PREVALENCE OF BETA THALASSEMIA TRAIT AMONG ANTENATAL WOMEN ATTENDING A TERTIARY CARE CENTRE

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements

For the award of degree of

M.D. (Branch-XIII)

BIOCHEMISTRY

GOVERNMENT STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI, TAMILNADU

MAY 2020

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This is to certify that the dissertation titled, **"PREVALENCE OF BETA THALASSEMIA TRAIT AMONG ANTENATAL WOMEN ATTENDING A TERTIARY CARE CENTRE",** is a genuine work done by **Dr.M.MENAKA DEVI** for the partial fulfillment of the requirement for M.D (Biochemistry) Branch XIII Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in May 2020, during the academic period 2017 - 2020.

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DECLARATION

I, **Dr. M. MENAKA DEVI Register No:201723052** solemnly declare that the dissertation titled **"PREVALENCE OF BETA THALASSEMIA TRAIT AMONG ANTENATAL WOMEN ATTENDING A TERTIARY CARE CENTRE"** is a bonafide work done by me during the period of October 2018-december 2019 at Government Stanley Medical College and Hospital, RSRM Hospital, Chennai under the expert guidance of **Prof. Dr. M.P. SARAVANAN,M.D,** HOD, Department Of Biochemistry, Government Stanley Medical College and Hospital, Chennai.

This thesis is submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the rules and regulations for the M.D. degree examinations in Biochemistry to be held in May 2020.

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Title of the Work : Prevalence of Beta Trait among antenatal women attending tertiary Care centre.

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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 11.05.2018 at the Council Hall, Stanley Medical College, Chennai-1 at 10am.

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

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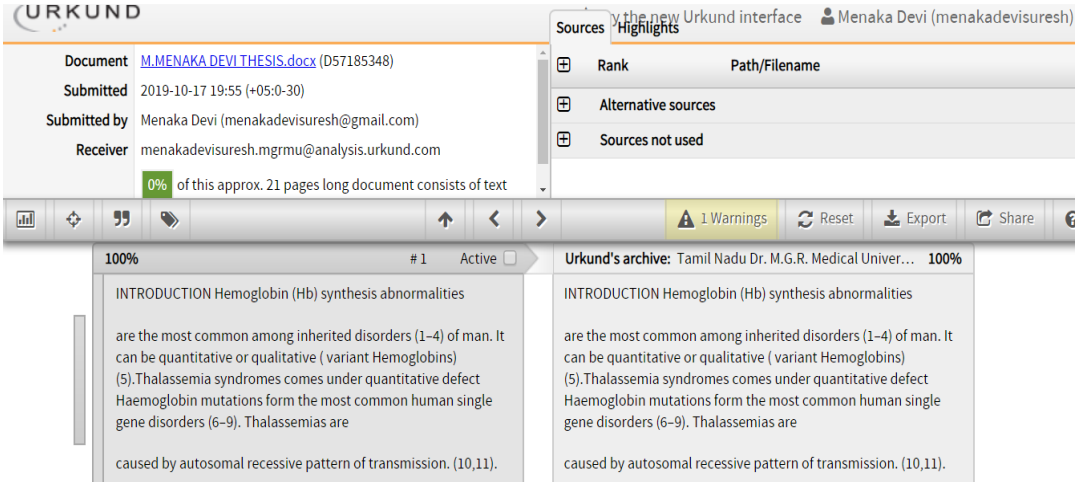

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ACKNOWLEDGEMENTS

First and foremost, I thank the Almighty. Without Him nothing would have been possible. With God's Grace, everything is possible.

I wish to express my sincere thanks to

Prof. DR.R.SHANTHI MALAR, MD., D.A., Dean, Government Stanley Medical College and Hospital and former Dean,

Prof. DR. S. PONNAMBALA NAMASIVAYAM MD., D.A., D.N.B., for permitting me to utilize the facilities of the hospital for the conduct of the study.

My heartfelt gratitude to **Dr. M.P.SARAVANAN M.D., Professor, HOD, Department of BIOCHEMISTRY,** for helping me to choose this study and for his constant encouragement, immense support, valuable and constructive suggestions, corrective comments and expert supervision.

My heartfelt gratitude to **Professor Dr. K.PRAMILA M.D., Professor, BIOCHEMISTRY, Institute of Child Health** for her support to perform HPLC analysis.

My heartfelt thanks to **Prof Dr. K.KALAIVANI, HOD, Obstetrics and Gynecology** for her support to conduct this study and for co-guidance.

I sincerely thank **Associate Professor DR.V.G.KARPAGHAVALLI M.D** for her valuable suggestion and corrective comments.

I would like to express my sincere gratitude to **Professors Dr. M.Vijayalakshmi, M.D, DR.R.SHANTHI, M.D** for their extra ordinary support, innovative ideas, valuable suggestions in my presentation in ethics committee and helping in the initial period of this present study.

I would like to express my gratitude to **Dr. V. ARUNA LATHA, M.D, PROF and HOD, Department of Pathology** for permitting to conduct the study in clinical pathology laboratory.

I would also like to thank **Associate Professor DR. Mythili M.D.,** for her support in carrying my dissertation work.

I wish to thank all Assistant Professors, Department of Biochemistry especially for their aid, keen interest, encouragement, and corrective comments during the research period, and I express my sincere thanks to my postgraduate colleagues for their enthusiasm and involvement for completing this study.

I thank all the staffs and technicians, Government Stanley Medical College and Hospital, RSRM Hospital for their cooperation and assistance.I am indebted to my parents, sister, husband,son and my friends for their care and moral support during the period of my study, without them it would be difficult for me to complete this study.

Last but definitely not the least; I would like to thank all the patients and their husbands who cooperated with me throughout my work.

INDEX

ABBREVIATIONS

1. **ARMS-PCR -** Amplifiction refractory mutations system
2. **Hb** –hemoglobin
3. **β-TT-**beta thalassemia trait
4. **CBC** –complete blood count
5. **CE**-capillary electrophoresis
6. **CE-HPLC** –cation exchange high performance liquid chromatography
7. **EDTA**-Ethylene diamine tetra acetic acid
8. **G6PD**- glucose 6 phosphate dehydrogenase deficiency
9. **IDA**-Iron deficiency anaemia
10. **IEF**-Immunoelectric focussing
11. **MCH**-mean corpuscular hemoglobin
12. **MCV**-mean corpuscular volume
13. **NESTROFT** –naked eye single tube red blood cell osmotic fragility test
14. **PCR**-polymerase chain reaction
15. **RBC**- red blood cell
16. **RFLP**-restriction fragment length polymorphism

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INTRODUCTION

Hemoglobin (Hb) synthesis abnormalities are the most common among inherited disorders of man.(1–4) It can be quantitative or qualitative (variant Hemoglobins)(5).Thalassemia syndromes comes under quantitative defect. Haemoglobin mutations form the most common human single gene disorders(6–9). Thalassemias are caused by autosomal recessive pattern of transmission.(10,11). The thalassemia mutations are endemic in Southeast Asia, the Indian subcontinent, the Mediterranean, the Middle East, and Africa. This is due to the protective effect of RBCs in thalassemia trait against developing severe falciparum malaria(12,13).

7% of world's population are affected with Haemoglobin (Hb) abnormalities (14). 7% of pregnant females carry a significant variant. About 1.1% of couples around the world carry a risk of having children with a haemoglobin disorder, of which 2.7 per 1000 conceptions are actually affected. Around 320,000 babies are born each year with a significant hemoglobin disorder. Majority of births (80%) occur in developing countries(15). Every year 3 lakhs infants are born with a major hemoglobinopathy.(16).3.4% of children mortality less than 5 years contributed by hemoglobin disorders in world. Sickle cell syndromes and thalassemia in this disorder constitute major public health problems. 50% of the world's population with β-TT are in Southeast Asia (12) .The frequency of beta-thalassemia trait in India is about 3 to 17%(17) with an average of 3-4%(2,18) . The frequency ranges from 3%-18% & 1.3% in North and South India, respectively(1).β thalassemia trait and

sickle cell trait form the most common hemoglobinopathy in India . In various regions of India, the prevalence of BTT is 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal(19). 52 different BTT mutations recorded until date in India (6,20).

The prevalence of BTT was 3.38 % among antenatal women in Gujarat(21).Every year around 7500 to 12000 β-thalassemia major infants are born in India (18,23), of which only 10-15% of thalassemia major child receive optimal treatment. The average yearly cost of regular blood transfusion therapy and iron chelation therapy would cost $5727(23).The cost of treating thalassaemic child increases annually as the child grows. So, identification of these disorders is immensely important epidemiologically (16).

The only cure for the child with thalassemia available today is bone marrow transplantation, which is beyond the affordability of most of the patients. Thus, the birth of a thalassaemic child causes physical and economic strain, not only on the affected child and its family, but also on the community. Thus, the emphasis must shift from the treatment to the prevention of such births in the future (17,24).

Screening of school children does not have the desired impact in many studies. Many of them did not know about their carrier status when they reached their adulthood. (18,25). Pre-marriage screening for haemoglobinopathies is not feasible (26) . Screening of pregnant women in antenatal clinics is possible(18). The diagnosis of thalassemia trait in a

pregnant woman identifies a couple at risk. Disorders of Hemoglobin synthesis should be screened in antenatal clinics to identify the family that would need a prenatal test(20) and advice them about the importance of genetic counselling (21).

Neonatal screening can only provide secondary or tertiary prevention for the affected children (27).In a populous country like India, routine screening of antenatal cases can be routinely done as it will be cost effective in preventing the number of homozygous births and reducing the financial burden on the health care system (2).

The approaches for prevention include mass screening for carriers, premarital counselling, antenatal screening, and prenatal diagnosis. In India, many studies have shown that antenatal screening followed by prenatal diagnosis is the most feasible for the prevention of birth of homozygous children(21).Without knowing the prevalence, screening programme is not possible. This study was conducted to know the prevalence of thalassemia trait in antenatal women who came for their routine check up in our hospital.

