Molecular Pathology of Beta Thalassemia

Hemoglobin is a tetramer consisting of two alpha like and two beta like globin subunits. These two subunits are encoded by physically linked clusters of genes (alpha like and beta like clusters) each of which is expressed sequentially, during development. The alpha like gene cluster is located on the short arm of chromosome 16 and encompasses a DNA region of 25 Kilobases (Kb) \( 1 \text{ Kb} = 1000 \text{ base pair bp} \). The beta like globin gene cluster is found on the short arm of chromosome 11 and covers a region of 50 Kbs. Alpha like gene cluster contains three functional genes (The embryonic and two adult alpha genes i.e. alpha 2 and alpha \( \psi \)). The two alpha genes encode identical poly peptides, only their 3 untranslated mRNA regions are quite divergent. Two inactive pseudogenes \( \psi \delta \) and \( \psi \alpha \) are also found in this clusters (Pseudogenes are genes that have sequences homology with the corresponding active genes but contain mutations that prevent their expression). The order of arrangement of alpha like cluster genes on the DNA primary structure is:

\[
\begin{align*}
5' & \quad \psi_5 \quad \psi_4 \quad \alpha_2 \quad \alpha_1 \quad 3' \\
\text{5Kb}
\end{align*}
\]

Fig. 1.
Beta like globin gene cluster contain five functional genes e.g. Embryonic Epsilon \( ^\epsilon \), the fetal \( ^\gamma \) and \( ^\alpha \) and delta \( ^\delta \) and one beta genes. Only one pseudogene \( \Psi \) \( B_1 \) is in the cluster. The order of arrangement of beta like cluster is:

\[
\begin{array}{cccccc}
\text{s'} & \epsilon & ^\gamma & ^\alpha & s & \beta & \text{s'} \\
\end{array}
\]

Fig. 2.

The human genome contains about \( 3 \times 10^9 \) base pairs of DNA and encodes 30,000 to 1000,000 gene products. It has 3 million Kb length. Each individual inherits 2 copies of this genome and the DNA is packaged as 23 pairs of chromosomes. With the development of recombinant DNA technology with southern blot the analysis of one DNA fragment at a time is possible. It has provided an index for human genome and made it possible to locate and isolate individual gene, gene segment or nucleotide sequence from the rest DNA. By the technique of cloning DNA it is possible to isolate individual gene or portion of gene to make an unlimited number of DNA fragments and to transcribe and translate the gene. The various genes and gene products can then be utilised for diverse studies of gene structure and function in normal and disease state. Using homogenous DNA hybridation technique and restriction fragment length polymorphism (RFLP) disease trait can be analysed both directly and by genetic linkage of polymorphic sites. The specific homogenous hybridization process were obtained and successfully employed to isolate genes. Introduction of cell fusion techniques
and southern blotting permitted the construction of physical
map of globin gene DNA in genome and their localisation on
the chromosomes.

Structure of a gene

A typical gene produces an mRNA that is translated
to a protein. A gene includes 3 coding blocks exon separated
by two introns or intervening sequences. Conserved sequences
important for gene function are found just before the mRNA
coding sequences at the exon-intron boundaries and at the end
of the mRNA coding sequences. The globin gene promoter includes
sequences at the 5' end that are essential for RNA polymerase
binding and for accurate and efficient initiation of RNA
transcription. Exon-intron boundary sequences are crucial for
the accurate removal of RNA transcribed from introns and for
the precise splicing of coding sequences to form a functional
mRNA molecule. Conserved sequences found at the 3' end of RNA
coding sequences involved in cutting the RNA transcript.
Adenosine is added to form poly A tract. The poly A tract is
important for nuclear to cytoplasmic mRNA transport and for
mRNA stability in the cytoplasm. Successive steps of the
biosynthesis from the nuclear DNA, transcription, maturation,
splicing of RNA, transport of mRNA to the cytoplasmic transla-
tion is essential for globin synthesis. Any of these steps can
be responsible for a thalassemia syndrome.
The thalassemia syndrome

The thalassemias are genetic disorders characterised by absent or deficient synthesis of the globin poly peptide chains. Alpha thalassemia characterised by diminished or absent alpha globin chain synthesis while beta thalassemias are characterised by diminished or absent beta globin synthesis. The recombinant DNA technology have led to the elucidation of molecular etiology of most of the common type of alpha and beta thalassemia in different human populations. 33 mutations producing beta thalassemia have been reported. Beta thalassemia may be due to point mutation or deletion. The beta thalassemia are due to 'Cis' mutation. A Cis acting mutation affects only the gene on the chromosome on which mutation occurs. A transacting mutation influences expression of both genes at a chromosomal locus.

