CHAPTER-I

INTRODUCTION
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1.1.0 INTRODUCTION

Physical Anthropology has utilized the genetically method to understand the affinities and variation within and between populations for the past many decades. This has helped us to understand the ethnic composition of different areas and also traced migrations of peoples. Inspire of a number of studies in this line, our knowledge is still far from complete, especially in the processes of fission and fusion of peoples over time and space. Besides, the emphasis that is being accorded for micro-evolutionary processes of genetic change within populations provides an opportunity to study certain problems like sickle cell anemia, which are highly prevalent in some specific populations. So keeping in view the sickle cell as a genetic health problem, a study of sickle cell anemia, in addition to a set of other genetic parameters among the Mahar (Neo-Buddhists) and its subgroups would well be in order. A study of this kind would obviously aim at understanding the genetic composition as well as to study the prevalence, spread and disease course of the genetic disorders, with special reference to sickle cell anemia; it would thus elucidate the tempo of micro-evolution with respect to this abnormal gene in this population.

Sickle cell anemia is a recessively inherited hemolytic disorder and is present all along and around the tropical belt (Fig. 1.1). The sickle cell gene is located on autosomal chromosome, number eleven and its homozygous genotype results in severe anemia leading to fatal patho-physiological conditions, with a wide variability in disease course.

The reasons for this variation are far from clear. First, it was assumed that the sickle cell anemia is the problem of Negros. But now, after decades of research, it is clear that a maximum number of world populations harbor this gene with a wide variation in occurrence. The other genotype that is the heterozygote is comparatively symptom less, though a few studies have reported a significant difference in some somatometric characters between the sickler heterozygote and the normal unaffected individuals (Reddy 1998).
The Sickle cell Anemia is a haemoglobinopathy. It is one of the major genetic disorders in the world. As well as our knowledge is increased about haemoglobin we come more near to Sickle cell hemoglobin. The Sickle cell hemoglobin (HbS) is a molecular abnormality of beta – globin chain with substitution of glycine in place of valine at 6th position (Bhatia 1986). It was first recognized in African population & was considered as Negroid character. However, it is well established that this particular gene is widely distributed in other parts of the world.

Presence of HbS in India was first reported in Southern India by Lehman & Cut bush (1952) and in Assam by Dunlop & Mazumdar (1952) simultaneously. Since there large data on prevalence of Sickle Cell Trait and Disease have been collected in different tribal population from Indian Subcontinent (Fig. 12). In India, HbS is considered predominantly a tribal character. But Pandey (1992) stated that not only tribal populations but all other population groups, schedule caste backward caste, Brahmins, Thakurs, Muslims etc. also possess the gene in varying frequencies.

Scientist is not unanimous in connection with the origin of Sickle cell gene. According to Single mutation theory a single mutation occurring in Neolithic time in the fertile Arabian Peninsula is favored by Lehman (1954), who postulated that the changing climatic conditions & conversion of this area to a desert caused a migration of peoples that could have carried the gene to India, Eastern Saudi Arabia & down to Equatorial Africa. This theory was also favored by Gelpi (1967).

Possibly a mutation which occurred in beta A gene, & beta S & beta C genes etc. came in existence independently. Current opinion favors the hypothesis that the beta S mutation occurred independently. Current opinion favors the hypothesis that the beta S mutation occurred independently several times on chromosomes manifesting a variety of different heliotypes, probably between 3000-6000 generations (70000-150000 years) ago (Sergeant et al. 1985, 1989).

1.2.0 TYPES OF HEMOGLOBIN'S

There are mainly three type of normal Hemoglobin: viz. Hemoglobin A or HbA - haemoglobin A2 or HbA2 Haemoglobin F or HbF. There is three another type of Hemoglobin which is predominant in postnatal life these are Hb Gower I- , Hb Gower II
Fig. 1.1: Prevalence of Sickle Cell disorder in tropical belt (from Gujarat to Andhra Pradesh) in India
Fig. 1.2: Prevalence of Sickle Cell disorder in India.
and Hb Portland Only chain contain 141 amino acids while rest chains are made of by 146 amino acids (Sergeant et al 1989.)

Sickle Cell Anemia is caused due to chain of hemoglobin A. In the sixth position of chain there is glutamine, but while it is replaced by valine the property of hemoglobin changed, and in the absence or lack of oxygen it takes solid form and due to this RBC takes sickle shape which is known as Sickle Cell. This type of hemoglobin is known as Sickle Hemoglobin's (HbS). Latter many Sickle Hemoglobin determined from C to Q. But this system of Nomenclature seen to be inadequate & it was decided that each new hemoglobin should be allotted a common name, usually the laboratory, hospital, town or district where the hemoglobin was found (Dacie and Lewis 1994).

Following are the main types of Sickle Cell Hemoglobin:

1. Sickle Cell Hemoglobin S or HbS.
2. Sickle Cell Hemoglobin C-George Town or HbC.
3. Sickle Cell Hemoglobin D Punjab or Hb D.
4. Sickle Cell Hemoglobin E or HbE.
5. Sickle Cell Hemoglobin O Arab disease.
6. Sickle Cell Hemoglobin J or HbJ.
7. Sickle Cell Hemoglobin Lepore Boston and Lepore Holland & Baltimore

1.2.1 Sickle Cell hemoglobin S or HbS:

HbS is typical among the Sickle Cell hemoglobin, it is widely distributed in the world, and it is caused by the substitution in hemoglobin A, at the B6 position of glutamine by valine producing the abnormal hemoglobin. There are two major consequences of the substitution first the de-oxy form of the hemoglobin is favored, that is HbS has a low, O₂ affinity. HbS is written as Hba₂ B⁶₂ glu-Valine.

Patient with Sickle Cell disease who have only HbS thus tolerate low haemoglobin well sufficient oxygen being available to the tissues at a partial pressure of 40 mm Hg from haemoglobin. The far more significant abnormality is the tendency for the de-oxy HbS molecules to combine together to produce an insoluble structure which
gel: within the red cell, block capillary flow and produce local ischemia, causing the painful "Crisis" of the disease. The initial crystalline formation is reversible process with restoration of the red cell to apparent normality following re-oxygenation. The recurrent precipitation of HbS does appear to damage the red cell more rigid membrane which permanently adopts the sickle cell shape the irreversible sickled cell (ISC). The rigidity of the ISC is possibly related to the entry of Ca++ ions into the membrane which fundamentally alters the properties of membrane fluidity (Levy 1979).

