Chapter 3

Observation & Result
3. OBSERVATIONS & RESULTS

3.1 The haemoglobinopathies and survey:

Adult blood contains a mixture of different Hbs. The most common Hb A, is made of two alpha and two beta globin chains. These are coded for by four alpha and two beta gene loci. 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. He was conclude from his experiment, the thalassemia are inherited disorders of Hb synthesis resulting from an alteration in the rate of globin chain production. He noticed that decrease in the rate of production of a certain globin chain impedes Hb synthesis and creates an imbalance with the other normally produced globin chains. He suggest that since two types of chains pair with each other to form normal Hbs, an 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 chains. The type of thalassemia usually carries the name of the under produced chains. The reduction varies from a slight decrease to complete absence of production.

Guiliotis was the first to confirm that when beta chains are produced at a lower rate, the thalassemia is termed beta+, whereas beta-0 thalassemia indicates a complete 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 utilization of delta chains by the excessive free alpha chains resulting from lack of adequate 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 membranes, intervening with cell membranes, intervening with normal cell division, and acting as foreign bodies, leading to destruction of RBCs. 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 countries 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.

Hemoglobin E
Hb E is commonest in south-east Asia, especially in Thailand, southern China, Assam, Burma, Bangladesh, Laos and Kampuehea. From these countries it has spread westwards to eastern and northern India, and south to Malaysia and
Indonesia. It is also found in Sri Lanka, the Maldives Islands and, rarely, in Saudi Arabia and Turkey. It was taken with workers from India and Indonesia to South Africa and the Caribbean, and in recent years refugees have brought it to North America, Australia and most western European countries from Vietnam. In Britain it is common among people whose families originated in Bangladesh, and is also found among people originating from the Caribbean or Southern China.

**Hemoglobin O- Arab**

This occurs in North Africa and the gulf area, Bulgaria and Romania. It is found rarely in Cyprus and Turkey.

**Alpha – zero thalassemia**

This is common in Southeast Asia, particularly in southern China, Thailand, Vietnam, Laos, Kampuchea, Malaysia and the Philippines. It is also found in parts of the eastern Mediterranean, particularly in Greece, Cyprus and Turkey. People from southern china have brought alpha-zero thalassemia to Malaysia, South Africa, the Caribbean and North and South America, while the influx of refugees from Vietnam in the late 1980s has increased its frequency in Western Europe.

**Alpha- plus thalassemia**

This is the commonest form of thalassemia in all tropical areas of the world. It is rarely found north of the Mediterranean or in northern Africa, but in Sardinia,
Cyprus, most of Sub-Saharan Africa 15 Southeast Asia in some populations, in Oman and some Pacific Islands, almost everyone carries it.

**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 Hbf73. Several reviews are available in India on haemoglobinopathy and thalassemia 2, 13, 23, 73–78, sickle cell haemoglobin 79–84, hemoglobin E85, haemoglobin D86, haemoglobin double heterozygosity 87 and various mutations detected in India 31, 47, 50–56, 58, 61, 88, 89. A map depicting distribution of cases of major forms of hemoglobinopathies in India.

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 79, 82, 84, 90. Verma 91, 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 92 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. Balgir87 detected three families of haemoglobin-D in Orissa. Subhedar
93 reported one case of Hb-J in a scheduled caste family from Nagpur. Labie
94 recorded 3 cases of Hb-K among 114 Hindus of lower caste and another of unknown identity in Pondicherry. De Traverse
95 demonstrated 3 instances of Hb-K among 101 South Indians in Chennai. Trincao
96 reported 2 instances of Hb-K in a survey of 1843 Indians in Goa. Verma
91 detected 18 cases of HbAK trait among the Hindus migrated from Poonch and Mussafrabad area of West Pakistan in Jammu. Sukumaran
97 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 Amritsar67. Three members of the family were found to have Hb-M levels of 7%, 33% and 50%. 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, present3. 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
96 detected 4 instances of Hb-Q in a survey of 1843 Indians in Goa. Sukumaran
98 recorded a new Hb-Qa (ref. 64) (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
99.