REVIEW OF LITERATURE

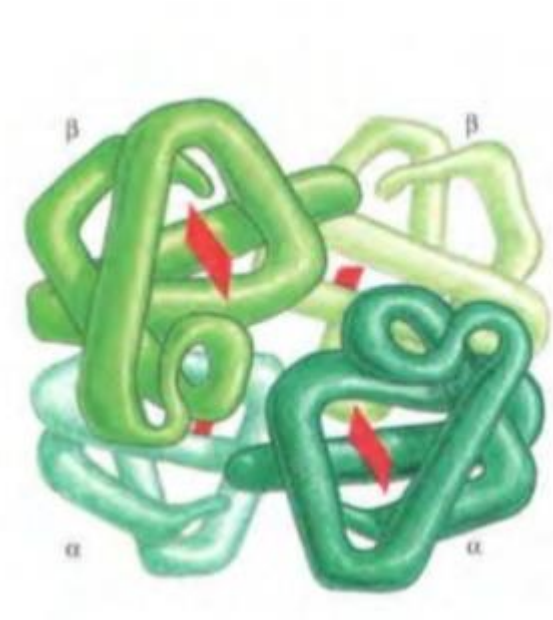
The term "thalasa" is a Greek word meaning the sea, and "emia" is the term for blood. Thalassemia was recognised as a clinical entity around the year of 1925, when a syndrome of splenomegaly and bony deformities was described in the children of Mediterranean origin.

HEMOGLOBIN STRUCTURE

Normal red blood cell contains hemoglobin molecules of approximately around 300 million (28,29).The Adult contains about three type of haemoglobin -HbA, HbF, HbA2. Human hemoglobin contains 4 globin polypeptide chains. It contains a pair of α globin chains with 141 amino acids and a pair of β globin chains with 146 amino acids. Normal adult blood has 96% of HbA, 3% of minor adult haemoglobin (HbA2), 1% of fetal hemoglobin (HbF). HbA has two α and two β chains (α2 β2). HbA_2 has two α and two δ chain (α2 δ2), and HbF has two α and two γ chains (α2 γ2).

The: α-globin genes present on chromosome 11 and β-globin genes present on chromosome 16. Normally each person inherits one β-globin gene and two α globin genes from each parent .Thus each individual should have four copies of the alpha gene and two copies of the beta gene (α2 β2). Heme which contains iron situated in a cavity formed by four globin chains bind with a single oxygen molecule. Thus one molecule of haemoglobin carry four molecules of oxygen(11).

Figure 1 Peptide chains of Hb



Source: Alberts et al,molecular biology of the cell,5th edition, page no - 144

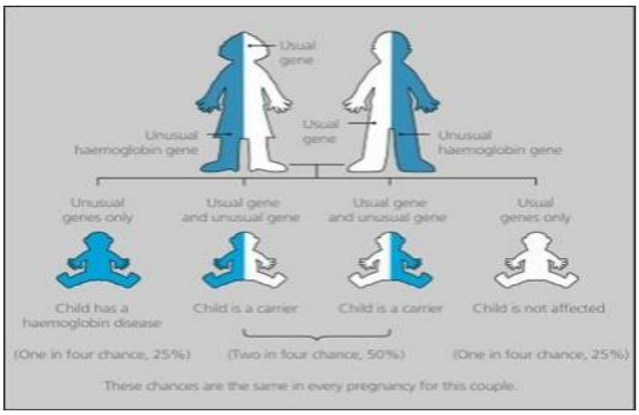
Fetal hemoglobin (HbF) present throughout the fetal life and replace embryonic hemoglobins Gower I, Gower II and Portland produced from the sixth week of gestation. After birth, synthesis of HbF reduces and gradually replaced by by HbA in the peripheral blood. Within the first two years of life, the characteristic hemoglobin phenotype of the adult with very low levels of HbF is found(30).

PATHOPHYSIOLOGY OF THALASSEMIA

Unpaired polypeptide chains of hemoglobin are insoluble and form inclusions which can damage red blood cells (RBC). Globin chains (α and β) form a tetramer which is soluble and prevent any cell damage. Normal globin

production is regulated in such a way that any new chain formed will have a partner to pair. In thalassemia syndromes, this regulation is impaired resulting in overproduction of either α or β chain and underproduction of other. This mismatch results in accumulation of unpaired chains and hence insolubility and precipitation of such globin chains. Depending upon whether the genetic defects or deletion lies in transmission of α or β globin chain gene, thalassemias are classified into α and β-thalassemias(9).Thus, patient with α-thalassemias have impaired production of α chains whereas patient with β-thalassemias have impaired production of β chains. Each of two main types of thalassemias may occur as heterozygous (minor) or homozygous state (major). The former is generally asymptomatic while the latter is severe congenital hemolytic anemia(11).Beta thalassemia is heterogeneous at molecular level. More than 23 molecular defects have been identified for β-thalassemia to date(31).

Figure 2 Autosomal recessive pattern of transmission



Courtesy: NHS Sickle Cell and Thalassemia Screening Programme handbook (Draft version 0.7) - Public Health England, page no-11

Beta thalassemia mutations are most commonly of the non deletion type with point mutations in the promoter region of the beta globin gene. Mutations which result in a mild or moderate reduction in beta chain production are referred to as β+, and those causing a complete absence of beta chain production from the affected gene are β0(32).

The imbalance between production of α and β globin chains of haemoglobin results in thalassemia. The reduced amount or absence of beta globin chains in thalassemia result in a relative excess of unbound alpha globin chain that precipitate in erythroid precursors in the bone marrow, this interferes with maturation of the red cells and its destruction in the bone marrow (ineffective erythropoiesis) and results in marrow expansion. The resultant hypertrophy of erythroid marrow is characterized by deformities of the bone of the face; it could also result in osteoporosis with pathologic fracture of long bones. The structural abnormalities of red cell membrane cause premature destruction of the red cells .The resulting anaemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the bone marrow which causes the bone deformities, disfigurement (frontal bossing, with enlarged maxilla and growth stunting). Prolonged severe anaemia along with increased erythropoietic drive result in extramedullary erythropoiesis and hepatosplenomegaly, it can also result in the formation of erythropoietic masses which may affect the spleen and the liver, the lymph nodes and spine. Haemolysis sometimes results in gallstones but this also occurs more commonly in thalassemia intermedia than major. Although individuals with

thalassemia intermedia are at risk of iron overload, secondary to increased intestinal absorption(12).Finally it results in reduced life expectancy.

Putative factors of ineffective erythropoiesis are suggested to be (1) oxidative stress induced by the excess of α-globin secondary to the α/β globin imbalance, (2) iron overload, and (3) endocrines and cytokine and environmental factors. Two key modifiers, an innate ability to produce fetal hemoglobin and coinheritance of α-thalassemia, both derived from family and population studies, affect the pathophysiology of β- thalassemia disorders at the primary level(33).

Beta thalassemia trait results when a β^+ or β^0 (mutation is paired with a normal beta globin gene (designated β/β^+ or β/β^0). This is generally asymptomatic and shows mild microcytic hypochromic anaemia on the CBC. In the beta-thalassemia trait, there is some degree of ineffective erythropoiesis, which leads to increased erythropoietic activity and increased iron absorption(32).

Patients with thalassemia trait are clinically well and are usually only detected through routine blood testing. However, the children of such patients could inherit the disease if the patient's partner also has the beta thalassemia trait.)(27)**.**

When there is a combination of β+ and β0 mutations (e.g. β^+/β^+, β^0/β^0, or β^+/β^0), the clinical phenotype is designated as either beta thalassemia

intermedia or beta thalassemia major. In intermedia, there is typically a moderate microcytic anaemia and occasional transfusion dependence.

Microcytic anemia in the case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (Hb) synthesis(34).

In major , patients typically have a severe microcytic anaemia and may present with severe illness and lifelong transfusion dependence(27).

The distinction between beta thalassemia intermedia and major is a clinical one, relying on the degree of clinical symptoms and the degree of transfusion dependence. Thalassemia intermedia was defined as a case of thalassemia with clinical severity intermediate between asymptomatic thalassemia minor and transfusion dependent thalassemia major(35). These conditions show a spectrum of disease including chronic hemolysis, ineffective erythropoiesis, spleenomegaly, jaundice, and iron overload due to transfusion dependence.

The laboratory diagnosis of beta thalassemia relies on this increase in HbA_2 (α_2 δ_2) .The increase in HbF is less consistent and diagnostically less reliable. Because of the normally high levels of HbF in early postnatal life, beta thalassemia does not become clinically apparent until a newborn is several months old. After six months of age, when most of the hemoglobin has switched from fetal HbF (α_2 γ_2) to adult HbA (α_2 β_2), the relative lack of beta chains will manifest as microcytic anaemia and an elevated HbA_2. IDA and thalassemias syndrome are the most common causes of microcytic

hypochromic anemia(7,10,10,34,36–45). Iron deficiency anaemia causes decreased Hb-A_2 bacause of decreased transcription and/or translation of the delta gene So coexistent iron deficiency anaemia and beta thalassemia trait can present with normal HbA_2 level(7).

It is essential to differentiate between the two, so as to avoid unnecessary iron therapy which is contraindicated in beta thalassemia and for the prevention of beta thalassemia major by genetic counselling. (37).

Disorders interfering with the formation or rate of production of hemoglobin (Hb) can induce a reduction in mean red cell Hb and corpuscular volume (MCV) with resultant hypocromia and microcytosis. Both genetic and acquired factors influence Hb levels(36). Low haemoglobin concentrations can be caused by genetic traits, such as sickle-cell anaemia and thalassemia, inadequate bioavailability of dietary iron, folate, or vitamin B12, infestations like malaria, schistosomiasis, hookworm infection, HIV infection(46). A full clinical history is essential to ensure the correct diagnosis(36).

