INTRODUCTION
The thalassemias are hereditary hemolytic anemia characterised by decreased or absent synthesis of one of the globin subunits of the hemoglobin molecule\(^1\). The term thalassemia was first used by Whipple and Brendford in 1932\(^2\). The word thalassemia has been taken from the Greek word "\(\theta\alpha\lambda\alpha\sigma\sigma\varepsilon\mu\alpha\)" the sea. In 1889 Von Jaksch\(^3\) of Praug described a young boy with anemia, leukocytosis, splenomegaly and fever in whom a subsequent biopsy did not show changes of leukemia. This was termed Von Jaksch's anemia. Cooley & Lee (1925)\(^4\) reported the description of the disease in 4 young children who had anemia, splenomegaly, enlargement of liver, discoloration of the skin and conjunctival sclera without bile in the urine. The red cells showed increased resistance to hypotonic solutions. There was moderate leukocytosis with nucleated red blood cell in the peripheral smear. A peculiar mongoloid appearance with enlargement of cranial & facial bones. Cooley's great contribution was to describe a series of children with clinical syndromes and to separate clearly the disorder from the heterogeneous groups of childhood anemia known as Von Jaksch's anemia. The first definite evidence that Cooley's anemia is genetically determined was provided by Caminopetros through his papers in
1936 & 1938. In 1940 Wintrobe & his Colleagues described typical thalassemia blood changes in 40 members of three Italian families. Several members showed splenomegaly and mild icterus. They had seen this condition in both parents of a child with Cooley's anemia. Valentine & Neel (1944) described mild form of Cooley's anemia as thalassemia minor. By 1949 (Neel et al.) it was apparent that thalassemia is not a single disorder but a complex syndrome resulting from the interaction of more than one and probably many genetic factors. Neel (1949) and Lamotte-Legnands (1950) suggested that human globin synthesis is controlled by a pair of genes and the sickle cell gene is a mutant of allele for the hemoglobin A gene. Ingram (1956) obtained evidence which suggested that globin consists of two half molecules. Adult hemoglobin consists of two pairs of identical peptide chains which were called alpha and beta chain. By the early 1960s technical advances in protein sequencing had enabled the complete amino acid sequences of \( \alpha, \beta, \gamma \) chain to be analysed. Subsequently delta chain was detected. Four different loci controlling the structure of \( \alpha, \beta, \gamma \) chain were postulated. In 1959 Ingram & Stretton extended the idea of genetic basis of thalassemia. They suggested two major class of thalassemia \( \alpha \) & \( \beta \) in the same way as there are two major types of structural hemoglobin variants for alpha and beta chains. They examined published pedigrees and explained quite elegantly the interaction between
beta thalassemia and beta chain variants and alpha thalassemia and alpha chain variants. They explained the synthesis of HbH as resulting from defective alpha chain synthesis allowing excess beta chains to polymerize. They believed that reduced rate of alpha or beta chain might be due to silent (undetectable) mutation of the gene. They also proposed an alternative explanation called the "TAP Hypothesis" in which they suggested the defect might not lie in the structural gene but in the area of DNA in connecting unit preceding it. This paper was a landmark which became the basis for all future work on genetics and biosynthesis of haemoglobin in thalassemia. Simple analytical techniques had been developed for analysing the level of HbA, A2, F, HbH & Hb Bart's. Hematologists in routine hospital laboratories could apply these techniques to study thalassemic patients throughout the world. It was soon realised that thalassemias are disease of the wide distribution and remarkable genetic heterogeneity. With the development of sophisticated technique for genetical study at DNA level recently more relevant information about thalassemia is continuing to be added. Thalassemia may present in the homozygous or heterozygous form as it is an autosomal co-dominant disorder. Complex situations arise due to interaction between different thalassemia gene with that of abnormal hemoglobin\(^{13}\). Such variations can be diagnosed by study of parents, siblings & children.
CLINICAL TYPES

Thalassemia can be classified on the basis of clinical severity as (1) thalassemia major (2) thalassemia intermedia (3) thalassemia minor and (4) thalassemia minima.

Thalassemia major

Clinically this is the most severe form of thalassemia and results from several types of genetic defect e.g. homozygous beta\textsuperscript{0} (no synthesis of beta chains) thalassemia, homozygous beta\textsuperscript{+} (decrease synthesis of beta chains) thalassemia. Homozygous state of Hb lepore (cross over of delta and beta gene) or double heterozygosity of beta\textsuperscript{0} and beta\textsuperscript{+} thalassemia. The child is quite normal at birth and for first few months of life. Between 3 to 6 months of life, anemia appears and gradually increases. Patient develops hepatosplenomegaly, overgrowth of molar bones, frontoperiptal bossing giving peculiar thalassemic facies and is susceptible to infection. Hemosiderosis develops as the age advances leading to various organ failure e.g. lack of puberty, spurt of growth and sexual development, diabetes, cirrhosis of liver & heart failure.

Thalassemia intermedia

The condition is usually a result of double heterozygosity of different varieties of beta thalassemia genes. It can also result from homozygous state of beta\textsuperscript{+} thalassemia,
rarely a heterozygote for beta\text{0} thalassemia may have a clinical picture of intermediate severity. The patient may have the symptoms of anemia, hepatosplenomegaly and occasionally leg ulcers. The patient may require occasional transfusion but he is not transfusion dependant. The clinical severity is thought to be intermediate only when the patient has splenomegaly causing mechanical symptoms or hypersplenism and/or causing significant symptoms and/or ankle ulcer for none of which there was any cause. Diagnosis is not possible unless appropriate investigations and family studies are carried out.