1.2.2 Sickle cell Hemoglobin C George Town or HbC:

Hemoglobin C is an anomaly in the beta Chain. According to Geralo and Rath (1966) in the sixth position of the beta, Polypeptide chain glutamic acid residues replaced by lysine resulting in HbC. It is first discovered by Pierce et. al. in 1962. It is frequently found in some populations of West Africa and their descendants elsewhere. In certain areas it is found in 20 percent. According to Gerald and Rath (1966) HbC may be written as a 2 B 6, 2 glu-lys

1.2.3 Sickle cell Hemoglobin D Punjab or HbD:

Hemoglobin D Punjab is widely distributed. The first case of SD Punjab disease was noticed in 1934. It is widely distributed but reaches its highest prevalence of approximately one percent among Sikhs in the Punjab. It also occurs in the Black populations in the Caribbean and North America as well as in English populations. Molecular formula of HbD Punjab is a2B2 121 glu- gln.

1.2.4 Sickle Cell Haemoglobin E or HbE:

The first discovery of Hemoglobin E was made in 1954. It is widely distributed in Southeast Asia and throughout the Indonesian archipelago, i.e., Thailand, Kampuchea, Laos, Vietnam, Malaya, Burma, Indonesia, Borneo, etc. It has been reported from Sri Lanka also. It is very common in some population of northeast India, especially Assam Meghalya and North Bengal (Das, 1994) c.f. Das, 1997.
Livingstone is of opinion that the distribution of HbE in the Indonesian archipelago may be correlated with the spread of agriculture, as in the case of West Africa, where HbS is correlated with malaria and Agriculture. Molecular formula of HbE is as a2B2 2glu lys

1.2.5 Sickle Cell hemoglobin O Arab disease

Hemoglobin O Arab was first recognized in an Arab family in Israel in which two siblings were heterozygous for HbS and HbO Are (Ramotet. al. 1967) Jamaica (Mither et. al. 1970), Kenya (Kendall and Barn 1973), in white population of United State (Gilman and Abel 1980) c.f. Sergeant, 1989.

1.2.6 Sickle Cell hemoglobin J

In the year 1963, Bolton and Harrison recognized hemoglobin J. It was identified by its mobility on electrophoresis. Except Pakistan the Hemoglobin J is found in comparatively rare instances in South India in Gujarat.

1.2.7 Hemoglobin Lepore Boston

This hemoglobin is widely distributed but at low frequencies. The Highest concentration appears to be around Naples in southern Italy but it has been recognized in Black population in the United State, Jamaica and Cuba. The other two lepore hemoglobin’s. Holland’s and Baltimore are rare and have not been reported in association with Hbs.

1.3.0 HEMOGLOBINOPATHIES AND ALLIED DISORDERS-

Abnormal hemoglobin syndromes, thalassaemia and glucose-6-phosphate dehydrogenase (G6-PD) red cell enzyme deficiencies are major hereditary hematological disorders which are of considerable public health importance. They produce hemolytic anemia of varying severity (Basu, 1978).

Hemoglobin, the respiratory pigment of erythrocytes, is a conjugate of the protein globin and the pigment heme. Heme is a metal complex consisting of an iron atom in the centre of a porphyrin ring. The porphyrin ring is formed from four paroles which are linked by methane bridges. The globin moiety of the hemoglobin molecule is composed
of four different kinds of polypeptide chains called Alpha, Beta, Gamma and Delta. Each kind of polypeptide chain differs with each other in number and arrangement of amino acids. 98 per cent of hemoglobin of normal adult people (HbA) consists of two alpha polypeptide chains, each consisting of 141 amino acids and two beta polypeptide chains, each consisting of 46 amino acids. The 2 per cent hemoglobin of normal adult human blood (HbA) contains two delta polypeptide chains instead of beta chains. The hemoglobin of fetal blood has two gamma polypeptide chains instead of beta polypeptide chains. At birth, the major hemoglobin is HbF and during first year of life, HbF is gradually replaced by HbA and HbA2. The protein structure for all three hemoglobin's is written as follows:

\[
\begin{align*}
\text{HbA} &= \text{A2 B2} \quad \alpha 2 \beta 2 \\
\text{HbA2} &= \text{A2 D2}\beta \quad \alpha 2 \delta 2 \\
\text{HbF} &= \text{A2G2} \quad \alpha 2 \gamma 2
\end{align*}
\]

Heme group is responsible for carrying oxygen and in a normal hemoglobin molecule; four heme groups are attached four polypeptide chains which in total consist of 574 amino acids. Synthesis of polypeptide chains are regulated by structural and operator genes.

In the hemoglobinopathies, the actual chemical structure of hemoglobin is abnormal, and the alteration in structure may affect the rate at which hemoglobin is synthesized in the body, or the fate of the red cells that contain abnormal pigment (WHO, 1966).

1.4.0 ABNORMAL HEMOGLOBIN'S-

The abnormal hemoglobin's result from substitution of one amino acid for another in the normal peptide sequence of one of sub-units of the globin portion of hemoglobin. These abnormalities in amino acid sequence arise through mutations of the genes that determine the structure of the polypeptide chain concerned. Examples of these changes are the electrophoretically detectable abnormal hemoglobin's like HbS, HbC, HbE, etc, occurring in the heterozygous or homozygous states. Abnormal hemoglobin's are
inherited as simple Mendelian characters (Chatterjea, 1968). Although, it is likely that all
the variants of hemoglobin A are allele-morphs, direct proof of this assumption has so
far been obtained for hemoglobin A, S, C and E only. The common mode of inheritance
in abnormal hemoglobin's attributed to the presence of specific gene which in
heterozygous state does not usually cause any significant handicap to the person
concerned. In homozygous state, this may, however, produce various grades of
hemolytic anemia.