**Thalassemia and other haemoglobinopathies:**

Alphathalassemia 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*: Chatterjea67 reported 15 cases of 
S-thalassemia, 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.*100 recorded a high incidence of S-thalassemia among Sorathis in Palghar (3.7%). Lele *et al.*101 identified one family of S-thalassemia in a survey of 100 students belonging to scheduled caste in Aurangabad.

**E-thalassemia:**

Chatterjea67 detected 526 cases of Ethalassemia investigated in Calcutta among Indian Hindus and the regional distribution was as follows: Bengalces (508), Oriahs (10), Biharis (4), Assamese (2), Punjabis (1), South Indians (1), and 48 cases among Bengalee Muslims and one in Bihari Muslims. Sarkar102 detected 14 cases of E-thalassemia from Calcutta. Kochhar and Kathpalia103 and Praharaj *et al.*104 reported solitary instances of E-thalassemia in a Kannada and an Oriah family. Dash105 demonstrated a case of E-thalassemia in Punjab. Ghosh106 described 7 cases of E-beta-thalassemia from Punjab and one case from Rajasthan. 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.*107 and Das108, respectively, in North-Eastern India. DNA haplotypes analysis showed a common origin of haemoglobin E mutation in Assam and in South-East Asia109.

**D-thalassemia:**

Chatterjea67 recorded 9 cases of Dthalassemia: 6 from Bengal, and one each from Bihar, Punjab and South India. Occasional cases of D-thalassemia have been reported in and around Delhi110. Lele *et al.*101 detected one case in a
Kunbi family from Aurangabad. Sukumaran 111 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 92.

**J-thalassemia:** Sanghvi 112 recorded one case of J-thalassemia in a Gujarati-speaking Lohana. Swarup 113 reported 4 cases of J-thalassemia in Bengalee Hindus.

**K-thalassemia:** Swarup et al. 114 reported an interaction of Hb E and K with thalassemia in a Bengalee family of Calcutta.

**Q-thalassemia:** Sukumaran 115 recorded one case of Q-thalassemia major and 2 cases of Q-thalassemia minor in Sindhi families in Mumbai.

**Haemoglobin Lepore:** Chouhan et al. 116 reported the only case of Hb Lepore in an

### 3.1.1 Misconceptions and Myths in the Care of the Patient with Sickle Cell Disease:

SICKLE CELL DISEASE is an inherited disorder that is characterized by altered amino acid composition of the globin chain. While the presence of sickle cell disease most commonly affects persons of African descent, it can also occur in people from the Mediterranean region. The gene frequency in African Americans is about 8%, but may be as high as 50% in equatorial Africa. Approximately 1 in 500 African Americans is affected with a sickle hemoglobinopathy. Currently, 220 patients with sickle cell disease and thalassemia (150 children and 70 adults) receive their care at Dr. Punjabrao Deshmukh Medical college. Of these, 80% Childrens and, and about 20% adult Hemolytic anemia and vaso-occlusion are the hallmarks of this disorder.
Vaso-occlusive events may occur acutely or may be recurrent. These events can be quite frequent and severe, and when they occur in vital organs they can be life threatening. There can be considerable variability in the clinical course of different patients with sickle cell disease, and even at different times in the same patient. A discussion of the mechanism and pathogenesis of vaso-occlusion is beyond the scope of this paper. However, there is increasing evidence that several factors, such as erythrocyte-endothelial interactions, vascular modulation, as well as hemostasis may play a role in the vaso-occlusive phenomenon. Genetic modifiers such as coinheritance of thalassemia and fetal hemoglobin expression may also influence clinical expression.