A small set of patients with beta-thalassemia trait develop iron overload. High levels of serum ferritin have been observed in beta-thalassemia trait even if the person did not have any transfusion (32). High serum ferritin levels are found in a large spectrum of genetic and acquired conditions, whether associated or not with iron overload. The precise diagnosis of hyperferritinemia is difficult and requires a detailed medical history, blood biochemistry and

sometimes, genetic tests. An increase in ferritin levels combined with an increase in transferrin saturation usually is a sign of iron overload(32).

At first, thalassemia is differentiated from Iron deficiciency anemia by seeing haematological parameters. Iron deficiency develops in sequential manner over a period of negative iron balance. These stages include the iron depletion phase, iron-deficient erythropoiesis, and finally IDA. As the state of iron deficiency proceeds, mean cell volume (MCV), mean cell hemoglobin (MCH) tend to decline and results in microcytic hypochromic anemias (34)**.** Iron-deficient erythropoiesis is characterized by the production of RBC with a decrease in Hb content, so a high percentage of hypochromic cells are present. In iron deficiency states, RBCs are continuously produced in the bone marrow, the iron stores progressively decrease, and they tend to be more microcytic. Because of their long lifespan, several cohorts of normocytic and microcytic RBCs coexist in the peripheral blood leading to anisocytosis.

β -Thalassemia trait is characterized by an increase in RBC count, as a result of the chronic increase in erythropoiesis, decreased level of MCV, MCH and anisocytosis. The underlying pathogenic anomaly in β thalassemia has no fluctuations, and as a result, the bone marrow produces a constantly uniform population of microcytic erythrocytes. The measurement of microcytic and hypochromic red cells shows different results in patients with uncomplicated β thalassemia and IDA. Because of the impaired globin synthesis, microcytes of β thalassemia have small volume and a high rate of microcytosis is present(34)**.** Hepcidin is a key regulator of iron homeostasis: it blocks iron

release from macrophages and hepatocytes and inhibits intestinal iron absorption. Its liver expression increases in response to iron overload and inflammatory stimuli. If hepcidin expression would be correctly regulated, it should be increased in β-thalassemia patients in order to decrease intestinal iron absorption. However, the opposite effect is observed. Indeed, two hepcidin erythroid regulators have been reported: the growth differentiation factor 15 (GDF15) and the twisted gastrulation protein homolog 1 (TWSG1). High concentrations of both proteins, members of the TGF-β superfamily, were evidenced in β-thalassemia serum compared to normal human serum. These proteins down regulate hepcidin secretion by hepatocytes (47).

TABLE 1 CLINICAL FEATURES, GENOTYPE AND BLOOD INDICES OF BETA THALASSEMIAS

Thalassemia syndrome	Genotype	Clinical manifestation	Blood cell indices
Beta thalassemia trait	(β^{+}/β) or (β^{o}/β)	Asymptomatic	Hb: normal to moderate anemia MCV<80fl, MCH<27pg Elevated or normal RBC count
Beta thalassemia intermedia	(β^{+}/β^{+}) or (β^{+}/β^{o})	Spectrum of clinical disease-chronic hemolysis, spleenomegaly, jaundice, not transfusion dependent, although occasional transfusion may be required.	Moderate to severe anemia MCV<80fl,MCH<27pg Elevated or normal RBC count
Beta thalassemia Major	(β^{o}/β^{o})	Transfusion dependent haemolytic anaemia, ineffective erythropoiesis , iron overload	Hb < 6g/dl Marked microcytosis and hypochromia

Source: Aker et al (2018) From Distinguishing iron deficiency anaemia from thalassemia trait in clinical obstetric practice, Journal of Pregnancy and Reproduction, volume 2(1): 2-6

MANAGEMENT

Management is dependent on suppression of excessive erythropoiesis and prevention of excess iron overload and thereby preventing the severity of anaemia.

Blood transfusion- Severe anaemia with haemoglobin < 7g% for more than 2 weeks is widely accepted as an indication to start blood transfusion. The goal should be aimed at selecting the individual for transfusion with pre transfusion Hb level of 9 to 10 g/dl and to maintain post-transfusion Hb level of 13 to 14 g/dl. Such regime generally prevents growth impairment, organ damage and bone deformities. Care should be taken to avoid faster transfusion exceeding 5 ml/kg/h and amount of transfused RBC should not exceed 15 to 20 ml/kg/day. The frequency of transfusion is usually every 2 to 4 weeks. Patients with thalassemia intermedia may survive without chronic transfusion but the development of hypersplenism may require splenectomy in such patients. Vaccination against Streptococcus pneumoniae, Hemophilus influenzae and Neisseria meningitidis may be required in such individuals. These individuals may develop iron overload from increased gastrointestinal absorption of iron even without transfusion and therefore chelation therapy is started when the serum ferritin concentration exceeds 300ng/ml.

Bone Marrow and Cord Blood Transplantation

Bone marrow transplantation (BMT) remains the only definitive cure currently available for patients with thalassemia.Cord blood transplantation is another option with a low risk of graft versus host disease.

Gene therapy

Gene therapy for thalassemia is not very successful and the future of

Prognosis

this therapy will depend on efficiency of gene delivery and various other factors such as viral titers, non-oncogenic insertion, the variable expression of globin genes and the variable contributions of the β-thalassemia phenotype.

Patients with thalassemia minor have excellent prognosis. Thalassemia intermedia may survive without blood transfusions till adulthood. However as the requirement for transfusion increases and associated complications like hypersplenism develop, the prognosis become unfavourable. Earlier thalassemia major was lethal by the age of 5 years without any treatment. However with the early initiation of blood transfusion and iron chelation therapy these children can survive up to second and third decades. (11)

Prevention

The prevention includes identification of individuals carrying Beta thalassemia trait and counselling them about partner selection and prenatal diagnosis so as to defer birth of a child with major disease. Secondly many trait

carriers have mild to moderate anaemia, which is misdiagnosed as iron deficiency anaemia and treated with iron that is absolutely unnecessary as well as harmful. For above reasons, trait carriers are needed to be detected(48).

Inherited disorders of haemoglobin synthesis are, therefore, an important cause of morbidity and mortality worldwide. They place a large burden to the patients, their families, and even their communities. They are generally not curable but can be prevented by genetic counselling, and prenatal diagnosis(49) . Premarital screening for β-thalassemia is not widely acceptable in India because of social and cultural habits. Antenatal screening is the important step to identify women at risk of producing a child affected with hemoglobinopathy. In India, many studies have reported the success of antenatal screening followed by prenatal diagnosis.(21). Thalassemia can be diagnosed prenatally by analysis of fetal deoxyribonucleic acid sample obtained by amniocentesis or chorionic villous sampling. Genetic counselling can be done to the individuals at risk of having affected children.

The genotype may not clearly predict the phenotype of fetus. Genetic counsellors need to be well versed in the current treatment options and local availability, so they can provide couples with the appropriate information to assist them in making a difficult decision.

THALASSEMIA IN PREGNANCY

The diagnosis of thalassemia trait is often overlooked or not considered in women with microcytic anaemia. The goal in treating iron deficiency in pregnancy is to raise the hemoglobin to 10mg/dl or above, but this will not be possible in many women with thalassemia trait. The ferritin level should be used to monitor iron status, and the risks of iron deficiency in pregnancy must be balanced with the risks of unnecessary iron therapy. Signs of iron deficient erythropoiesis start to begin at a serum ferritin level of 25-40 ng/ml. So, it is best to start screening for iron deficiency in these patients and to maintain ferritin at 40-50 ng/ml. Therefore referring patients for intravenous iron when their hemoglobin levels are not rising with oral iron replacement subjects them to the potential of future iron overload, unnecessary cost, and inconvenience(29).Correctly identifying women with thalassemia trait has important implications for genetic counselling and avoiding unnecessary iron therapy.

There are several physiological changes occurring in pregnancy that may contribute to the variation in thresholds of serum ferritin-defining iron deficiency in pregnancy. These include (i) increased overall iron consumption compared to non-pregnant states (ii) second trimester plasma volume expansion (i.e. haemodilution)(iii) physiological rise in acute phase proteins secondary to pregnancy (iv) changes in inflammatory measures in the final trimester of pregnancy and (v) the uncertainty in the degree of increased iron consumption and iron requirements in multiple pregnancies(50).

Thalassemia trait in a pregnant woman should be suspected when there is a microcytic (MCV < 80 fl) and/or hypochromic (MCH < 27 pg) anaemia and any of the following: failure to respond to iron therapy, anaemia which predates the pregnancy and is not consistent with IDA, normal or elevated red blood cell count (RBC), or member of a high risk ethnic group(29).Clinical implications of identifying thalassemia trait in a pregnant woman are of clinical benefit to her and her family.

Patients with thalassemia trait should not receive iron supplementation. Although these patients are not transfusion dependent, they may still develop iron overload later in life, which can lead to pulmonary hypertension and thrombosis [58].

LABORATORY DIAGNOSIS

Screening of thalassemia minor is the only method to prevent the occurrence of homozygotes in the society**(21,39)**. Prenatal diagnosis is the only effective way to prevent the birth of a fetus with severe thalassemias, which include hemoglobin Bart's hydrops fetalis and thalassemia major**(51)**.

In all cases, measurement of Hb is followed by complete blood cell count (CBC) looking mostly for anaemia, microcytosis and, hypochromia(14). In some developing countries, the Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), a cost effective, rapid and reliable screening test for detection of β-thalassaemia trait, is largely used as a first approach(14)**.** The screening of thalassaemia trait in the areas with limited laboratory facilities is

often done by NESTROFT. Despite its sensitivity and rapidity, in around one out of four cases of iron deficiency anaemia, this test leads to a false positive result**.**

Cation exchange HPLC offers accurate and precise detection of Hb variant and thereby aiding in prevention and management of various hemoglobinopathies(5,35) .