(1) Beta-thalassemia due to point mutations

Beta thalassemia are caused by deficient (B+) or absent (B°) production of beta globin, a very heterogenous disorder caused by many defects in the beta globin gene. By recombinant technology and DNA polymorphism 33 different mutations have been detected in different site and stage of beta globin production. Out of which 17 are different splicing mutations, 10 nonsense mutations (Mutation that blocks translation of mRNA into protein) and 6 different transcription mutations. Certain cases of abnormal beta globin secondary to single nucleotide substitution can produce mild thalassemia
syndrome. HbE mutation produces beta thalassemia as the nucleotide change responsible for the amino acid substitution is also responsible for activation of cryptic donor RNA splicing site which affects the splicing mechanism and produces abnormal RNA molecule and also abnormal hemoglobin. So many mutations produce the final result of reduced or absent beta globin synthesis. Therefore there are many combinations of genes which produce the phenotype of beta thalassemia major. The frequency of genetic compounds e.g. beta thalassemia individuals with two different beta thalassemia alleles is greater than previously thought. Prenatal diagnosis using DNA from amniotic cells has to rely on analysis of linkage between the beta gene and DNA polymorphism in the beta globin gene cluster. Use of oligo nucleotide probes specific for each mutation may provide a definite method of prenatal diagnosis using fetal DNA.

(2) Association of haplotypes with beta gene mutations

Initial evidence of linkage between chromosome haplotypes and beta gene mutations arose from study of beta thalassemia among Mediterraneans. The close association of mutations with haplotypes evident in Mediterranean suggested a systemic strategy for the characterisation of molecular basis of beta thalassemia world population. A sizable number of (about 40)
beta thalassemia bearing chromosomes are haplotyped in each at high risk ethnic group. Since the mutations to other common beta hemoglobinopathies such as \( S, E, \) and \( C \) have occurred in specific ethnic groups, one would expect similar associations of these mutations with specific haplotypes.

Deletions in the beta globin gene cluster.

Deletions of different size involving the beta globin gene cluster produce different syndromes. Beta\(^0\) thalassemia, hereditary persistence of fetal hemoglobin syndrome (HPPH-Pan cellular type), \( g \) thalassemia, \( \delta \) thalassemia and hemoglobins due to hybrid gene (Lepore, Kanya). All the deletions can be detected by restriction endonuclease analysis. The deletion of 619 nucleotides which involves the 3' region of the beta globin gene\(^{56}\), account for almost 36% of the beta thalassemia gene in Asiatic Indians\(^{57}\).

Nondeletion defects:

In some syndromes associated with raised fetal hemoglobin production, deletion of the gene is not detected by genomic blotting analysis. As in the deletion forms of HPPH, it appears that the elevated \( \gamma \) globin expression is in cis to the mutation. The recent works of Collins et al (1984)\(^{58}\) suggests a novel mechanism in a condition known as Black \( \gamma^b \) thalassemia.
Heterozygotes make about 20% hemoglobin F essentially all of the \( Y \) gene. Beta chain synthesis is present though reduced for the affected chromosome. Collin et al (1984)\(^{58}\) have observed a single base substitution (C - G) 202 bp upstream from the \( Y \) gene. This change creates a sequence which is an inverted form of the distal element PUC PUCCC related to the herpes-thymidine kinase gene promoter sequence and the 21 bp repeats of SV40. They propose that this substitution is an up-promotor mutation. Recently Ottolenghi et al (1984)\(^{59}\) found a C-T mutation 196 bp upstream from the \( A \) gene in a Mediterranean patient with nondeletion HPPH and elevated, \( A \) chain production. Observation of substitution upstream from the gene of interest at 202 in \( Y \) HPPH and at -196 in \( A \) HPPH increases likelihood that these substitutions are the HPPH mutations.