The acute, painful “crisis” accounts for most of the hospital visits of adult sickle cell patients. Because of the risk of overwhelming infections in the pediatric patients, episodes of fever account for a considerable number of pediatric visits. In most patients, there is no obvious precipitating factor for a painful crisis. In some patients, infection, extremes in temperature, and physical or emotional stress may precipitate a crisis.

The pain typically involves the back, extremities, chest and abdomen but may occur anywhere. It is important to note that, in many cases, pain can and does occur without objective physical signs. The severity and frequency of pain can vary considerably among patients and sometimes in the same patient. Interestingly, the pain rate (i.e., the numbers of episodes per year) has been shown to be a good measure of clinical severity and a predictor of early death in patients over 20 years old. About a third of all patients with sickle cell disease have recurrent vaso-occlusive crisis, and only a very small percentage (5 – 10%) of patients experience more than 10–20 events per year. Although acute,
recurrent, painful episodes are often the predominant feature of this disease, it
should be underscored that sickle cell disease is not a one-dimensional disease.
The frequent need to use narcotics to treat severe pain often leads to drug
dependence in some patients which results in their stigmatization by some health
care professionals. What role, if any, do these factors play in cultivating
misconceptions, myths, misallocations and distrust? The acute painful episode
is frequently the principal complaint that leads the sickle cell patient to
interface with the health care system. Perhaps the best approach to understanding
the origins of these myths and misconceptions, stereotyping and
stigmatization would be to focus on the issues of pain and pain management. It
might be helpful to examine certain elements that may be a source of the
friction with this particular patient group. Both the patient and health care
provider must understand the management of sickle cell pain. Negative
attitudes about narcotics and fears of addiction may bias both providers and
patients, which results in the failure to use opioids appropriately. In addition,
patients may not have the skills needed to cope with a chronic, incurable
disease. Feelings of helplessness, which often surface in early childhood or adolescent
years, can lead to maladaptive behavior. This is frequently manifested during
encounters with the health care system. Several fundamental questions should
be addressed:
- What is the knowledge and understanding of sickle cell disease among health
care providers?
- Do health care providers have a basic understanding of the pain of sickle cell disease?
- What is the patient’s understanding of his/her disease?
Since this is a disease that affects the entire family and support system of the patient, does the health care provider understand the psychosocial background of the patient?

In this observation, I will attempt to address how and why these issues may impact on the provision of adequate and effective treatment for this group of patients, and propose strategies that may assist the medical community in developing a more candid relationship with the sickle cell patient.

Understanding the Pain of Sickle Cell Disease:
Health care providers often fail to appreciate that there are different types of pain syndromes that occur in patients with sickle cell disease. Table 1 lists the different types of pain that a sickle cell patient might experience. It is important to recognize and appreciate these differences, because acute painful episodes are unpredictable in onset and severity.

Sickle Cell Pain:
Many health care providers mistakenly believe that all pain is the same and, therefore, can be treated the same. But, pain research focused on the neurobiology of pain has established that the pathophysiology of pain in sickle cell disease is quite different from that of other pain syndromes. Ballas et al. have discussed this in detail. The standards and guidelines in the management of pain in patients with cancer and other pain syndromes are well established and have been widely published. These guidelines are helpful tools in the management and appropriate.
Table 3.1.a: Showing Types of Sickle Cell Pain.

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td>Painful crisis</td>
<td>Avascular necrosis (hips/shoulder)</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>Vertebral collapse</td>
</tr>
<tr>
<td>Right upper quadrant pain</td>
<td>Leg ulcer</td>
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<tr>
<td>Cholecystitis</td>
<td>Arthropathy</td>
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<tr>
<td>Hepatitis</td>
<td>Arthritis</td>
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<tr>
<td>Priapism</td>
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<tr>
<td>Dactylitis (Hand Foot Syndrome)</td>
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<tr>
<td>Splenic sequestration</td>
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</tbody>
</table>

use of opioids and other analgesics for chronic pain of a finite duration. They do not, however, address the issue of the recurrent or chronic pain that is unique to sickle cell disease. The frequent, recurring and often chronic pain experienced by sickle cell patients sets it apart from many other pain syndromes in that this disease is lifelong and for most patients incurable.