The recommended strategy is to use a combination of cation-exchange high performance chromatography (CE-HPLC), capillary electrophoresis (CE) which give high throughput. Difficult cases may demand further investigations requiring specialized protein and/or molecular biology techniques(14).

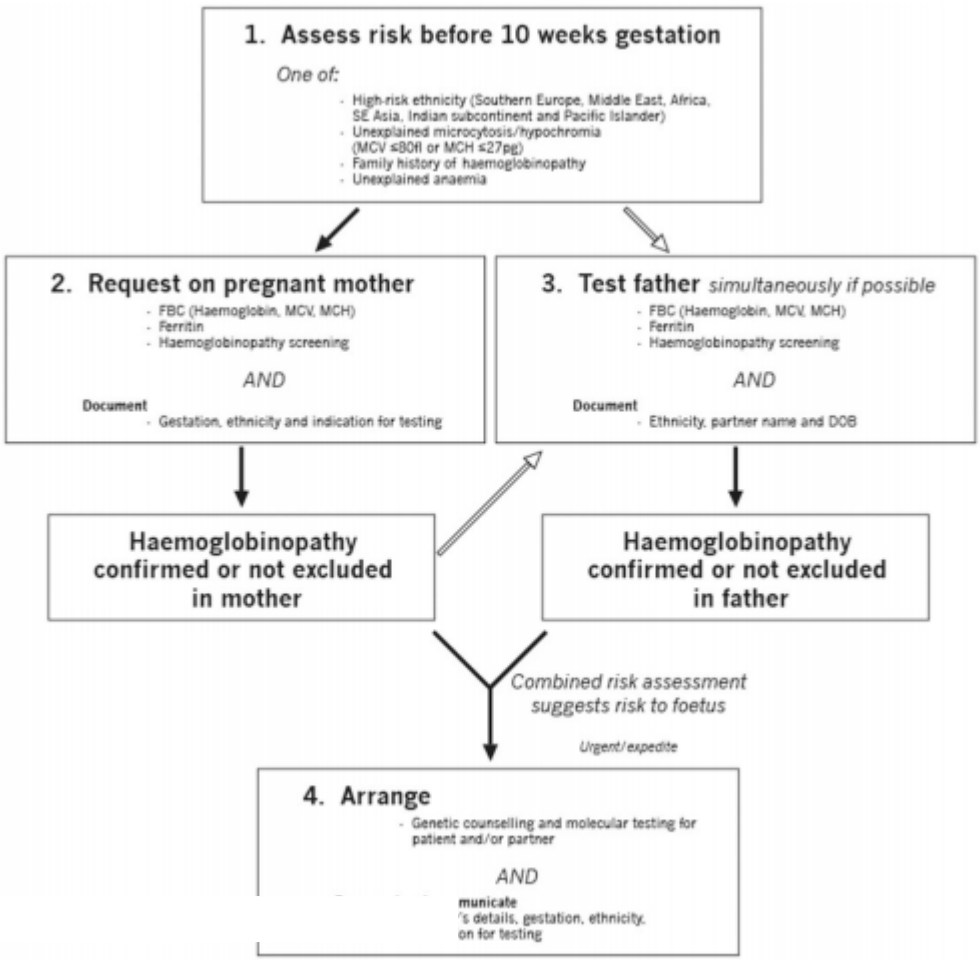
Genetic testing -sequencing of the beta globin gene may be diagnostic; PCR methods may be necessary to target the most common deletions as well as non-deletional mutations. Genotyping for known alpha- and beta-gene mutations was done with gap-PCR and ARMS(10,52). In cases of some rare mutations, the genotyping was done with the help of other techniques such as RFLP and ARMS-PCR.(52). Other patients may benefit from allele specific oligonucleotide hybridization (ASO) or reverse dot blot analysis (RDB).

Cation exchange high-performance liquid chromatography (CE-HPLC) is one of the best method used for screening and identification of various hemoglobinopathies. It has the advantage of quantifying Hb F and Hb A along with Hb variant screening in single and highly reproducible system. The simplicity of the automated system with internal sample preparation, superior

resolution, rapid assay time, and accurate quantification of Hb fractions establishes CE-HPLC as an ideal methodology for routine clinical laboratory(53).

Together with a complete blood count, the CE-HPLC is effective in categorizing hemoglobinopathies as traits, homozygous disorders and compound heterozygous disorders(54).

Figure 3 selective antenatal screening algorithm



Courtesy: Orly Lavee1 and Giselle Kidson-Gerber, Antenatal haemoglobinopathy screening: Patterns within a large obstetric service. Working towards a standard of care Obstetric Medicine 2015, Vol. 8(4) 184–189! The Author(s) 2015

INDIAN SCENARIO OF BETA THALASSEMIA TRAIT

The ten year cohort study conducted by Balgir RS provided the data base on the pattern of spectrum of hemoglobinopathies in Orissa. One thousand fifteen cases of anemia were analysed (1994 to 2003). Hemoglobin electrophoresis was carried out on CAM (cellulose acetate membrane) in Tris EDTA-Borate buffer at pH 8.9 and quantification of A_2 fraction of hemoglobin by elution method. The value more than 3.5% of A_2 fraction of hemoglobin was taken as cut off point for beta thalassemia trait and more than 10% as Hb E. Hb electrophoresis in acidic medium (pH 6.2) was also carried out to confirm Hb D or E band. Sickle cell trait (29.8%), sickle cell disease (7.5%), sickle cell-beta-thalassemia (1.7%), beta-thalassemia trait (18.2%), thalassemia major (5.3%) were the most common hemoglobinopathies observed (28).

Mamtani et al conducted a study in Nagpur, India (1997 to 2004) in a predominantly Sindhi population. In a community survey, a total of 1563 subjects were screened for β-thalassemia trait. NESTROFT was done for all samples. Measurement of HbA_2 was done first by identifying presence of HbA_2 in cellulose acetate electrophoresis (at pH 8.4).Then a nine scenarios was made based on varying cut-offs of MCV and HbA_2 analyses. For the purposes of screening and counselling, possession of β-thalassemia was defined as a positive NESTROFT test and/or HbA_2 level exceeding 3.5%.But the author had not conducted any tests for diagnosis of iron-deficiency anemia. Their bias-corrected estimates suggested that NESTROFT can miss 7% of the β-thalassemia trait cases. However the authors observed that in spite of this

limitation the performance of NESTROFT was superior to HbA2 and MCV. Through this study the author strongly supports the use of NESTROFT as a screening tool for β-thalassemia trait—especially in high prevalence and low-resource settings(55).

Sinha et al studied the prevalence of anemia in young pregnant women, correlated with RBC indices and studied the significance of identification of hemoglobinopathies. Of the 120 pregnant women that were screened, 11.6% had hemolytic anemia, 50% were thalassemia trait. MCV< 76 fl was observed in 88 (73.3 %) cases. MCV< 27 pg had 100 % sensitivity and 28.7 % specificity for screening of beta-thalassemia trait. NESTROFT had comparable sensitivity but lower specificity (14.9%). Thus, moderate to severe anemia was observed in most pregnant women. Hemoglobinopathies should be screened in antenatal clinics to identify the couples that would need a prenatal test. They suggested that a lower MCV/RBC with RDW in the normal range may be useful in screening for thalassemia trait in pregnant women to identify the couples that would need a prenatal test(20).

Chopra et al (2005-2006) studied the pattern of haemoglobinopathies amongst the referred patients of anaemia in a tertiary hospital of Armed Forces in India. A total of 1032 patients were studied for anaemia investigation. Haematological indices and haemoglobin electrophoresis was done in all cases. Out of 1032 cases, 774 (75%) were normal and 258 (25%) cases had abnormal haemoglobin pattern. 82% of microcytic hypochromic anaemia had reduced serum iron and elevated total iron binding capacity (TIBC), whereas 85% had

decreased serum ferritin levels. Spectrum of haemoglobinopathies prevalent was β-Thalassemia trait (17%), followed by sickle cell trait (2.3%). (56).

Mulchandani et al conducted a cross-sectional study in 446, young, apparently healthy, unrelated (by blood) Sindhi individuals before marriage or before reproduction to study the prevalence of Beta thalassemia trait (βTT) in Sindhi community of Nagpur City and the association between βTT and some epidemiological factors like age at menarche in females, past history of diagnosis and treatment of anaemia and the current haemoglobin concentration. Blood samples were processed for Beta thalassemia trait (βTT) using two stage approaches. Two screening tests - Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) and RBC indices were performed on all samples and those positive for either one or both screening tests were further investigated for HbA2 level estimation by Haemoglobin electrophoresis. HbA2 level of > 4.5 % was taken as confirmatory of β thalassemia trait. The prevalence of βTT in Sindhis of Nagpur was found to be 16.81 %. (48).

Singh and Gupta studied the efficacy of naked eye single tube red cell osmotic fragility test (NESTROFT) as a screening test for beta thalassemia trait, and to standardise a saline concentration which could give best results with minimum error and maximum sensitivity and specificity. Five concentrations (0.35 percent, 0.36 percent, 0.37 percent, 0.38 percent and 0.39 percent) of buffered saline solutions were used. NESTROFT was done to three groups of subjects: 24 normal individuals, 87 subjects with genetically -proven beta-thalassemia trait and 13 patients with proven iron deficiency anaemia. The

results demonstrated that 0.36 percent was the best saline concentration for NESTROFT. It could detect 97.7 percent of heterozygous beta-thalassemia patients, Specificity of NESTROFT with 0.36 percent saline was also higher at 83.3 percent, This test with 0.36 percent saline concentration was also positive for three (23.08 percent) patients with iron -deficiency anaemia. . NESTROFT with 0.36% buffered saline showed a very high negative predictive value (90.9%). They concluded that subjects who are positive for NESTROFT should undergo further investigations to confirm the diagnosis and this test may be considered as the single screening test to be used in areas where availability of laboratory resources and economic resources are limited. (17).