Although \( \delta \beta \) thalassemia is usually due to genetic deletion, Pirastu et al (1984)\(^{60}\) have recently reported that multiple mutations produce this syndrome in Sardinia. In particular the nonsense 39 mutation was present on a beta thalassemia chromosome, implying the existence of a second nondeletion defect responsible for absent delta gene expression. Whether other nondeletion beta thalassemia or HPPH syndromes are associated with beta thalassemia gene is not reported. George et al (1986)\(^{61}\) studied a Chinese family in which beta thalassemia and delta beta thalassemia were found in simple and compound
heterozygous states. There was nondeletions beta-thalassemia associated with increased expression of both \( \gamma^G \) and \( \gamma^A \) gene.

HEMATOLOGICAL PARAMETERS USED FOR DIAGNOSIS OF BETA-THALASSEMIA

Pathophysiology of thalassemia

The pathophysiological changes in beta thalassemia is reflected on the hematological parameters and clinical syndromes. Selective deficiency of one or more polypeptide chains have two immediate sequences. They are (1) decrease hemoglobin synthesis and (2) imbalance between alpha and nonalpha chain production. Due to decrease hemoglobin synthesis there is red cell hypochromia and raised RBC counts. In the absence of adequate complementary globin chains with which to bind, chains with normal amount of synthesis precipitate within the cytoplasm. The precipitates damage the cell membrane and leads to premature cell destruction. In beta thalassemia absent or decrease beta chain synthesis leads to excess of alpha chains which precipitate and damage the erythroid cells in the bone marrow leading to intramedullary destruction instead of normal maturation to red cells. This ineffective erythropoiesis leads to increase erythropoietic activity. The inclusions in beta thalassemia consists solely of alpha chains and some attached hem. The clinical phenotype is also influenced by the rate of globin chain proteolysis and by the availability of \( \gamma \) chain. Cells having relatively greater \( \gamma \) chain synthesis have a smaller free alpha chain pool, fewer inclusion bodies and longer survival.
Hemoglobin levels in beta thalassemia trait

Deficient or absent synthesis of beta globin results in decrease hemoglobin synthesis. Carriers of beta thalassemia trait can have varying degree of anemia. Usually they are asymptomatic and some are symptomatic. Agarwal et al (1982) found mean hemoglobin level to be lower in symptomatic beta thalassemia trait than asymptomatic group.

RBC counts in BIT

In response to shortened cell survival, there is erythroid hyperplasia and a physiologically appropriate increase in red cell production. In 1931 Greppi first reported the increased red cell count and microcytosis in the primary hemolytic icterus with increased osmotic resistance. Hammond et al (1964) described increased RBC counts in thalassemia trait cases.

RBC morphology in peripheral smears

The abnormal red cell morphology is more marked in thalassemia major and intermedia. The striking feature is anisocytosis, hypochromia, microcytosis, target cells, basophilic stippling and normoblasts. In thalassemia minor or heterozygous beta thalassemia trait the peripheral picture is in contrast to the hemoglobin level. These
include microcytosis, hypochromia, target cell poikilocytosis. Nucleated RBCs are not seen. Cooley and Lee (1925) demonstrated normoblast in the peripheral blood. In 1935 Micheli et al reported the presence of morphological abnormal cells in the patients of thalassemia major. Different Indian workers have reported the RBC morphology in beta thalassemia intermedia and in beta thalassemia trait.

Osmotic fragility

Reitti (1925) distinguished the primary hemolytic icterus from other congenital hemolytic anemia by demonstrating increased osmotic resistance. Bianco et al (1952) studied the increased osmotic resistance in 417 thalassemia minor cases. Chatterjea et al (1965) Mehrotra (1968) have studied the osmotic fragility of RBCs by different graded strength of buffered saline. Mehta et al (1971), Agarwal et al (1982) & Jain et al (1984) studied osmotic fragility with 0.4% strength of buffered saline. Osmotic fragility test along with other investigations has added significant role in the diagnosis of BTT cases. Many tests for the quantitative estimation of hemolysis of RBCs in hypotonic solutions have been reported by William et al (1959), Schhbethe et al (1960) and Danon (1963) quoted by
Valbonesi et al (1983)\textsuperscript{73} have developed a simple and rapid nephalometric test for the quantification of the osmotic fragility of red cells. They claimed this technique can help to differentiate heterozygous beta thalassemia patients from patients of iron deficiency. Kattamis et al (1981)\textsuperscript{124} developed a simple inexpensive one tube osmotic fragility test with 0.36% buffered saline. They have reported this to be a suitable screening test for heterozygous beta thalassemia. All 72 beta thalassemia heterozygote except one was positive by simple tube method. 33 normal samples were negative for the one tube osmotic fragility test which is quite encouraging.