Believe the Patient:

The report of pain by any patient must be considered accurate. If the patient believes that he or she is not being taken seriously, and the physician is suspicious of the patient’s motives in seeking care, an adversarial relationship is quickly formed. Although this principle of trust in one’s patient is crucial, it does not exclude a need for caution. Because the pain that some patients experience is chronic, the misuse of opioids does become a potential risk.
Tolerance and sometimes dependency can become real problems. One contributing factor may be the lack of understanding of pain management by both the provider and the patient. To circumvent these problems, the use of adjuvant non-opioid analgesics, pain diaries, patient care contracts and non-pharmacologic methods (relaxation and diversion methods) have proven helpful for some patients. Patients who exhibit problem behaviors and those who tend to overutilize emergency room services should be identified. It is essential that each patient be evaluated and assessed on an individual basis.

**Failure to Assess Pain Accurately:**

Accurate pain assessment is critical for adequate treatment of acute and chronic pain. Improper assessment of pain and failure to monitor the response to therapy are major factors in the failure to relieve pain. Patients are very different from one another in their clinical presentations.

A patient who has infrequent painful episodes and who is considered “opioid naive” may require much less analgesic to relieve pain than someone with frequent episodes, who might be “opioid tolerant.” Pain scales that allow a thorough evaluation and assessment of pain should be used; they may help to alleviate conflicts over appropriate dosage.

**“Good Sickler”/”Bad Sickler”:**

For many patients with sickle cell disease, pain is part of their daily lives. This can indeed lead to misuse of the opioid therapy prescribed. Such misuse, in turn, can lead to tolerance, dependency and overutilization of hospital services by a very small subset of sickle cell patients. This group of patients generally
accounts for many hospital emergency room visits. These patients, often labeled “drug seekers,” can induce the staff to form false beliefs about all sickle cell patients. Such stereotyping interferes with the adequate and effective treatment of pain for other sickle cell patients. The failure by health care providers to distinguish between addiction, dependence and tolerance is a major component in the failure of effective management of the sickle cell patient with pain. It can lead to conflict and adversarial relationships.

Strategies to Improve the Provider-Patient Relationship:
Most hospitals subscribe to and display a patient’s bill of rights. Recognizing a patient’s rights and clarifying patient responsibilities are paramount for improving the provider-patient relationship.

Patients have the right to:
- Considerate and respectful care.
- Confidentiality and privacy.
- Current information regarding their management.
- Participate in the planning of their care (unless it is judged unsafe for them to do so).
- Informed consent.
- Refuse treatment.
- Voice grievances about care or services.
- Continuity of care.

What role does the patient play in helping to dispel the myths and misconceptions about patients with sickle cell disease? A relationship that is grounded in mutual respect requires balanced input and therefore obligates the patient to uphold certain responsibilities.
Patients’ responsibilities include:

- Providing, wherever possible, accurate and complete information about their symptoms and medications to the various health care workers involved in their care.
- Complying with instructions given related to care and medications provided.
- Asking questions and participating in the planning of their care.
- Understanding the management of pain and the careful use of opioids and analgesics.
- Understanding the importance of establishing a trusting relationship with their provider.

**Changing Attitudes and Behaviors:**

As health care providers, our attitudes and beliefs influence the relationships that we form with our patients. These beliefs affect the care and management we provide. Attitudes and beliefs about pain and pain management, particularly the use of opioids, are typically negative.