Ajit C Gorakshakar and Roshan B Colah (2009) conducted a cascade screening study where the parents of children with β-thalassemia major receiving blood transfusions regularly at various centres in Mumbai City in western India were contacted. After screening 691 extended family members, as many as 151 carriers were identified. Red cell indices, Hb A_2 estimation was done by cellulose acetate electrophoresis (pH 8.9). Majority of the affected children (index cases) were from "high risk" communities and 44 families were screened. Among these, 25 siblings of index cases were also screened, and 10 of them were β-thalassemia heterozygotes. Similarly, 490 children from high-risk communities were screened and 96 were β–thalassemia heterozygotes. In all, 151 of the 691 individuals screened were β-thalassemia carriers (21.9%).As compared with other approaches, the percentage of β-thalassemia carriers identified was 5-6 times higher using this cascade screening approach(18).

Sirichotiyakul et al compared (2009) the accuracy of the osmotic fragility test (OFT) and MCV calculation for screening for the alpha-thalassemia 1 and/or beta-thalassemia trait. In this cross-sectional study, blood samples collected from 328 apparently healthy pregnant women were tested for OFT (using a glycerol 0.45%, phosphate-buffered, sodium chloride solution) and MCV testing. A polymerase chain reaction was also performed to diagnose alpha-thalassemia 1 carriers. Quantitative HbA_2 test was done to diagnose beta-thalassemia carriers. Sensitivity and specificity were 95.0% and 86% for the OFT; and based on the cut-off point of 78.1fl derived from the ROC curve, they were 93% and 93.4% for MCV calculation. Since MCV seems to provide fewer false-positive results, it may be the first choice wherever an automated hematology analyzer calculating MCV is available(57)**.**

Sachdev et al conducted a study (January to August 2008) in a, Clinical Reference Lab, Gurgaon, Haryana, India where a total of 2600 cases referred from New Delhi, Haryana, U.P, and Jammu and Kashmir and some from Nepal for Hb variant analysis .The tests were performed on an instrument BIO RAD 'VARIANT' (beta thalassemia short program) utilizes the principle of high performance liquid chromatography (HPLC). Of these, 327 cases displayed abnormal hemoglobin fractions on HPLC. 12.5% hemoglobin variants detected. A cut-off of over 3.9% was taken for diagnosis of beta thalassemia trait. A total of 232 cases (8.9%) of beta thalassemia trait were diagnosed. Thalassemia major and intermedia constituted approximately 0.6% of cases .(5)

Chandrashekar et al.(December 2009 to November 2010)carried out a hospital based study in Chennai during which 543 abnormal chromatogram patterns were seen. The commonest disorder encountered was β-thalassemia trait (37.9%), followed by HbE trait (23.2%), homozygous HbE disease (18.9%), HbS trait (5.3%), HbE β-thalassemia (4.6%), HbS β-thalassemia (2.5%), β-thalassemia major (2.3%). The average value of HbA_2 in β-thalassemia minor was 5.4%. (54).

Patel et al (2009) screened 32,857 students from different school and colleges in South Gujarat.Samples having MCV (≤ 78), MCH (≤28) and/or positive solubility test were investigated for Hb electrophoresis on cellulose acetate membrane (pH 8.6). Hb A_2 level ≥3.5% was considered as diagnostic for β-TT. High performance liquid chromatography on Biorad Hb variant system was done on samples having doubtful results. Overall prevalence of β-TT and SCT in South Gujarat was 4.4% and 1.3% respectively. Incidence of mild to moderate anemia was higher in β-TT and SCT (sickle cell trait) compared to non-β-TT or non-SCT subjects.(22)

Bhukhanvala et al conducted a study (2012) in antenatal women, followed by prenatal diagnosis in Surat, South Gujarat. Measurement of Red cell indices, solubility test, cellulose acetate electrophoresis tests were done and results were confirmed by HPLC. Husbands were also screened for hemoglobinopathies. The couples at risk were again counselled and referred to the National Institute of Immunohematology, where mutations in parents and fetuses were identified by molecular analysis. Out of 3,009 women, 37.04,

52.6, and 10.3 % were in the first, second, and third trimester of pregnancy, respectively. Among those having hemoglobinopathies, 102 (3.38 %) had the β-thalassemia trait, 46 (1.5 %) the Sickle cell trait, and 26 (0.86) had hemoglobin variants like Hb D Punjab, Hb E, Hb D Iran, Hb Q India, Hb J Paris-I, and Hb O Indonesia. Out of the 14 couples at risk of having an affected child, 11 (78.5 %) couples opted for prenatal diagnosis. Three foetuses had homozygous β-thalassemia and hence the pregnancies were terminated. (21).

An exploratory study by Kulkarni et al was conducted in a Primary Health Centre, south Bangalore, India, for a period of 3 months (June – August, 2010) to find out the prevalence of the Beta Thalassaemia trait among the pregnant women who attended the antenatal clinics and husbands of the NESTROFT positive women were also tested using the NESTROFT. Out of the 210 pregnant women who were tested, 18 (8.5%) were thalassaemia carriers, 39% of the carrier women had histories of one or more abortions, of which 85.7% had first trimester spontaneous abortions. This study does not included the analysis of HbA_2 and iron status(24).

Philip et al conducted a study to determine the prevalence of hemoglobinopathies in patients with microcytic hypochromic anemia and to assess the suitability of using high performance liquid chromatography (HPLC) routinely for screening antenatal cases and patients with anemia. 4335 cases received from Mar 2007 to Nov 2011 were studied for various hemoglobinopathies and variants on BIO RAD 'VARIANT' analyzer. Of the 4335 cases studied, 2119 were antenatal cases, 1710 patients with other

disorders and 506 family studies. Of these, 688 cases displayed abnormal hemoglobin fractions on HPLC of which 140 were antenatal women.They found a high prevalence (15.8%) of hemoglobinopathies amongst microcytic hypochromic anemia and antenatal cases. (58).

The objective of the study by Piplani et al (2013) was to evaluate the validity of "NESTROFT" (Naked Eye Single Tube Red Cell Osmotic Fragility Test) as a useful screening tool in the diagnosis of beta thalassemia trait in Northern India and to compare its findings with studies done in other parts of India and the World. This study was conducted on 150 subjects in the department of haematology in a tertiary health care center in north Indian state of Punjab. In Group I, 111 cases diagnosed as microcytic hypochromic anaemia were selected. In Group II, 39 individuals (the family members of known cases of beta thalassemia major) were selected. Complete haemogram, NESTROFT and HbA_2 levels by electrophoresis were done. Of the 111 cases in Group I, 20 (18%) gave positive results with NESTROFT while 91 cases (82%) tested negative. In Group II, out of 39 cases, 30 (76.92%) tested positive with NESTROFT while 9 gave a negative result. In Group I, out of 20 NESTROFT positive cases, only 3 had HbA_2 levels more than 3.5%. In Group II, all the 30 NESTROFT positive cases had HbA_2 levels more than 3.5%. The test showed a sensitivity of 100%, specificity of 85.47%, a positive predictive value of 66% and a negative predictive value of 100%. (1).

Verma et al (2014) conducted a prospective hospital based study in 30 patients with concomitant IDA and β-TT. Patients with HbA_2 levels >3.7%

with low serum ferritin <10ng/ml for females and <16ng/ml for males, normal random blood sugar levels, and no evidence of other hemoglobinopathy were included in the study. All the patients had a complete blood count, serum iron studies, and thalassemia screening using BIORAD hemoglobin Variant testing system. The patients received oral iron therapy in appropriate dosages for a period of twenty weeks, after which all the investigations were repeated. All patients except two were adults with a marked female preponderance. Oral iron therapy led to statistically significant improvement in hemoglobin, red cell indices and marked change in serum iron, ferritin, and HbA_2 levels. There was a significant reduction in the total iron binding capacity levels.Hence, iron deficiency should be identified and rectified in patients with suspicion of beta thalassemia trait(45).

A retrospective, single-center, cross-sectional study was conducted by Mukhopadhyay on consecutive 10,407 participants for hemoglobinopathies in West Bengal by CE-HPLC in the Thalassemia control unit (TCU) of the department of pathology of Institute of Post Graduate Medical Education and Research, Kolkata during 2010–2013. Haematological parameters, Red cell morphology and platelet counts, the subjects with normal as well as with abnormal haemoglobin by HPLC were assessed. Out of 10,407 subjects, 8,898 (85.5 %) were diagnosed as normal, 579 (5.6 %) were as β-thalassemia trait (β-TT) and 522 (5.0 %) were detected as HbE carrier on HPLC study and ten additional variants were encountered (16).

AIM AND OBJECTIVES

AIM:

The aim of the study was to find out the prevalence of beta thalassemia trait among Antenatal women attending a tertiary care centre.

OBJECTIVES:

1. To Screen all Antenatal women by RBC indices and NESTROFT test for beta Thalassemia trait
2. To measure serum Ferritin in microcytic hypochromic anaemia to rule out iron deficiency anaemia and to measure the percentage of the Hb variant by HPLC among the women with positive screening test

MATERIALS AND METHODS

EQUIPMENTS USED:

1. Beckmann coulter AU 480
2. Sysmex XN 100
3. Cobas e411 autoanalyzer
4. Bio-rad-D10-HPLC

REAGENTS USED:

1. Erba Mannheim reagent for-urea
2. Saturated picric acid and 0.75 N NaOH for creatinine
3. Ferritin Cobas kit
4. HbA_2/HbF Dual reagent Kit-Bio-Rad D10
5. 10% Buffered saline.