As the single tube osmotic fragility test (NESTROFT) is inexpensive, simple and sensitive for the screening test of thalassemia, this test has been introduced in the study along with the dilution at 0.4% buffered saline.

Mean corpuscular volume (MCV)

In thalassemia MCH is low and it takes long time for intracellular concentration of hemoglobin to reach a critical level to initiate cessation of nuclear activity. Cell division continues till this stage. Size of RBC is reduced due to repeated cell division\textsuperscript{14}. Cappelline (1959)\textsuperscript{74}
has described the microcytosis of RBC and its association with quantity of HbA2. He analysed the hematological data of MCV with HbA2 and found the overlap to be much less. The mean corpuscular hemoglobin (MCH) distribution follows the pattern of MCV. The determination of MCV and erythrocyte osmotic resistance provides the most suitable basis for distinguishing a subject with thalassemia minor from an unaffected individual after exclusion of iron deficiency and other causes of microcytosis. Silvestoni et al (1957) found after extensive hematological & biochemical study of microcythemic individuals that the diagnosis of thalassemia minor continues to rest upon a complete clinical, hematological examination rather than biochemical studies of hemoglobin. Hammond et al (1964) concluded that from time of Cooley and Lee to the present, the hereditary and microcytic manifestation of thalassemia carrier have been the most constant feature. Pending the discovery of the fundamental basis of thalassemia, the definition, in the classical sense should be limited to those phenotype which show carriers with hereditary microcytosis and the associated phenomenon of increased osmotic resistance. Chatterjea et al (1965) found MCV to be low in cases with Hbe-thalassemia. Aksoy et al (1978) found low MCV in 20 cases of different type of beta thalassemia intermedia along with clinical hematological
parameters based on genetical study. Mehta et al (1932) studied BTT cases with mild anemia having Hb about 9-12 gm% and peripheral blood smear showing microcytosis, hypochromia, anisocytosis and target cells. There was correlation of HbA2 and MCV studied with comparison of iron status. In pregnancy with beta thalassemia trait the RBC size does not increase as that of size in cases of normal pregnant woman. The physiological mechanism is impaired by defective globin chain synthesis as well as iron deficiency. Pearson et al (1983) described MCV to be a reliable screening test for thalassemia trait.

Discriminant factors

On examination of blood smears for morphology, microcytosis, hypochromia, anisocytosis, poikilocytosis, target cells are seen in both iron deficiency anemia and BTT cases. There is difficulty in diagnosis with MCV, Hb & RBC counts. Various formulae have been devised to differentiate the iron deficiency and beta thalassemia trait. The formulae have been designated as discriminating factors (DF). England and Fraser (1973) formulated the discriminating factor DF1 as MCV-TRBC-(5 x Hb) - 3.4 < 0 e.g. in thalassemia trait the value will be negative and positive in iron deficiency anemia. Mentezer (1973) reported another discriminating factor e.g.
ratio of MCV/TRBC and claimed to be equal with DF. The ratio is 13 in thalassemia and 13 in iron deficiency anemia.

Mentezer mentioned that co-existing disease which influences red cell count invalidates both DF and MCV/TRBC results. Another discriminating factor (DF3) $\frac{RBC \times Hb}{MCV}$ was devised by Arnold et al (1932) where the result is 0.8 in thalassemia trait and 0.8 in iron deficiency anemia. Jonson et al (1933) described the accuracy of the discriminant factors calculated based on MCV, TRBC, MCH & Hb in 93 patients with microcytosis known either due to iron deficiency or thalassemia and prospectively in 26 patients with microcytosis in whom globin chain synthesis ratio was used for diagnosis. Discriminant factors can be taken for screening test as it is not involved with sophisticated technique.