Denial of the importance or presence of pain and fears of addiction are the basis for this bias. How do we change attitudes and behaviors? One obvious answer is education. Curricula for physicians, nurses and other health care professionals have been developed by the International Association for the Study of Pain, and teaching materials are available.
The Difficult Patient:

As previously mentioned, a small subset of patients is difficult to manage and triggers negative reactions in some health care providers. Possible solutions for dealing with these patients include:

• Providing continuity of care by designating a single physician or health care team.
• Offering psychosocial support (i.e., family, other patients, support groups and psychiatric intervention).
• Designing patient care contracts that set limits.
• Designating a single pharmacy for prescriptions.
• Being consistent with the plan of care.

Factors Affecting the Prescription of Analgesic Medications

Factors Corrective Strategy

Denial of the existence Educate; change policies to of pain require measurement of quality assurance of pain; file complaints.

Denial of the importance Monitor and record pain; of pain educate about long-term effects of pain.

Unfamiliarity with Pain management education. effective use of analgesics

Fear of adverse effects Educate about extent and (e.g., respiratory management of side Effects. (depression and addiction)

Fear of scrutiny by Ensure that regulatory agencies regulatory agencies are sensitive to management.

Family views Examine underlying anxieties; give information.

Patient/family fear of Patient/family education tolerance, addiction directed at false beliefs, and drug abuse.
3.1.2 Consanguinity:

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 conservative, 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.

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.

**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 (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 (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 proscription.

**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.

**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.

**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. 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 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 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 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.
3.2 Blood analysis:

**NESTROFT**

A total 856 peoples were screened, NESTROFT was positive in 125 cases of β-thalassemia trait (True Positive, TP). There were no False positive. It was negative in 236 cases. (false negative, FN) and 325 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 568 cases of sickle cell trait and 235 cases of sickle cell disease and 256 cases of β-thalassemia major. However, none of the normal subjects showed positive NESTROFT test.

3.2.1 Estimation of Foetal haemoglobin:

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

3.2.2 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
- Color
- Inclusions
- Peculiarities
- Relationships
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 burr cells.
Sample 2

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

![Peripheral blood smear showing abnormality of blood cell.](image)

Sample 3

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

![Peripheral blood smear showing abnormality of blood cell.](image)
Sample 4

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 3.2 d: Peripheral blood smear showing abnormality of blood cell.

Sample 5

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

Figure 3.2 e: Peripheral blood smear showing abnormality of blood cell.
Hematopathology:

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.

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

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
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

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
Hematocrit (PCV):

The hematocrit, also known as the "Het", "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

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.

Mean corpuscular hemoglobin concentration (MCHC):

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

Platelets:
Platelets are cell fragments formed in the bone marrow that circulate throughout the blood stream. A normal platelet count ranges between 150,000 and 450,000.
WBC counting:
The total WBC count was invariably done using an automated method.

Estimation of Foetal haemoglobin:
The overall incidence and the degree of raised haemoglobin-F values in thalassemia and sickle cell anemia are shown, no inclusion bodies were seen in the red cells in any case. Hb-F showed heterogenous intraerythrocytic distribution in all the cases. The effect of age at onset of the disease on the rise of Hb-F is shown. There were no significant differences between the regularly transfused and untransfused groups.

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 and sickle cell anemia population of the region.
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 thalasemic population under study showing following frequency.

Sex ratio of people suffering from thalassemia and Sickle cell anemia:
In the present study the total number of males and females suffering from thalassemia and sickle cell anemia 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.

The male:female ratio was found out to be 64%:36% in thalassemia affected population.

Electrophoretic pattern study:
Electrophoretic pattern by using cellulose electrophoresis was done. Different pattern of electrophoresis including HbA₂, HbS, HbD were observed.
Fig. 3.3.1 a : Showing level of erythrocytes in thalassemic patients.

Fig. 3.3.1 b : Showing level of erythrocytes in sickle cell anemic patients.
Fig. 3.3.2 a : Showing level of haemoglobin in thalassemic patients.

Fig. 3.3.2b : Showing level of haemoglobin in sickle cell anemic patients.
Fig. 3.3.3 a : Showing mean corpuscular volume in thalassemic patients.