1.5.0 SICKLE CELL ABNORMAL HEMOGLOBIN (HBS)

The brief characteristics of sickle cell abnormal haemoglobin (HBS) are as
follows:
This is the most commonly found abnormal hemoglobin in India. It is one of the
important abnormal variants of adult hemoglobin (HbA). Sickle cell hemoglobin (HbS) is
a molecular abnormality of beta globin chain with substitution of Glutamic acid by Valine
at 6th codon position as shown by Vernon Ingram in 1957. This particular mutation
grossly affects the solubility and crystallization of this hemoglobin under conditions of
hypoxia. The first report of sickle was made by the Chicago physician, J.B. Herrick, who
in 1910 discovered these 'peculiar elongated and sickle-shaped' cells in the
blood of an anemic student from the West Indies (c.f. Sergeant, 1989). Linus Pauling,
outstanding chemists, showed in 1949, a significant difference between the
electrophoretic mobilities of hemoglobin derived from erythrocytes of normal
individuals and from those of sickle cell anemic individuals. Sickle cell disease is an
autonomic hereditary disorder and it has been found to occur in people with two widely
different clinical conditions.

1.5.1 Sickle cell disease (HbSS) Homozygous

Sickle cell disease (HbSS) resulting from homozygous condition, is a hemolytic
anemia (shortened life span of red cell) leading to severe and often fetal anemia. The
disease is further characterized by enlarged spleen, painful crisis, organ damage,
impaired mental functions, increased susceptibility to infections and ultimately early
death under certain conditions. A fresh blood-film usually shows some red cells with a peculiar bizarre sickle – shape. The patients tend to have shorter trunk shorter with long legs and an overall aesthetic (weak) built, their liver is usually found to be enlarged, chronic leg ulcers are present. Sickled cells have been found to increase blood viscosity and impede normal circulation in small blood vessels. Affected patients suffer from hemolytic anemia and recurrent episodes of abdominal and musculoskeletal pain (Vogel and Motulsky, 1982). Sickling needs slight acidosis and neither in vitro nor in vivo can sickle cells be formed when the blood pH is within the alkaline side of the normal range. Red cells carry about 95 per cent or more hemoglobin S and the rest is HbF. Sickle cell anemia patients have usually low hemoglobin levels (6-10g. per cent).

The unique aspect of sickle cell anemia is the infraction by sickle cells. These sickling infarcts appear in different ages – in very small children growing bones of the hands particularly affected (dactylitis), later stages the spleen and in puberty, tops of femur are affected, in adults chest or abdomen is affected.

1.5.2 Sickle Cell Trait (HbAS): (Heterozygous)

Found in heterozygous state, carries are perfectly fit, healthy and suffer from no anemia. But compared to normal, these persons are more prone to infectious and vaso-occulsive episodes under conditions of hypoxia and stress. Sickle cells trait cells survive the usual 100 to 120 days, blood looks normal, but when it is deoxygenated by sealing a drop of blood under a cover slip, preferably after the addition of a reducing substance such as sodium met bisulphate (Na2 S2 O5), the red cells show sickling. HbS forms about 25 per cent to 45 per cent of the total hemoglobin and HbA 75 per cent of the total hemoglobin and HbA about 75 per cent to 55 per cent. The level of sickle cell hemoglobin in sickle cell trait being too low, sickling occurs only when the patient suffers from considerable anoxia e.g. on flying in unpressurised aircraft, in severe pneumonia or during anesthesia. Disease crisis is primarily due to precipitation of HbS resulting with sickle shape under deoxidized condition. Red cells sickle when they reach a critical level of deoxygenating. Evidence has been brought forth that HbF protects the sickling behavior because HbF chains do not combine with HbS precipitate. Sickling has also
been observed to be combined with other abnormalities of hemoglobin production such as thalassaemia or hemoglobin C.

Since the sickling phenomenon in vivo greatly depends upon the amount of HbS, the heterozygote HbA + S with less than 50 per cent HbS are free from the clinical problems. While those with greater than 50 per cent like SS, S and C, S-thalassaemia have normally greater risk of sickle cell crisis (Bhatia, 1986).

1.6.0 THALASSEMIAS

Thalassaemia represents a group of hereditary disorders of hemoglobin synthesis with varying severity. The production of normal hemoglobin (HbA) is inhibited due to anomaly in the orderly synthesis of one or the other polypeptide chains of the hemoglobin molecule. Indeed, the thalassaemias are often classified as hemoglobinopathies. They differ, however, from the other disorders of hemoglobin formation in that no abnormal hemoglobin chains are formed. Rather, the rate of adult hemoglobin formation is diminished and as a consequence, various combinations of normal polypeptide chains may exist in abnormal quantity. With the advance in the knowledge of the structure of the hemoglobin molecule, it is now possible to distinguish a great majority of thalassaemias. The two commonly found thalassaemias are beta-thalassaemia in which beta chain synthesis is reduced and alpha-chain synthesis is affected. Both alpha and beta thalassemias may exist either in the heterozygous state (when the gene for thalassaemia is inherited from either father or mother) or in the homozygous state (when the gene is inherited from both parents).

1.6.1 Beta-Thalassaemia

A. Homozygous state is known as thalassaemia major or Cooley's anemia. It is a serious inherited childhood anemia:

i. Severe anemia occurs requiring frequent blood transfusions and often terminating fatally in early childhood.

ii. Presence of red cells of very varying size and many distorted forms (Poikilocytes) and target cells.

iii. Elevation of fatal hemoglobin (HbF) (50-90%).
iv. The percentage of hemoglobin A2 is very variable. HbA2 level is found to be elevated in the parents of patients of thalassaemia minor.

v. Erythrocyte count is much reduced (1,000,000-3,000,000 cells per Comm.) in homozygote, whereas it is elevated in heterozygote.

vi. The serum iron concentration is characteristically higher than normal.

vii. The stained blood shows a marked hypochromia, many of the cells showing only a thin rim of hemoglobin.

viii. There is usually retardation of growth and mental development in homozygote.

ix. There is marked pallor, patchy skin pigmentation, chronic leg ulcers and abdominal distension.

x. Bones of cranial vault are often grossly thickened and changes in the facial bones produce the characteristic appearance known as the Mongoloid faces.

xi. Bone pain and recurring bouts of fever occur from an early age.

xii. Splenomegaly is found early in the course of the disease and is progressive.

xiii. Affected infants fail to thrive and gain weight normally and become progressively anemic.

B. Heterozygous state is known as thalassaemia minor or Cooley's trait. People with thalassaemia trait are perfectly healthy:

i. Mild to moderate anemia occurs.

ii. Elevation of HbA2 (2.5% to 5%) is observed.

iii. Erythrocyte counts are less reduced.

iv. Fetal hemoglobin (HbF) if detectable at all is present in small amount in majority of the cases.