Iron status in heterozygous thalassemia

Studies of iron absorption in beta thalassemia trait have shown results ranging from normal to increased absorption. There are reports of iron deficiency as well as iron overload in cases with BTT. These divergent results can be explained by the observation that in beta thalassemia there is variable degree of ineffective erythropoiesis. Ineffective erythropoiesis increases iron absorption. In a population group with similar food habits lower incidence of iron deficiency in carriers of BTT than in noncarriers would imply better iron absorption in carriers. Mehta et al (1982) reported that
Iron deficiency was significantly less in heterozygous beta thalassemia than control group. Its cause might be due to increased iron absorption. High incidence of IDA even with heterozygous beta thalassemia may be due to low iron content of and/or poor iron availability from the diet consumed by the population from which the subjects of study come. Alternatively these patients may have minimal ineffective erythropoiesis and therefore very little advantage in the form of increased iron absorption.

Economidou et al (1980) found serum ferrin level to be higher in pregnant woman with beta thalassemia than healthy woman. Mehta (1982) concludes that beta thalassemia protects its carrier from development of iron deficiency. Protection would depend upon the degree of ineffective erythropoiesis resulting from the trait.

Iron overload is a constant and most important complication in thalassemia. Serum ferritin concentration accurately reflects body iron stores. Serum ferritin level is higher in HbE thalassemia. Most striking feature is that serum ferritin level increases after splenectomy in thalassemia is associated with increased iron absorption and increases transferrin saturation. Cause might be due to splenectomy itself or increased iron absorption. Further increase in iron overload after splenectomy in thalassemia should be taken into
account in considering removal of this organ. Hemosiderosis is uncommon in BTT. Intermedia cases can develop hemosiderosis even though they receive little blood transfusion. This can be attributed to increase iron absorption from the gut. Mehta et al (1981) reported 3 cases of hemosiderosis out of 11 thalassemia intermedia cases.

Fargion et al (1982) studied the iron status of 140 Italian subjects with BTT. Serum iron, transferrin saturation, serum ferritin and urinary iron excretion had been studied. 44% of man and 21% of woman had serum ferritin level higher than normal range. Serum ferritin was not sensitive index among those measured for detection of iron overload.

Vatanavicharn et al (1983) measured $^{59}$Fe absorption in 16 patient of beta thalassemia/HbE disease and 5 normal control using a total body counting technique. The average iron absorption in the patient was 62% in contrast to 16.5% in the normal control. 6 of the 9 splenectomised patients had absorption value $\geq 65\%$. Absorption of iron from food represents a further possible source of iron loading in these patients.

Fargion et al (1985) have characterised HLA antigens in subjects with BTT with and without iron overload. 50% of the cases with iron overload are carriers of HLA-A3, the HLA antigen tightly linked to the IH allele. This is a considerable number
of these subjects beta thalassemia and IH coexist. This association exerts a synergistic effect in inducing iron overload. Simon et al (1983) suspected the co-existence of BTT and idiopathic hemochromatosis (IH) gene.

HbA2 level in IDA and HBT

Raised hemoglobin A2 level is an important diagnostic criterion for beta thalassemia trait cases. Josephson et al (1958) reported reduction in HbA2 in iron deficiency anemia cases. Mehta (1982) studied 102 subjects of HBT (HbA2 < 3.5%) to determine their iron status and compare HbA2 levels among HBT with and without iron deficiency. Ben-Basset et al (1974) reported decreased synthesis of alpha chain in iron deficiency anemia. This may explain decreased level of HbA2 in IDA. However in another series 22 cases of HBT associated with iron deficiency there was no significant reduction in HbA2 levels compared to HBT without iron deficiency. It would appear from the study that iron deficiency does not mask the diagnosis of HBT based on HbA2 level. Saraya et al (1984) reported from their study that HbA2 level was not reduced in BTT and it did not effect for the diagnosis of heterozygous beta thalassemia.