Fig. 3.3.3 b : Showing mean corpuscular volume in thalassemic patients.
Fig. 3.3.4 a : Showing hct in thallasemic patients.

Fig. 3.3.4b : Showing hct in sickle cell anemic patients.
Fig. 3.3.5a: Showing mch in thalassemic patients.

Fig. 3.3.5b: Showing mch in sickle cell anemic patients.
Fig. 3.3.6 a: Showing mchc in thallasemic patients.

Fig. 3.3.6 b: Showing mchc in sickle cell anemic patients.
Fig. 3.3.7 a: Showing level of platelets in thalassemic patients.

Fig. 3.3.7 b: Showing level of platelets in sickle cell anemic patients.
Fig. 3.3.8 a: Showing level of monocytes in thalassemic patients.

Fig. 3.3.8 b: Showing level of monocytes in sickle cell anemic patients.
Fig. 3.3.9 a: Showing level of leukocyte in thalassemic patients.

Fig. 3.3.9 b: Showing level of leukocyte in sickle cell anemic patients.
Fig. 3.3.10 a: Showing level of eosinophils in thalassemic patients.

Fig. 3.3.10 b: Showing level of eosinophils in sickle cell anemic patients.
Fig. 3.3.11a: Showing level of basophils in thalassemic patients.

Fig. 3.3.11b: Showing level of basophils in sickle cell anemic patients.
Fig. 3.3.12 a: Showing level of lymphocytes in thalassemic patients.

Fig. 3.3.12 b: Showing level of lymphocytes in sickle cell anemic patients.
3.3.13: Electrophoretic pattern of thalassemia major.

3.3.14: Electrophoretic pattern of thalassemia major with HbD.
3.3.15: Electrophoretic pattern of thalassemia major.

3.3.16: Electrophoretic pattern of sickle cell anemia.
3.3.17: Electrophoretic pattern of sickle cell anemia.

3.3.18: Electrophoretic pattern of HbD.
3.3.19: Electrophoretic pattern of Family affected with thalassemia major.

3.3.20: Electrophoretic pattern of Family affected with sickle cell anemia.
3.3.21: Electrophoretic pattern of unidentified disease.
3.3 Biochemical analysis:

3.3.1 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.

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 0.8 to 1.4 mg/dl and in urine 90 to 150 mg/dl.

3.3.2 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 bloodstream. Thus the serum alkaline phosphatases is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.494-495.

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.

3.3.3 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 reticuloendothelial 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 steriobilinogen 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 anemia, 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 destruction 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 (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
3.3.4 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\textsuperscript{19,197}.

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 occurs 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{201,202}.

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, hypercorticoïdism, 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 and sickle cell anemia 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 and sickle cell anemia the K+ was non-significantly increased, did not show any variation as compared to control subject.

### 3.3.5 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. 3.3.22 a: Showing level of creatinine in thalassemic patients.

Fig. 3.3.22 b: Showing level of creatinine in sickle cell anemic patients.
Chapter 3: Observation and Result

Fig. 3.3.23 a: Showing level of Alkaline phosphatase in thalassemic patients.

Fig. 3.3.23 b: Showing level of Alkaline phosphatase in sickle cell anemic patients.
Fig. 3.3.24 a : Showing level of serum bilirubin in thalassemic patients.

Fig. 3.3.24 b : Showing level of serum bilirubin in sickle cell anemic patients.
Fig. 3.3.25 a: Showing level of serum sodium in thallasemic patients.

Fig. 3.3.25 b: Showing level of serum sodium in sickle cell anemic patients.
Fig. 3.3.26 a: Showing level of serum potassium in thalassemic patients.

Fig. 3.3.26 b: Showing level of serum potassium in sickle cell anemic patients.
Fig. 3.3.27 a: Showing level of serum urea in thalassemic patients.

Fig. 3.3.27 b: Showing level of serum urea in sickle cell anemic patients.