1.6.2 Alpha Thalassaemia

It is not common and only rarely causes any illness in children. There are two clinical forms of alpha-thalassaemia.

A). Hemoglobin Bart's hydrous syndrome,

B). Hemoglobin H disease.
In alpha thalassaemia major, both lines of alpha chain production are affected, the condition is lethal and produces hydrous foetalis with the miscarriage of a non-viable child during pregnancy. Alpha thalassaemia minor is difficult to diagnose, the heterozygous state is harmless. It is recognized by the presence of Hb-Bart's in a newborn and also by the occasional presence of small quantities of HbH in adult heterozygote.

1.7.0 G6-PD RED CELL ENZYME DEFICIENCY

The brief characteristics of G6-PD enzyme Deficiency are as follows:

Glucose-6-phosphate dehydrogenase is an important enzyme of the red blood cell and its deficiency is inherited as an X-linked recessive trait. The gene responsible for the G-6-PD deficiency is located on X- chromosome. Males who carry the gene (being hemizygous) show full expression (strongly affected) and suffer from hemolytic episodes but expression in female heterozygote varies greatly from low to intermediate. The female homozygote, however, are strongly affected.

Glucose – 6- phosphate dehydrogenase deficiency is one of the most common of inherited red cell enzyme defects which renders individuals vulnerable to drug induced hemolytic anemia. This enzyme is necessary as a catalyst in biological oxidation reduction reaction of glucose-6-phosphate – one of the stages in the metabolism of carbohydrates. Deficiency of enzymes concerned in red cell metabolism results in sensitivity to sulpha drugs (sulphonamides), Nitro furans, Antipyretics, Naphthalene, Sulphozone , Favabeans etc. and produce varying degrees of hemolytic anemia among the G-6-PD deficient persons. Favism, a hemolytic conditions produced by eating Favabeans etc. and produce varying degrees of hemolytic anemia among the G-6-PD deficient persons. Favism, a hemolytic condition produced by eating Favabeans (Vicia fava), is found among population living in the Mediterranean area is caused by G-6- PD deficiency.

It is estimated that more than 150 million people suffer from G-6-PD deficiency all over the world, thus exerting a great strain on the preventive facilities of public health departments. More recently it has become apparent that a very extensive genetic polymorphism exists for the enzyme, G-6- PD. A particular variant may be described in
terms of (1) quantitation of its enzymatic activity, (2) its electrophoretic mobility, and (3) its biochemical characteristics.

Not all G-6-PD variants that are found result in severe clinical hemolysis. In fact, some persons with G-6-PD variants rarely show any sign of hemolysis. The detailed molecular biological studies show that like abnormal hemoglobin's (e.g. HbS, C, E etc), many of enzyme variants occur as a result of point mutation in the structural gene, usually through amino acid substitutions (Baxi, 1985). A systematic investigation into the mechanism of hemolysis by Alvin and co-workers (Beutler, 1972) led to the discovery of an intracellular defect of deficiency of enzyme glucose-6-phosphate dehydrogenase. Further biochemical investigations revealed that deficiency subjects had a highly unstable reduced glutathione (GSH) system which is responsible for protection of erythrocytes against oxidative insults (e.g. due to drugs, chemicals etc). Lack of generation of reduced equivalents in the form of NADPH as a result of G-6-PD deficiency severely affects the ability of deficiency red cells to counter the oxidative damage. Under these circumstances, the hemolysis occurs. Over 250 variants of G-6-PD enzyme have been reported (McKusick, 1982). In Indian population groups, the association between G-6-PD deficiency and neonatal jaundice has been well established (Deshmukh and Sharma, 1968a, Jolly, 1986).

Due to the lethal effect of Sickle cell gene Biochemist, geneticist, Medical specialist & Anthropologist give attention to this problem, because healthy personnel is the first & chief need of society & country. Many researchers take interest on this topic so many mysteries open by scientists of different field.

Sickle cell Anemia or trait is caused due to abnormal hemoglobin. The Hemoglobin is a blood protein consists of by Haem (a large organic molecule having an iron atom) & globin (a protein made up of several common amino acids). In this way the hemoglobin molecule is consist of by polypeptide chains. The types of polypeptide chain vary with different stages of intra-uterine development & are designated by the Greek letters-alpha (a), beta (beta), Gamma (y), delta (s), epsilon (e) & zeta (ζ).
1.8.0 MALARIA AND SICKLE CELL TRAIT

The first observations on malaria and the Sickle Cell trait were from Northern Rhodesia where Beet (1947) noted in two communities that malarial parasites occurred less frequently in blood films from individuals with the sickle cell trait (Alison 1954) first drew wide attention to this association and concluded that persons with the sickle cell trait developed malaria less often and less severely that those without the trait.

HbS offers protection against Plasmodium falciparum infection probably because of deoxy HbS aggregates with RBC interfering with intra-erythrocyte schizgony and their subsequent removal by macrophages (Bhatia 1986).

Other genetic disorders possibly giving protection against Malaria are HbC, HbE, HbF, Thalassaemia, Spherocytosis and Ovalocytosis (Bhatia 1986). Number of the studies has been carried out on the selective advantages of sickle cell gene for malarial infection. In India, the first report on this aspect was published in 1974 among Mahar of Aurangabad where a good correlation between the endemecity of Malaria and the incidence of HbS trait was observed (Sharma et al 1974).

If, at all selective advantage for any of these genetic markers exists, it must below for Plasmodium Vivax (Bhatia 1986). Attempts to estimate the correlation Coefficient for the frequency of HbS and endemecity of the malarial infection in different districts of Madhya Pradesh failed to show a significant correlation (Bhatia 1986). It is possible that if HbS offers any advantage it must be only limited, thought the severity of the infection may be reduced by its presence, for which studies are not available in India. It is also likely that selective advantage may be more significant or Plasmodium falciparum than Plasmodium Vivax (Sharma et al 1974).

1.9.0 GENETIC MARKERS

The General Introduction of genetic markers like A1A2BO, Rh (five antis era), and H blood group are given below.