Hemoglobin A2 estimation

Globin synthesis rate measurements reinforced by genetic evidence constitute the most definite method for identification of thalassemia trait. Because such determinations
are only available in research laboratories, elevation of the percentage of HbA2 offers the most practical approach to the diagnosis of carrier state for beta thalassemia. Kunkel & Wallerius (1955) first described and named HbA2. An estimation of HbA2 more than 3.5% has been taken as the diagnostic criterion for diagnosis of beta thalassemia trait. For quantitation of HbA2 many methods like Starchblock electrophoresis, Starch Agar gel electrophoresis, Cellulose acetate electrophoresis, Filter paper electrophoresis, DEAE Sephadex, DEAE cellulose microchromatography and radial immunodiffusion technique have been accepted as the standard method, ICSH tentative standard and EP (dated 11.2.1977). HbA2 estimation by paper electrophoresis with elution has given reliable results.

In India filter paper electrophoresis with elution has been accepted as an economic and reliable method. Efremov et al (1974) have reported a simple microchromatography method for HbA2 estimation from a hemolysate containing or without containing HbS. The procedure makes use of DEAE cellulose slurry kept in ordinary laboratory glass ware pastuer pipette. It has been reported whole blood sample, red cell hemolysate and blood collected in filter paper can be used without dialysis and can be analysed with comparable accuracy. Moors et al (1979) investigated the utility of microchromatography...
as a routine HbA2 essay and as a screening method to detect beta thalassemia trait carriers and patients with iron deficiency. Microchromatography in Pasteur pipette tends to overestimate low and normal HbA2 concentrations and underestimate high HbA2 concentration. The results obtained with commercial columns were in good correlation with those obtained HbA2 values in Starchblock electrophoresis.

Yawson et al (1974) studied HbA2 in 11 normal and 42 beta thalassemia trait by simple visual assessment of electrophoretogram. They compared the results with microchromatography method and opined that visual method of HbA2 estimation without elution is unreliable.

Ghosh et al (1985) estimated the HbA2 by microchromatography method in 34 hematologically normal subjects and 65 beta thalassemia trait. The HbA2 level obtained in beta thalassemia could separate clearly from the normal control. They compared the conventional paper electrophoresis and A2 estimation by densitometry. This method could not distinguish clearly between healthy control and beta thalassemia trait. They concluded that microchromatographic estimation of HbA2 offers a simple quick method for the epidemiological study of beta thalassemia traits.

Saranya et al (1984) studied the HbA2 levels by microchromatography in cases of normal control & beta thalassemia trait cases with or without iron deficiency anemia.
They found that diagnosis of beta thalassemia was not affected by concurrent iron deficiency.

Krishnan et al (1986)\textsuperscript{110} evaluated the HbA2 values in the two methods of electrophoresis and microchromatography and found that both the methods are equally reliable and provides an adequate discriminating gap between values obtained in normal persons and in beta thalassemia carrier states.

**Hemoglobin F**

With the introduction of alkaline denaturation technique, presence of a large amount of alkaline resistant fraction of hemoglobin (Fetal hemoglobin HbF) was clearly demonstrable. This added a precise diagnostic criterion for the identification of homozygous form of thalassemia\textsuperscript{111}. Singer (1951)\textsuperscript{111} developed the alkaline denaturation technique for estimation of HbF. Micheli et al (1935)\textsuperscript{112} detected blood abnormalities in both parents of a patient which would now be diagnosed as "thalassemia minor". In 1935 & 1953 he proposed that multiple normal alleles at the same locus might account for the varying expressions in heterozygotes. Zuelzer et al (1961)\textsuperscript{113} and Malamos (1962)\textsuperscript{114} have demonstrated that increased fetal hemoglobin may occur in various combinations. The hereditary persistent fetal Hemoglobin, Hb lepore and delta chain deficiency are listed as thalassemia like conditions.
Persistant fetal hemoglobin syndrome shows no clinical abnormalities. The lepore trait is characterised by microcytosis, reticulocytosis and elevation of fetal hemoglobin and is known to interact with beta thalassemia. Fessas (1962)\textsuperscript{115} has reported an individual believed to represent the thalassemia with delta chain deficiency who had mild anemia, microcytosis and a complete absence of HbA2.