Thalassemia minor

This results from heterozygous state of any one of the beta thalassemia genes. This condition is usually asymptomatic although about 80\% of persons have mild degree of anemia. Rarely there may be minimal splenomegaly, jaundice or leg ulcers. These cases are detected when their blood sample is examined in a population survey, family study or investigation is done for refractory anemia. Mild anemia does not respond to hematinics.

Thalassemia minima

Thalassemia minima refers to a condition in which the impairment of beta chain synthesis escapes the attention
by clinical evaluation or conventional hematological assessment. The presence of the silent carrier state may be established by demonstrating a decrease in the beta/alpha globin chain synthesis ratio by peripheral blood reticulocyte.

GEOGRAPHICAL DISTRIBUTION

The thalassemias are found in broad belt extending from the Mediterranean basin to India and the orient. The beta thalassemia gene is particularly prevalent in Italy & Greece. The carrier state found were 20% of population in Ferrara near PO river delta area, 11-34% of Sardinia, 10% of Sicilian, 5-15% in South and Central area of Greece, Cyprus and Malta. It is less frequently encountered in northern and western part of Africa. In South East Asia and Southern China beta thalassemia is less frequent than alpha thalassemia. In North America, thalassemia is noted primarily in persons of Italian and Greek descent and in blacks. Beta thalassemia in Jamaica may have its origin in both the African and Oriental immigrations. Sporadic cases have been reported in north Europeans having no apparent Mediterranean or oriental ancestry. It has been reported from Kurdish Jews in Saudi Arabia & Pakistan.

In India thalassemia is the commonest of all the inherited hemolytic anemia. Amongst the thalassemia syndromes, beta thalassemia in India is the most significant one. Agarwal et al (1982) reported 97.2% of beta thalassemia from a study.
of 292 cases with symptomatic thalassemic syndrome. Various studies have been done in India to know the frequency of beta thalassemia alone and associated with hemoglobinopathies in different parts of India. The frequency of beta thalassemia reported in India are 3.7% from Calcutta, 0.6% from Kerala & 1.75% from Rajasthan. On community survey the thalassemia carrier state detected was 4.2% in Chitrapur Saraswat, 1.1% in Gowd Saraswat & 13.6% in Lohana. Mehta et al (1972) reported 14.9% in Bhanushali community. Variable incidence of beta thalassemia trait case has been found in several groups as 10.7% in Cutchhi Lohana, 17.2% in Halai Lohana, 6-8% in Sindhi Lohana and 5.2% in Punjabi Khatri. Jain et al (1984) reported 3.7% of beta thalassemia in Bohra Muslim and 0.4% in non-Bohra Muslim in Rajasthan. Genetic interaction of HbE & beta thalassemia is expected and it has been well documented from India. Chatterjea (1962) reported 3.7% from Bengal. The other reports as documented are 2 cases of E-thalassemia from Bombay, 0.132% from Uttar Pradesh and 0.144% out of 2075 Indian soldiers. Sixteen cases of E-thal from Bombay and 10 cases from Delhi. Out of 10 cases, 9 cases were from Bihar. Interaction of beta thalassemia with sickle cell was reported first in India from Bombay. Cases of sickle cell thalassemia has been reported from Nagpur and Bengal. Sukumaran (1961) reported 3 cases of sickle cell thalassemia. Parekh et al (1957) reported a case of thalassemia with sickle cell gene.
The beta thalassemia gene is distributed in different parts and in different caste and tribal groups of India. The average frequency of this gene is 3-4%^36. The heterozygous beta thalassemia is usually asymptomatic and the carriers don't seek medical advice. Magnitude of the problem of thalassemia can be judged by the presence of nearly 20-25 million persons harbouring this gene of which nearly 3-4 million are in the active reproductive age and continue to produce thalassemia carriers or the disease. Nearly 0.75 million couples with both partners being thalassemia carriers are high risk couples who may produce children with thalassemia major^36. These high risk couples are of public health concern. The beta thalassemia trait should be diagnosed before or during early phase of pregnancy and the carrier status of husband should also be determined. The high risk couples should be advised for the prenatal diagnosis of thalassemia major. If the fetus has thalassemia major, the couple can be offered termination of pregnancy. Prenatal and genetic counselling can help to minimize the risk of thalassemia major. For this, screening test for detection of beta thalassemia trait should be available to young people before marriage. In Cyprus the frequency of thalassemia has reduced due to carrier detection, marriage counselling and prenatal diagnosis of thalassemia^37.

Advancement in laboratory technology like globin chain synthesis^38, Isoelectric focussing^39, DNA recombinant technology with southern blot^40, DNA gene mapping with the help
of DNA probe have made possible to diagnose different variants of thalassemia and hemoglobinopathies. These techniques are being utilised for prenatal diagnosis of thalassemia and sickle cell anemia and several other genetic disorders. However all these techniques have not been employed in India due to many limitations. Globin chain synthesis and isoelectric focussing is being done in very few laboratories only.

Certain hematological parameters have been taken into account to diagnose beta thalassemia. The parameters are Hb%, RBC morphology, RBC count, mean corpuscular volume, certain discriminant factors based on red cell indices, RBC osmotic fragility, HbA2 estimation and fetal Hb estimation. The diagnostic criterion for beta thalassemia trait is raised HbA2 3.5% . Abnormal finding of individual parameter is present in about 80-85% of individuals with beta thalassemia trait, but any one of these abnormalities is present in about 97.2% of persons heterozygous for beta thalassemia gene.

The title of my work is "Study on diagnostic problems in beta thalassemia trait". I will utilise the commonly used hematological parameters for diagnosis of beta thalassemia cases in particular beta thalassemia trait cases. I will evaluate the diagnostic values of different hematological parameters which will be used for diagnosis. The derived knowledge can be utilised for overcoming the diagnostic problems for detection of beta thalassemia trait cases.