1.9.1 ABO blood group system:
ABO system was discovered by Land Steiner in 1900 and consists of four basic groups having naturally occurring antibodies. These antibodies react at all temperatures possess large amounts of H antigen which is an important basic substance from which
A and B antigens are derived. A, B and AB individuals therefore possess less of H antigen on red cells.

1.9.2 Bombay (Oh) phenotype:
"Bombay blood group was first reported in 1952 (Bhende et all). The phenotype (Oh) is characterized by the absence of A, B and H antigens on red cells. The sera of these persons possess anti-A, B and an extra antibody anti individuals are non-secretors of ABH and a majority of them are Le (a+). It is now well established by facility studies that oh group individuals may possess formal A/B genes but the corresponding antigens are not expressed on red cells because of the absence of basic H antigen on which A/B genes act. These individuals are therefore genetically termed as Oh. Incidence of on pinenotype is 1: 7600 in the population of Bombay.

1.9.3 Rh Blood Group System:
The Blood Group System: The discovery of Rh by Land Steiner and Wiener (1940), associated with the earlier report of an irregular antibody by Levine and Stetson (1939) later identified as anti-Rh antigen is absent in 15% of white people and 5% of Indians. Occurrence of several antigens, some of them antithetical, led the British scientists to postulate three closely linked genes C-c, D-d and E-e in the Rh system giving rise to eight different combinations.

Rho (D) Positive genes Rho (d) Negative genes-
CDe - R1
cDE - R2
cDe - Ro
CDE - Rz
cde - r
Cde - r'
cdE - r''
CdE - ry

However for the routine blood bank purpose Rho (D): is important since more than ninety per cent of the immunizations to Rh occur due to Rho (d) antigen. Since every human being will have any two of these eight gene complexes, thirty six possible genotype combinations could occur. Number of phenotypes would however
depend on the type and number of antisera used for testing. The gene frequencies, calculated statistically (Mourant, 1954) from the observed phenotypes, vary in different populations.

1.10.0 REVIEW OF LITERATURE

The brief review of literature of Sickle Cell Disorder, Thalasaemia and G6-PD enzyme Deficiency are as follows: --

1.10.1 Sickle Cell Disorder

Too much attention have been paid by scientists of different field to throw light on the Sickle Cell Anemia as well as on the Sickle cell gene and other genetic disorders but it is still mysterious for us. Though too much advancement take place in the field of genetics and genetics engineering but our knowledge is still incomplete and series of mysteries is indefinite.

Synthesis of Artificial DNA, cloning techniques are glorius technological achievement it in the field of Genetic engineering, but we are still suffering from many lethal gene i.e. disorders.

The history of Sickle Cell disease goes back to 1846 when Levy performed autopsy after execution for murder. At autopsy spleen was wanting. In 1898 Hodenpyl reported the case of a 32 year old black male who presented with pains all over the body Pleuritic symptoms and jaundice and was noted to have sears on the ante Rica surface on both legs. He died in hospital at autopsy no trace of the spleen was recorded (Serjeant, 1989).

The first generally accepted report of the disease in North America appeared in the November 1910. When Dr. Herrick of Chicago described a young Negro student from Grenada in the West Indies in a paper entitled 'Peculiar Elongated and Sickle shaped Red Blood Corpuscles in a Case of Severe Anemia. The illustration of the blood film left no doubt that this patient has a severe form of Sickle Cell disease, probably homozygous Sickle Cell disease.
Beside this one three another cases was described on Black man and woman on the basis of these four case reports, Mason (1922), concluded that, this was a new diseases and was the first to use the term 'Sickle cell Anemia.

Lehman and Cut bush (1952) was the pioneer worker who first reported presence of HbS in Southern India. Dunlop and Mazumdar (1952) reported the Sickle Cell trait in Assam. Since then large data on the prevalence of Sickle Cell trait and disease have been collected in different tribal population from Indian Subcontinent. In India HbS considered predominantly a tribal character. However according to Pandey et.al. (1993) reported among non tribal populations such as Brahmins, 'Thakur, Gupta, Agrawal, Maheshwari, Jain, Patel, Chaurasia, Muslims etc. also possess the gene in frequency.

Many reported studies on sickle cell indicate a selective advantage of the sickle cell heterozygote in a malarial environment and it is most widely prevalent in India, first reported from Nilgris (south India) among the tribal groups and tea garden workers in Assam (Lehman and Cut bush, 1952). Foy et al (1956) observed that the distribution of the sickle cell gene is maximum among the major tribe’s ranges from 7-35 per cent. Kar et al (1985) reported 11.5 percent frequency of sickle cell on hospitalized patients in Burla medical college, Orissa. In India, presence of HbS is considered a predominantly tribal character but, in contrast to its wide spread among tribes, the gene is almost absent among caste populations except for some lower castes (Foy et al 1956).

Naidu and Mathew (1978) reported a high frequency of 28.30 per cent sickle cell trait in a scheduled caste of Visakhapatnam town. In an analytical discussion about the sickle cell trait in India, Mukerjee (1985) stated that the trait is mostly confined to the tribes and low caste populations of western southern and central India, among whom the frequency peaks up to 35 per cent. However, recent studies have shown that lower and middle castes also possess this gene in varying frequencies. Kate (1985) propounded that in Maharashtra, the sickle cell gene is common among the low socio-economic groups like that tribes and scheduled castes.

Among the Mahar (Neo-Buddhist) in Nagpur and Aurangabad regions of Maharashtra, the frequencies of sickle cell trait reach to as high as 18-24 per cent (Shukla and Solanki 1958, Deshmukh and Sharma 1968, Das et al 1961 and Solanki et
al 1967). However, lower frequencies of 1-6 per cent of sicklers have also been reported from Aurangabad, Poona and Raigad regions of Maharashtra (Mukherjee and Das 1990, Lele et al 1962 and Negi, 1976).


The rate of sickling and its disease course are supported to depend greatly on both genetic and environmental cause. Lehman and Cutbush (1952) say that there is a remarkable correlation between high sickling and low social status. Other factors like endogamy, high fertility, and low mortality also effect the distribution of this gene. The disease course and its severity is also determined by the co-existence of a and Beta thalassaemia as well as G-6PD deficiency. A number of studies have been carried out on the selective advantages of sickle cell gene for malarial infection. In India, the first report on this aspect was published in 1974 among the Mahar (Neo-Buddhist) of Aurangabad where a good correlation between the endemicity of Malaria and the incidence of HbS trait was observed (Sharma et al 1974).

