.
Electrophoretic pattern of family III showing thalassemia major and Sickle cell anemia

Electrophoretic pattern of family IV showing HbD (Punjab) very rare in Amravati.
Electrophoretic pattern of family V showing increase amount of HbA$\alpha$.

Electrophoretic pattern of family VI showing load of thalassemia.