Normally fetal hemoglobin is present in high percentage at birth and gradually it is replaced by adult Hb. At the age of 1 year it comes to 1%. There is switch on from gamma chain to beta chain to produce adult hemoglobin. In beta thalassemia there is interaction of substances and gama chain combines with alpha chain to produce more Hbf. Agarwal et al (1982)\textsuperscript{116} found Hbf level in different conditions like (1) in delta beta thalassemia trait (Hbf 5 - 15%) and heterozygous distribution of Hbf in red cells (2) in HPFH trait (Hbf 15 - 30%) and homozygous distribution of fetal Hb in red cells, (3) in thalassemia trait Hbf > 2%.

STUDIES DONE IN INDIA RELATED TO BETA THALASSEMIA

Studies on beta thalassemia have been reported from India since 1938\textsuperscript{70}. Mukherji et al (1938)\textsuperscript{117} and Napier et al (1939)\textsuperscript{118} had reported thalassemia from Calcutta. By that time the diagnostic criteria for thalassemia was evidence of hemolytic anemia, characteristic red cell morphology and bony
changes. After the demonstration of high level of alkaline resistant fetal hemoglobin (HbF) in 1951 by Singer et al, Chatterjea et al (1965) processed a number of samples from patients suspected for thalassemia and confirmed the findings. They found HbF raised significantly in homozygous cases but not in heterozygous parents. These findings were corroborated with HbF bands in paper electrophoresis strips. In 1956 Chatterjea et al reported first HbE in India and subsequently reported double heterozygous state in which gene of thalassemia and HbE were present together. In 1956 a search by Sidoo et al showed 5 cases of beta thalassemia in Vancour from 80 individuals of Sikhs. Interaction of thalassemia with sickle cell gene with a presumptive diagnosis was reported from Bombay in 1957. Parekh et al (1957) reported a case of hemolytic anemia which presented evidence of thalassemia with sickle cell gene. Cases of sickle cell thalassemia were reported from Nagpur and Bengal. In 1960 Sukumaran found 4 cases of HbD thalassemia among Gujarati speaking and Sindhi speaking Lohana communities in Bombay. Sukumaran et al (1961) described three cases of HbE thalassemia in a muslim family. In the same year (1961) they reported 3 cases of sickle cell thalassemia in two Gujarati speaking families. Sharma et al (1963) reported two cases of HbE thalassemia from Bombay i.e. one in a local muslim family and the other a resident of Gonda District of Uttar Pradesh. Sharma et al (1971) conducted a hematological and
genetical studies in Saraswat & Lohana community and reported thalassemia carrier state as 4.2% of 240 Chitrapur Saraswat, 1% of 98 Goud Saraswat and 13.6% of 103 Lohana individuals. Mehta et al (1972)\textsuperscript{23} reported 14.9% of beta thalassemia trait of 599 individuals from Bhanushali community of Bombay. Pillay et al (1972)\textsuperscript{20} surveyed 1000 random patients in Trivandrum Medical College Hospital and reported beta thalassemia to be 0.6%. Sinha et al (1973)\textsuperscript{28} studied 2075 soldiers who represented personnels from all other Indian and found a case of HbD thalassemia. Bhatia et al (1976)\textsuperscript{24} reported a variable incidence of beta thalassemia trait which has been found in several groups as 10.7% in Cutchi Lohana 17.2% in Halai Lohana, 6-8% in Sindhi Lohana and 5.2% in Punjabi Khatri. Mehta et al (1980)\textsuperscript{29} studied 16 cases of HbE thalassemia. Interaction of HbE produced a varying severity of E thalassemia. 14 cases were beta\textsuperscript{+}, 1 case beta\textsuperscript{0} and family studies in one patient revealed the interaction with delta-beta thalassemia a rare situation. In 1981 Mehta et al\textsuperscript{15} reported 11 cases of beta thalassemia trait with an intermedia clinical severity. In many instances intra familial variation in clinical severity was seen amongst members with the same trait. 3 cases of hemosiderosis were reported by them. In 1982 they studied heterozygous beta thalassemia and observed hemoglobin to be lowered in symptomatic group than asymptomatic group.