Previously it was thought that the Sickle Cell trait was considered to be localized in certain isolated pockets in south India. But Sukumaran et. al. (1950) Fay et. al (1958) clearly indicates that the trait has a much wider distribution, in India(c.f. Goutam, 1998). Shukla and Solanki's investigation (1958) amongst Mahar, Kunbi and Teli of Nagpur region in Central India, Negi's (1974) investigation amongst the Mahar of Nagpur reveal a high frequency of Sickle Cell trait n the Mahars.

Kate (1985) Propounded that in Maharashtra, the Sickle cell gene is common amongst low socio-economic groups like tribal and scheduled cast groups. This gene is present amongst the major tribal known as Bhil, Pavara, Tadvi, Madia, Caud and Kat Kari and Pardhans ranges from 7.00 to 35.00 percent. Similarly amongst Scheduled caste groups it ranges from 5.00 to 25.00 percent.

Boal (1985) studied 4 generations of several families of Kui people of Orissa showing evidence that HbSS patients with unusually high HbF content have a better chance of survival.
Sundari Devi et. al. (1985) reported that the tribal from the Orissa border of Andhra Pradesh posses 3.00 percent Sickle cell disease. 6.00 percent have shown to be Sickle cell trait and 43.00 percent have shown to behaving increased fetal hemoglobin. Nayudu (1985) indicates a higher incidence among. Valmiki, (31.5 percent ) followed by the Konda Kammari (27.00 per cent) Koya Dora (17.3 percent) Konda Reddy (13.4 percent) Koya (12.1 percent) Konda Kapu (6.8 percent) and other (4.2 percent) Schedule caste had an incidence of (16.1 percent).

In two separate reports subsequently by Murthy (1971) and, Naidu and Mathew (1978) on Rellis a scheduled caste of Visakhapatnam town show 28.30 percent of Sickle cell trait respectively (c.f. Goutam 1998).

Kar et.al. (1985), studied on 9822 hospitalized patient (adult and children )in Burla Medial collage, Orissa were screened for Sickling test in 743 out of 1090 Sickling positive cases found was confirmed by Hb electrophoresis. Sickle cell gene was detected in 11.1 percent of the patient.

Deka (1985) reported haemoglobin E which occurs in appreciable frequencies among the authochothonous population of North-East India.

A critical discussion about the Sickle cell trait in India. Mukherjee (1985) states that in India, the trait is mostly confined in the tribes and low caste population of Western, south central and South Central India and the frequency ranges up to 35 per cent. There are different opinions about the origin of Sickle cell in India. To some observers the Malaria Sickle cell hypothesis has limited validity in India. In Africa etc. differential fertility is one of the factors responsible for the maintains of Sickle Cell Polymorphism. Effect of hemoglobin on the growth pattern of children and adult of various world populations has been observed.

454 blood samples from Yanadi tribe of South-eastern past of Andhra Pradesh tested by Reddy and Ramachandraiah (1985) for the Sickle cell gene and none of the mare showing the trait.

Reddy and Ramachandraiah (1985) studied the distribution pattern of HbS gene in some tribal population of southern India is examined and discussed. Up till now more than 60 tribal population studies are available from the four southern states of India in
Tamilnadu Karnataka and Kerala the HbS gene is quite high (32 to 41 percent) in some tribes, whereas in others it is found to be totally absent.

Ramesh and Murthy (1985) as a part of anthropogenetic survey of Telengana tribes, during 1975 – 79 blood samples were collected from 1002 individuals belonging to eight tribal populations. These samples were analyzed for hemoglobin variation by standard electrophoresis method and Sickling tests. Gene HbS was present in all the populations except Yerukula. The frequencies of this gene exhibited a great variation ranging from as low as 0.25 per cent in chenehu to as 18.32 percent in Pardhans.

There are various studies done by doctors and clinical specialist they have noted many disorders among the patients who have abnormal hemoglobin. Rath (1985) found that complications during the pregnancy are common among sickler women. Anemia Maternal death, prenatal mortality is common and they also have found ocular disorder among Sicklers. According to Rath et. al. (1985) clinical heart failure was more frequent in patient with Sickle Cell anemia as compared to others. Surgical complications noted by Patel et. al. (1985) in Sickle Cell Disease. Amongst the important surgical complications aseptic bone necrosis, Haematuria, Osteomyeltis and obstructive jaundice are frequent. Chronic leg ulcers are more frequent amongst children. Most of the cases were below 40 years of age and about 75 percent cases had moderate to severe anemia.

A clinical pathological study was done in 550 adult and 193 children in V.S.S Medical Collage Hospital, Burla India during the period 1978 to 1982 by Kar et. al. (1985). Amongst them 65.8 per cent children and 43.2 percent adults were homozygous Sickle cell Anemia, and rest was Sickle cell trait. The incidence of SS-disease in adults decreased with advancing age. Sickle cell crises were noted in 199 adults and 105 children patients. Vaso-Occlusive Crisis, haemopolyysis, epileptic fits, Haematuria were other complications noted during the study among both SS and Sickle cell trait patient.

The incidents of sicking disorder among malaria patient were 11.4 percent noted by Kar et. al. (1985). G-6PD deficiency was found in 4.9 percent of Malaria patients. Out of 61 Malaria patients 23 had Sickle Cell disease. Three of them were SS and twelve were SCT, Falciparum Malaria was seen in 6AS and one SS case. One of the
falciparum Malaria patients had both –AS, G-6PD deficiency. One case of G-6PD deficiency and one child of SCT had cerebral malaria.

Samal (1985) noted following clinical presentation during crisis among sicklers. Pallor 100 percent, Muscular- skeletal Pain 76.19 percent, Splenomegaly 83.33 percent, Hepatomegaly 66.33 percent, Pyrexia 54.76 percent, Jaundice 47.61 percent, Abdomen ache 19.44 percent, Hand foot Syndrome 7.41 percent, Bleeding episodes 7.4 per cent, Diarrhea with dehydration 19.04 per cent, Upper respiratory 14.28 percent, Lower respiratory 11.90 percent, Urinary Tract infection and 7.47 per cent Malaria Precipitated Crisis.