STUDY CENTRE:

1. RSRM Hospital, Government Stanley medical college, Chennai –1

2.24 hours Biochemistry and Pathology Lab, Government Stanley Medical College hospital

DURATION OF STUDY:

12 months (October 2018– November 2019)

STUDY DESIGN:

Descriptive study

STUDY POPULATION:

Antenatal women age ranges from 18 to 40 years attending OPD irrespective of gestational age and parity

SAMPLE SIZE:

150

SAMPLING METHOD:

Non random sampling- convenient sample

INCLUSION CRITERIA:

1. Antenatal women (age 18 to 40 years)

2. Irrespective of gestational age and parity

EXCLUSION CRITERIA

1. H/o recent blood transfusion (Within 6 months) –chances of missing carrier state because of dilution by transfused blood and possibility of transfusion with HbE carrier, since Hb E and A_2 have retention time, it will lead to increased HbA_2 level.(59)

2. Recent H/O blood loss –leads to iron deficiency anaemia

3. Known case of epilepsy on antiepileptic-–increased breakdown and decreased absorption of folic acid, which in turn cause megaloblastic anaemia.(60)

4. Alcoholic-because of low-folate diet and because of an inhibition of intestinal absorption, metabolic use, and hepatic uptake and storage of folate.(60)

5. Known HIV patient on drugs – zidavudine and stavudine impair DNA synthesis, resulting in delayed nuclear maturation. In this situation more haemoglobin synthesis occurs in less mature erythroid precursors, and the synthesis of delta chains is relatively greater in less mature cells; therefore, the increased synthesis of delta chains leads to higher HbA2 values.(61,62)

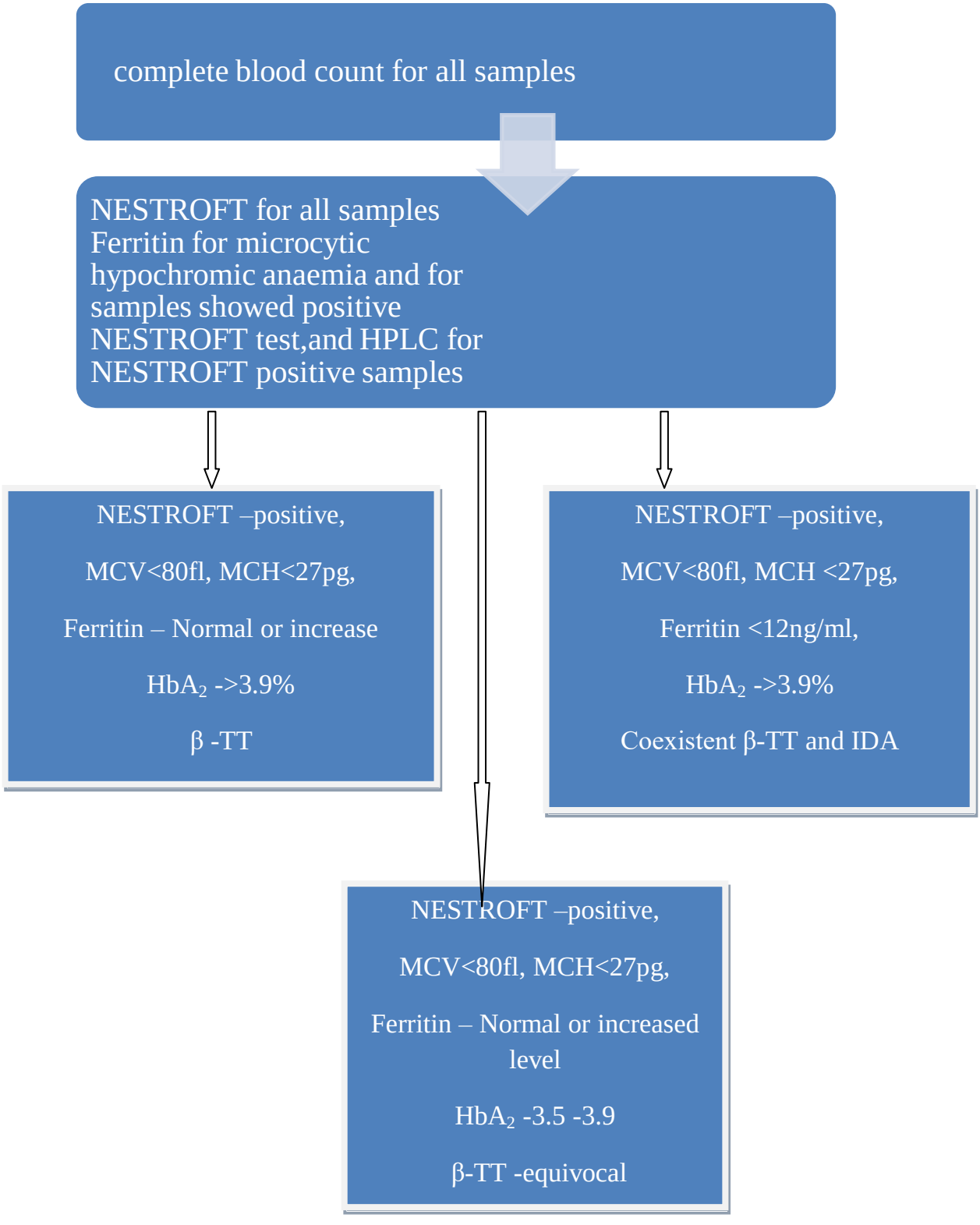
STUDY PROCEDURE

SAMPLE COLLECTION AND PREPARATION:

The data in the form included name, age, sex,religion ,occupation, income, parity , address, and telephone number .After getting the informed consent from the patient under aseptic precaution, 2ml blood collected in EDTA tube, and 3ml in red top tube. Anticoagulated Venous blood analyzed for CBC by a Coulter automated cell counter on the same day of collection. NESTROFT was also done on the day of collection.HbA_2 was measured by HPLC (Biorad D10) within 4 days of collection.

Blood in the red top tube allowed to clot and centrifuged at 2000-2500 rpm for 15 minutes. Routine investigations -blood sugar, urea and creatinine were analyzed. Serum sample separated immediately from the cells and samples refrigerated at -20ºC for serum ferritin analysis.

FIGURE 4 PLAN OF STUDY



LAB INVESTIGATION

1. Complete blood count -5 part hematology cell counter.

2. Peripheral smear

3. NESTROFT test- Naked Eye Single Tube Red Blood cell Osmotic Fragility Test- test tube based turbidity test using 0.36% buffered saline.

4. Serum ferritin - ECLIA

5. HbA_2 measurement - HPLC method

FIGURE 5 SYSMEX XN1000

PRINCIPLE:

This analyzer utilizes the both Coulter's principle (based on electrical resistance) to find out size and volume of cell and flow cytometry to determine the granularity, diameter and inner complexity of the cells.

The CBC count was done on the Sysmex XN 1000, fully automated differential cell counter. The 2-level controls were run every day in the cell counter, and the counter was maintained according to the manufacturer's instructions.

At the laboratory, the samples were subjected to a number of hematological procedures:

COMPLETE BLOOD COUNT:

1. Hemoglobin (135±15g/l)
2. Red cell count ($4.3 \pm 0.5x10^{12}$/l)
3. Hematocrit (0.41 ± 0.05 %)
4. Mean corpuscular volume (MCV) – 92 ± 9 fl
5. Mean corpuscular haemoglobin (MCH) – 29.5 ± 2.5 pg
6. Mean corpuscular haemoglobin concentration (MCHC) – 330 ±15 g/dl
7. Red cell distribution width (RDW) – 12.8 % ± 1.2%

Source: Barbara J Bain, Dacie and Lewis practical hematology-11th edition, page no-14

PERIPHERAL SMEAR FOR BLOOD PICTURE

PERIPHERAL SMEAR: Peripheral smear was done by slide method. A drop of blood was placed in the centre 1-2cm from one end. Another slide was used as a spreader, holding the same in 30-45◦ near the drop of blood. The spreader was moved backwards so that it makes contact with the drop of blood. The spreader was then moved forward rapidly over the slide. A thin peripheral blood film was prepared. It was dried and then stained using Leishman's stain. Then distilled water is poured over the stained film to dilute the amount of stain. The slide is washed after 1-2 minutes, dried and examined under oil immersion lens of the microscope.

NESTROFT

PREPARATION OF THE REAGENT:

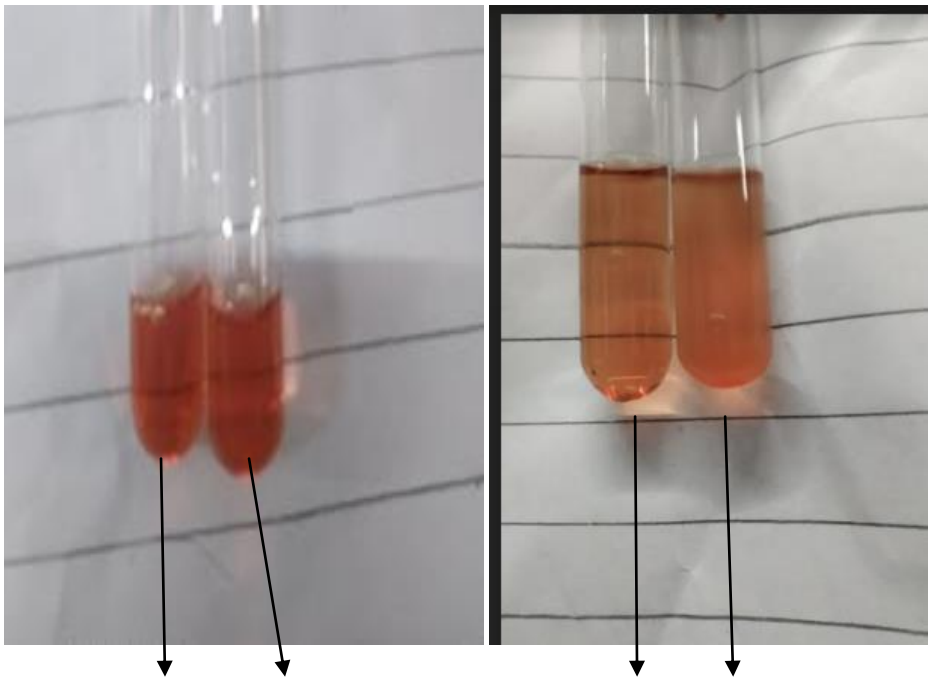
Stock solution of 10% buffered saline (pH 7.4) was prepared by taking NaCl- 90 g, Na_2HPO4 -13.65 g and Na_2 $HPO4.2H_20$ -2.4 g and dissolving them in distilled water. The final volume was then adjusted to one litre.1% solution was prepared from the above by 1 in 10 dilution with distilled water.0.36% buffered saline is prepared by diluting 36ml of 1% saline with 64ml distilled water , to make 100ml.