Clinical picture of thalassemia intermedia was seen in 14 cases. Agrawal et al (1982)\textsuperscript{116} did the genotypic analysis of 292
unrelated cases from Bombay with symptomatic thalassemia syndromes and classified according to the nature of the thalassemia gene/genes and the type of abnormal hemoglobin. Beta thalassemia was commonest and constituted 97.2% cases. The other cases were delta-beta thalassemia 4.8%, HPFH- 1% and HbH disease 0.7%. Mehta (1982) studied the interaction between nutritional anemia and thalassemia and observed that beta thalassemia trait protects its carrier from development of iron deficiency. Protection would depend upon the degree of ineffective erythropoiesis resulting from trait. In 1983 Mehta et al estimated the Delta-Amino Levulinic acid(ALA) levels in the urine and serum samples of control and thalassemia group ( heterozygous and homozygous ). They found overlapping of results in 3 groups and concluded that amniotic fluid ALA levels might not be useful for antenatal diagnosis of homozygous beta thalassemia. Jain et al (1984) studied abnormal hemoglobins and thalassemia in hospital populations of Rajasthan. From 2790 cases, 0.07% were HbD-thalassemia, 0.64% of homozygous beta thalassemia and 1.75%, heterozygous beta-thalassemia. They had used the commonly used parameters for laboratory studies. HbA2 was done by paper and cellulose acetate electrophoresis and quantitated by photovolt densitometer. Jain et al (1984) investigated 520 male Bohra Muslims and 500 nonBohra Muslim for evidence of G6PD deficiency, thalassemia and abnormal hemoglobin. They got 3.7% of beta thalassemia in Bohra Muslims and 0.4% of beta thalassemia in nonBohra Muslim.
Saraya et al (1984) studied the contribution of iron deficiency in the pathogenesis of anemia of beta thalassemia in areas where both are prevalent. They found iron deficiency in thalassemia and normal subjects to be similar in incidence. Respective proportion of iron deficiency was higher in females and had contributed to anemia. They suggested that iron should be supplemented in geographical areas with high prevalence of iron deficiency and heterozygous beta thalassemia. They had used the microchromatography technique first in India for estimation of HbA2. Ishwad et al (1984) reported HbD thalassemia from two koli families in Bombay. They have presented the hematological, biochemical, genetical and clinical data of the cases. Pati et al (1985) reported and discussed the unusual features in 10 patients of HbE thalassemia. 9 were Beta+ and all required periodic blood transfusion and/or splenectomy. They reported 5 cases from Bihar State where only 1 case had been reported earlier. HbA2 was done in microchromatography method. In 1985 Ghosh et al studied HbA2 levels in 34 normal subjects and 65 beta thalassemia traits by microchromatography. HbA2 level obtained by this method separated beta thalassemia heterozygotes from normal control. They used a conventional method of A2 estimation by paper electrophoresis and densitometry. This method could not distinguish clearly between healthy controls and beta thalassemia trait. They concluded that microchromatographic estimation of HbA2 offers a simple, practical and economic method for the
epidemiologic study of beta thalassemia traits. Mehta et al (1987) studied 239 parents of children of various hematological disorders for beta thalassemia trait (BTT) and iron status to know whether beta thalassemia trait protects against iron deficiency. BTT was found in 147 parents and 92 were non-beta thalassemic (control). They observed that presence of iron deficiency does not mask the diagnosis of BTT based on HbA2 estimation. It seems that beta thalassemia trait provides protection against nutritional iron deficiency. They suggested that the high prevalence of BTT in India may be due to the advantage that carriers have in the form of reduced prevalence of iron deficiency among them. Pavri et al from Institute of Immuno Haematology ICMR Bombay have started the technique of isoelectric focussing and globin chain synthesis in India for the diagnosis of thalassemia and antenatal diagnosis by fetal cord blood sampling at 18 weeks of pregnancy (Unpublished data - personal communication with Dr. Pavri).

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Aim of the present work: -

To assess the diagnostic values of various parameters which are usually used in most of the laboratories in India for the diagnosis of beta thalassemia trait.

Contribution from the study: -

The knowledge which will be derived from this study can be utilised for mass screening of the population for beta thalassemia trait and prevent the thalassemia syndromes by genetic counselling to the people at or before reproductive age in the long run.

In the light of derived knowledge antenatal screening for beta thalassemia trait in pregnant woman can be done at early pregnancy. After subsequent diagnosis of carrier status of the husband, the high risk couple can be advised for prenatal diagnosis of the fetus in the selected centres and the birth of children with thalassemia major can be prevented.