Das and Rao (2001) are of the opinion that HbS among Indian tribes with incline from the north west to central provinces and a decline from southern to eastern province in our country. The high average values are recorded among the Dravidian speakers in Southern as well as in central zone. The decline towards ester zone can be explained by the possibility of HbE replacement among the Tebeto- Burman linguistic tribe. Among the Thotis (ST) 12.84 % were found to be affected by sickle cell disease, in which 9.32 % were heterozygote and 2.52 % were homozygote (Elizabeth et al, 2001).
In another study Saraswaty et al (2001) found 6.8 percent heterozygote of sickle cell haemoglobin among the Kolam primitive tribe of Andhra Pradesh. Goutam and Sharma (2002) conducted a study on sickle cell gene and Malaria among the Mehra (Jhariya) a neigouring caste of Mahar, Nagpur. They found 43 percent sicklers in the study population, in which the frequency of HbS gene was found to be 0.245.

The sicklers (23.25 %) were more frequently affected by Malaria as compared to normal individuals (17.54).

1.10.2 Review of literature of Thalssaelia -

Thalssaelia is one of the most important haemoglobinopathy in India (Chatterjea, 1966). The first reported thalassaemia case in India in two years of Bengalese boy (Muikerjee, 1938). Now it has been reported from all regions in India and aboard and is clearly wide spread. Several studies have been done on particular this abnormal hemoglobin in different part of India on different population. The highest percentage of thalassaemia is reported from Sindhi, Punjabi, Liana, and Bengalese up to 20 percent (Das, 1997, Das, 2000, Chatterjea 1966, Sukumaran et al 1974).

More over, Double Heterozygote HbS– thalassaemia trait frequency (3-7%) reported from Bengal (Chatterjea, 1966, Sukumaran et al 1974, Shan bag and Bhatia, 1974).

In India thalassaemias are most frequently occurring red cell disorder among the different endogamous ethnic groups (Sukumaran, 1975). Beta thalassaemia with its wide and complicated spectrum of mutations and clinical severity accounts for about 80 percent of genetic disorders and responsible for maximum infant mortality. In Indian populations an overall frequency of 3 percent of Beta thalassaemia trait is estimated by ICMR(1991) c.f. Das, 1997. There is marked variation among the regional and ethnic groups all over the country (Das, 2002). Two major zones were earlier identified with variable distribution of ethnic groups. Among the scheduled caste population, the range of beta thalassaemia trait is from 0.0 percent to 4 percent (Choubsia, 1991) c. f. from Das, (2000). While the scheduled tribe population have a range of 0.0 percent to 9 percent (Rao and Gorakhshakar, 1990, Choubsia, 1991). In
contrast, the highly ranked castes, religious and linguistic groups especially those residing either in north western or in east and north eastern part of India, the frequency of beta thalassaemia trait has been reported to reach as high as 18 percent (Jain, 1984, Bhatia et al, 1976, Sukumaran, 1975, Choubisia, 1991, Flatz et al, 1972).

A majority of these studies are based on hospital data population based data sets are lacking. But it can be definitely said that the north western and the north eastern parts of India have a higher load of this disorder.

1.10.3 Review of literature of G6PD -

In India not most populations have been tested for G6PD deficiency. Generally the tribes have high frequency than castes population (Roy Choudhary, 1983). A Number of tribes are also noted among whom the prevalence rate exceeds 15 percent (Malhotra, 1995).

Some tribes among them both sickle cell trait and G6PD deficiency occur in high frequencies. In India, the commonest G6PD is the B+ or B- (Baxi, 1974) other variants reported are G6PD (Kerala), G6PD (West Bengal) (Azevedo et al. 1968) G6PD (Jammu) (Bealetter, 1975), G6PD Porbandar (Cayans et al 1977) G6PD Insulin (Mideraetal, 1995 c.f. Reddy, 1995 and Das, 1997).

High frequencies of G6PD deficiency in human population is due to selective advantage it provides against falciparium Malaria. (Motulshkey, 1954, Allison, 1960) other studies done on G6PD deficiency in Mahar population of Nagpur and Aurangabad district the frequency reach up to 11.00 percent (Solanki et al 1967, Deshmukh and Sharma, 1968).


This acute drug sensitive haemolytic anaemia is not uncommen to Indian population (Roy Choudhary, 1983, Bhasin et al 1994, Das, 2002). A wide variability in G6-PD deficiency occurrence across the ethnic groups with a tribal origin is observed when compared with that of the caste people. Among the Mediterranean people, it is also reported to be in high frequencies.
In most of the south Asian countries, the frequencies of GD- gene varies, but it is less than 0.10. Indian populations show a GD- gene frequency range of 0.0- 0.19 (Mourant et al, 1971) c.f. Das, 1997, which is also supported by WHO report (1966). From north to east, the GD- gene frequency increases with an intermediate range in the central zone in India, the western and southern part of India show comparatively lesser frequencies. The frequency is highest among the scheduled tribes followed by caste community and the least is among the scheduled caste populations (Bhasin et al, 1994). Bhasin (1994) also reports a high frequency of GD- gene in northern part of India among caste population. Saraswaty et al (2001) found a higher percentage of G6-PD deficiency among the Kolams. Though, the difference between the pre-natal and offspring's generations was statistically non significant. Their was an increase of this deficiency in the offspring generation.

1.11.0 STATEMENT OF THE STUDY

"A GENETIC STUDY AND POPULATION STRUCTURE AMONG THE NAO- BUDDHISTS OF NAGPUR CITY WITH SPECIAL REFERENCE TO HAEMOGLOBINOPATHIES"

1.12.0 DELIMITATIONS OF THE STUDY

1. The study is limited to Nagpur city, Maharashtra, India.
2. The study is limited among Neo- Buddhist (Mahar), a scheduled Caste.
3. The study is limited mainly Neo- Buddhist dominated different locations in Nagpur city.
4. The study is limited only on population structure and haemoglobinopathies with special reference to sickle cell disorder in addition to a few serological parameters.
5. The study is limited only up to Neo- Buddhist related subgroups or sub divisions.
1.13.0 NEED AND SIGNIFICANCE OF THE STUDY

Although high prevalence of the Sickle Cell disorder, including thalassaemia and G6PD deficiency and other related disorders apparently a Global Phenomena. It was first recognized in African population and was considered as Negroid character. However, it is now well established that this particular gene is widely distributed all over the world. Presence of HbS in India was first reported in Southern India. Since then large data on the Prevalence of Sickle Cell trait and disease have been collected in different tribal population from Indian subcontinent. The distribution of Sickle Cell trait in India can be used as a useful genetically marker to distinguish population in term of their ethnic affinity (Ahmed and Chaudhary 1980). It helps us to understand the human evolution, in different sequences. Sickle Cell gene have lethal effect, when it is in homozygous condition, by extensive study of trait and intensive genetic counseling, humanity may be free from this curse.