PRINCIPLE OF NESTROFT:

Microcytic red cells are resistant to lysis when exposed to hypotonic solutions.

PROCEDURE OF THE NESTROFT

FIGURE 6 NEGATIVE AND POSITIVE RESULT OF NESTROFT



Distilled water, Buffered saline DW BS

Two millilitres (2 ml) of 0.36 % buffered saline was taken in one tube (10cm x 1cm diameter) and 2 ml distilled water was taken in another tube. 20µl anticoagulated blood (taken in EDTA tube) was added to each of the tubes. The tubes were left undisturbed for half an hour at room temperature. After half an hour, the contents of both the tubes were shaken and then held against a white paper on which a thin black line was drawn .The line would be clearly visible through the contents of the tube which contained distilled water due to complete lysis of the blood cells. If the line was visible through the contents of

the tube which contained buffered saline, the test would be considered as negative, while the test would be considered as positive when the line was not visible. At the end of 30 minutes, the DW tube was seen to be homogeneously pink with no sediments. In the BS tube the negative test showed similar findings as DW tube where as in a positive case, a clear supernatant and a sediment at bottom was observed .(63)

ESTIMATION OF SERUM FERRITIN

METHODS

Electro-chemiluminescence immunoassay (**ECLIA**) for in vitro Quantitative determinations of ferritin in human serum was performed using Automated Cobas e411. Analysis is based on electro chemiluminescent technology using ruthenium complex and the measuring cell. 'Electro' means electrical stimulation 'chemi 'refers to chemical reaction and luminescence indicates production of light.

TEST PRINCIPLE

Sandwich principle:

Total duration of assay: 18 minutes

1st incubation: 10 µl of sample, a biotinylaed monoclonal ferritin – specific antibody, and a monoclonal ferritin – specific antibody labelled with a ruthenium form a sandwich complex.

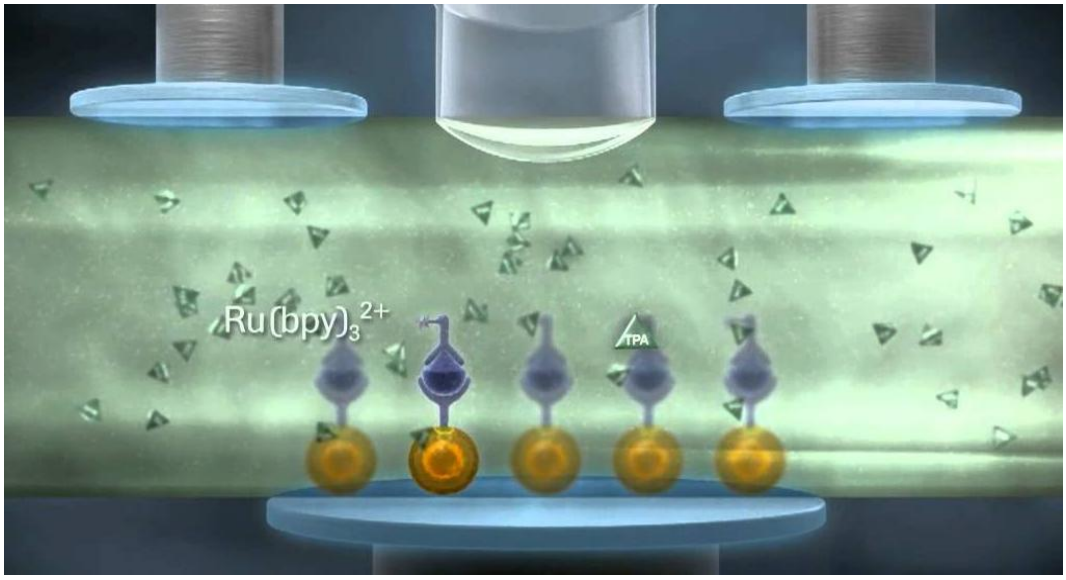
2nd incubation: after addition of streptavidin –coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and strepravidin.

The reaction is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a multiplier

EQUIPMENT:

- Cobas e 411 Analyzer

FIGURE 7 ELECTROCHEMILUMINESCENCE REACTION



This picture depicts about TPA enabling ruthenium to reduce to its base state with release of light

REAGENTS –working solutions

The reagent rack pack is labeled as FERR

M streptovidin-coated micro particles (transparent cap), 1 bottle, 6.5ml; streptavidin-coated micro particles 0.72mg/ml; preservative

R1 Anti- Ferritin-Ab-biotin (gray cap) , 1 bottle , 10ml: Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0mg/l;phosphate buffer 100 mmol/L ,pH 7.2;preservative.

R2 Anti-ferritin-Ab-biotin-Ru (bpy) $_3^{2+}$ (black cap), 1 bottle, 10ml: monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0mg/l; phosphate buffer 100 mmol/l, pH 7.2; preservative.

REAGENT:

- Ferritin Elecsys from Roche Diagnostics

STORAGE AND STABILITY:

- Store at 2-8 °C. Do not freeze.

REFERENCE RANGE:

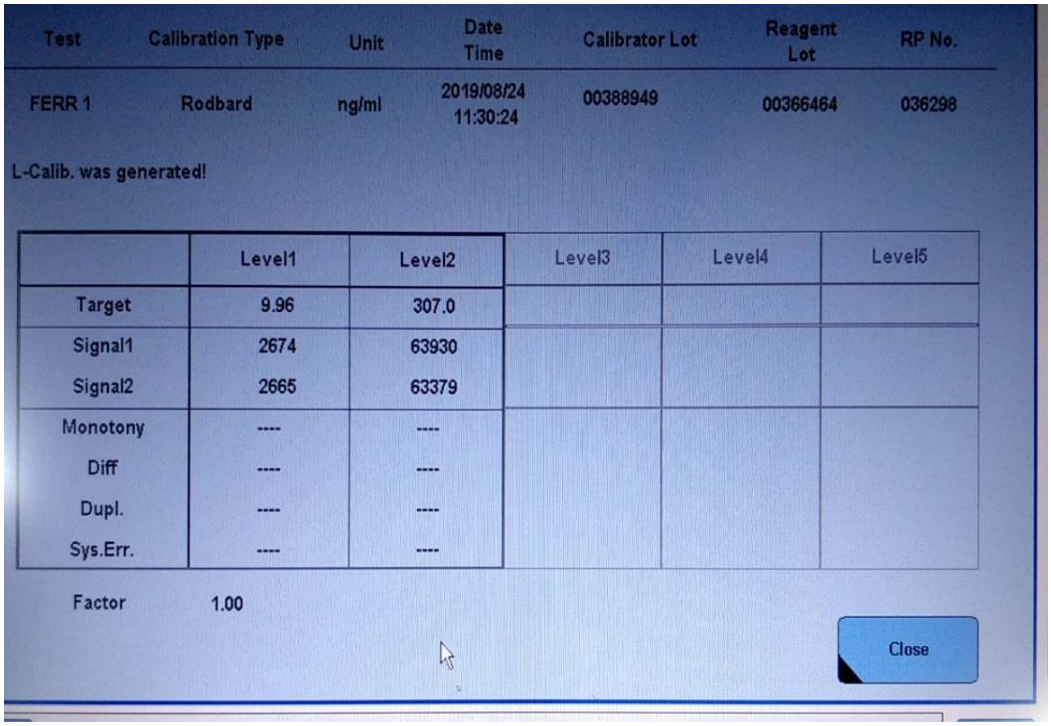
- 6.5-147.1ng/ml(15-49 years female)(64)

  Ferritin value < 12ng/ml was considered as having iron deficiency anemia.(50)

FIGURE 8 PICTURE OF COBAS e 411 – ANALYSER

This instrument works based on the principle of electrochemiluminescence reaction.

FIGURE 9 CALIBRATION CHART OF FERRITIN



Test	Calibration Type	Unit	Date Time	Calibrator Lot	Reagent Lot	RP No.
FERR 1	Rodbard	ng/ml	2019/08/24 11:30:24	00388949	00366464	036298

L-Calib. was generated!