The work of Bhatia and Rao (1987), Pande et. al. (1992), Kate, 1985, Kar et. at. (1986), Kondrashin (1992), Kuliev et. al. (1991), Kulozik et. al. (1986, 987, 1988), Reddy et. al. (1997, 1998) and many others show that too much attention have been paid for the study of HbS in different tribal population of Madhya Pradesh Maharashtra in the same way the prevalence of HbS is studied in tribal population of other state of India, While except tribal population other communities of Indian sub continent is also possess the gene of HbS, less attention have been paid for the study of the gene in these population.

Healthy population is fundamental requirement of the society as well as country. If there is any disorder which influences the health of personnel in any extent, of any population, it should be properly studied and well planned efforts should be done for removal of this type of disorder. Now, we well acknowledged with lethal effects of HbS gene Pandey (1992) studied that Schedule caste population possesses a high prevalence of Sickle Cell gene 33.54 per cent.

A step is taken through this investigation to find out the level extent of HbS, its impact on the health Causes of the disease and its selective benefit etc. Hence their health can be increase among them towards this type of genetic disorder by well planned health strategies. These types of studies will prove helpful for removal of
disorder as well as for the improvement of health status of the targeted population, society and country.

There is an urgent need to improve the health status of such under privileged groups like. It is a well known fact that health has become a major instrument of over all socio-economic development and creation of a new social order.

In view of the National Health care policy (NHP) studies on the nutritional status and genetic disorder of the Schedule Tribes, scheduled caste and Minority Communities have assumed extreme importance. Information of this type is very scanty, but they are essential for formulating welfare programmes. An attempt has therefore made in present study to assess the prevalence of Sickle Cell trait, its impact on health and other disorder.

The earlier studies on the Mahar has ignored the existence of subgroups, subgroup endogamy and the localization of some specific subgroups as if they have no influence on the genetic constitution in so far as the process of micro-evolution is concerned. The regional variation that is observed among the Mahar in terms of genetic constitution may also need to be seen from the point of geographical distribution of the subgroups and their origin. Thus a glance at the earlier reported studies on the sickle cell anemia among the Mahar (Neo-Buddhist), pose more questions and the variation in their frequencies could either be due to:

- The selective advantage enjoyed by this gene, probably leading to its uneven distribution among them across the state.
- The variable disease course due to the sickle cell gene in association with other genetic disorders, viz., α and Beta-thalassaemia, as well as G-6PD deficiency.
- Above all, the different subgroups of Mahar (Neo-Buddhist) might have the sickle cell gene in variable frequencies (but earlier studies have thus far ignored this aspect).

So, the need for an in-depth genetic study among the Mahar (Neo-Buddhist) subgroups, covering a few genetic markers, would be immense. This would elucidate, in addition to the ethnic composition of the Mahar (Neo-Buddhist) vis-à-vis its subgroups, an explanation as to how variation of the sickle cell anaemia has been brought about.
This study may also throw light on the disease course of sickle cell anaemia, which would be of great help to a future course of action in this regard. Moreover, it would probably be the first study of its kind delineating the sub castes/subgroups among the Mahar (Neo-Buddhist), with reference to genetic constitution including sickle cell anemia.

The available knowledge of hemoglobinopathies and allied disorders is inadequate in India. Coordinated investigative studies must be undertaken among various endogamous groups of India in different ecological and social settings with a view to delineate the nature and extent of prevailing hemoglobinopathies disorders. Attempts should also be made to determine probable susceptibility or resistance of hemoglobinopathies with diseases and also to evaluate the causes of variation of the incidence of hemoglobinopathies disorders in particular ecological settings. Social and public health aspects of hemoglobinopathies pose a significant problem which should be dealt with on a priority basis.

1.14.0 Objectives of the study:
The main objectives of the present study are as follows:

1. To study the intra subgroup variation in terms of population structure.

2. To identify subgroups among the Neo-Buddhist (Mahar) on the basis of marital pattern (Only Bawne, Kosare, Barke and Ladwan) subgroup are studied.

3. To study the prevalence, spread and the heterogeneity of sickle cell disorder and

4. To study a few other genetic loci, they include G6-PD deficiency, Beta-thalassaemia and two sero-genetic loci covering A1A2BO, and Rh (D) blood groups.
1.15.0 Picturesque of the study -

The thesis is divided into seven chapters for the convenience, first introduction, Second area and people, third material and methods, Chapter fourth deals observation and result of demographic profile and population Structure, Chapter fifth deals with observation and results of sickle cell disorder, Thalassaemia, G6PD Deficiency and A1A2BO, Rh (D) & Rh-5 blood group System. and sixth deals with discussion and finally seventh being Summary and conclusion.

The chapter first presents introduction of sickle cell disorder thalassaemia and G6PD deficiency and its co-relation with each other and with Malaria, Type of sickle cell hemoglobin review of literature, aims and objectives of the study. The second chapter presents area and people of the study; first of all in this chapter the area of the study is given. It includes little bit about Maharashtra, district Nagpur its tahsils, population, climate, tribes and scheduled castes etc. Second part of this Chapter presents target population Mahar (Neo-Buddhist) as a whole its sub castes, aspects of habit and habitat, social organization, social control, occupation and economic structure, custom of marriage, religion, language etc. Third chapter deals material and methods of the study, it includes place wise distribution of the samples, Sex-wise distribution of the samples, No of couples collected from each group & second part of this chapter includes methods of the study, serological investigation from collection of blood samples to organized camps to brought back to laboratory analysis and statistical analysis are given. Chapter fourth deals observation and results of demographic Information and population structure. Chapter fifth deals observation and results of sickle cell disorder, Thalassaemia, G6PD deficiency and A1A2BO, Rh (D) & Rh-5 blood group System. Chapter sixth deals discussion of the study in brief. Chapter seventh summarized and concluded the study in brief.