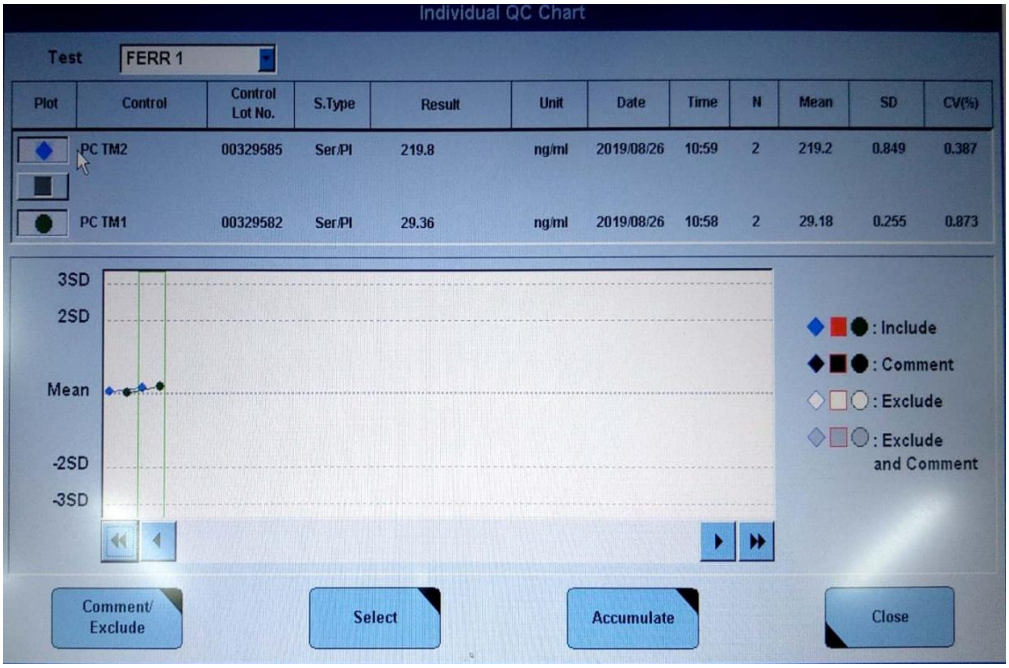
	Level1	Level2	Level3	Level4	Level5
Target	9.96	307.0			
Signal1	2674	63930			
Signal2	2665	63379			
Monotony	----	----			
Diff	----	----			
Dupl.	----	----			
Sys.Err.	----	----			

Factor 1.00

Close

Results are determined via calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the reagent barcode.

Figure 10 chart of both level of controls



Two levels of controls were run in the analyzer. Samples were analyzed in two batches. Control values in both days were within acceptable limit.

HPLC: BIORAD-D10

A recorder pack containing the elution buffers, calibrators, calibrator diluents, whole blood primer, and sample vials were provided with each kit.An Hb A_2/F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run.

TABLE 2 Hb A_2 AND Hb F IN NORMAL AND BETA THALASSEMIA TRAIT

Genotype	HbA_2 (%)	HbF (%)	MCV (fl)and MCH(pg)
Normal	2.3-3.5	<2	80-100 and 27-32
Beta thalassemia trait	4-8	0.5-4	Reduced

Values of HbA_2 between 3.5 and 3.9% are considered equivocal and require detailed evaluation before a diagnosis of β-TT can be made. Subjects with HbA2 level between 4.0 and 9.0 % were diagnosed as β-TT

1–2 ml of whole blood sample was collected in EDTA tube and was stored at 2–8 C. 5 µl anticoagulated whole blood samples were mixed with 1.0 ml of haemolysis reagent to each sample tube and were analysed within 4 days. The prepared samples separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing two buffers of different ionic strengths to elute the different haemoglobins.

FIGURE 11 BIO-RAD D10

HPLC PRINCIPLE

With HPLC, the positively charged Hb fractions are separated based on their ionic interactions with a negatively charged stationary phase in a chromatography column, followed by their elution by a mobile phase with phosphate buffers of differing pH and ionic strength. The adsorbed positively charged hemoglobin molecules are eluted from the column into the liquid phase at a rate related to their affinity for the stationary phase. A dual-wavelength filter photometer analysed the haemoglobin elution from the cartridge by detecting the absorbance changes at 415 nm and the secondary

filter at 690 nm corrected the baseline for effects caused by mixing buffers with different ionic strengths. Hemoglobins are identified by their retention time and quantified by computing the area under the corresponding peak in the elution profile. Different peaks of different haemoglobins in defined windows with their retention time, relative percentage and area displayed in a chromatogram of absorbance versus time Hb A_2 values ranging from 2.0 to 3.9%, and the Hb F values up to 1.3% were considered normal, which were provided by the manufacturer. The average retention time of HbA_2 in the HbE disorders was 2.76, 2.7, and 2.71 in HbE trait, HbEE disorder, and HbE β-thalassemia, respectively. The hemoglobin fall into windows which are defined by their retention times. Hemoglobins with retention times outside the windows are detected as unknown peaks.

HPLC INTERPRETATION:

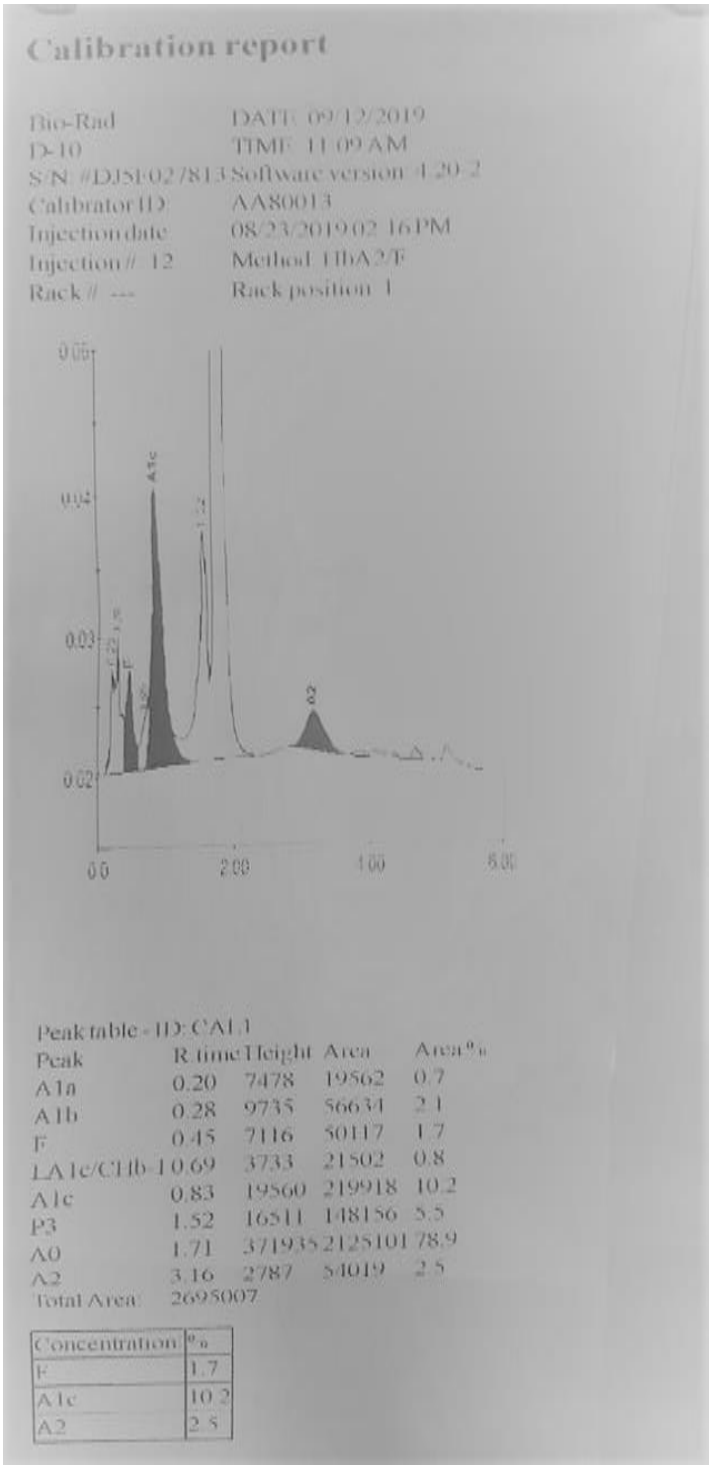
The total area acceptable was between—one to four million. Sample ratio was increased in case of low total area and vice versa.(5).The blood samples collected in EDTA vacutainer were diluted and injected in to the analytical cartridge of D-10 analyzer (Bio-Rad Laboratories, Hercules, CA). Phosphate buffers of increasing strength are then pumped in to the cartridge and the hemoglobin elute out based on their ionic interactions with the cartridge. A chromatogram for each sample is obtained using the HbA_2/HbF/HbA1c dual program(54).The average retention time of HbA1a was 0.21 (range from 0.19 to 0.25). The average retention time of HbA1b was 0.28 with a range from 0.25 to 0.35, and HbA1c average was 0.79, with a range of

0.71–0.97 minutes. The retention time of HbF was 0.43, ranging from 0.41 to 0.51 in all cases where HbF was normal or minimally elevated.HbA_2 retention time ranged from 2.74 to 3.09 with an average of 2.94.(54)

Hemoglobin separates into major and minor hemoglobins when subjected to CE-HPLC. The order of elution of the various components is HbA1a, HbA1b, HbF, LA1c/CHb-1, LA1c/CHb-2, HbA1c, P3 (Hbd component), HbA0, and HbA_2. The minor hemoglobins A1a, A1b, A1c, F1, and the P3 component are posttranslational modifications of the globin chains(54).An elevated HbA_2 with an average value of about 5%, along with microcytic hypochromic indices, is characteristic of β-thalassemia trait. In β-thalassemia major, in addition to a markedly microcytic hypochromic blood picture, there are elevated HbA_2 and elevated HbF ranging from 10 to 90% [5]. HbE trait is diagnosed by the presence of a high HbA_2 (E+A2), approximately 30% [6]. Homozygous HbE patients have approximately 90% HbE+A_2 with minor elevation of HbF [6]. HbE+A_2 levels of 40–60% with marked elevation of HbF are seen in HbE-β-thalassemias . Now CE-HPLC and CE allow a direct measurement of the Hb F fractions. Increased Hb F level may indicate a β-thalassaemia trait but it could also be the result of a hereditary persistence of foetal Hb (HPFH) or of another condition, such as diabetes mellitus, or pregnancy (14).

FIGURE 12 CALIBRATOR 1 REPORT

Calibrator 1 shows HbA_2 retention time at 3.16 and area under curve - 2.5%.



Calibration report

Bio-Rad
D-10
S/N: #DJ51027813
Calibrator ID: AA80013
Injection date: 08/23/2019 02:16 PM
Injection #: 12
Rack #: ---
Method: HbA2/F
Rack position: 1

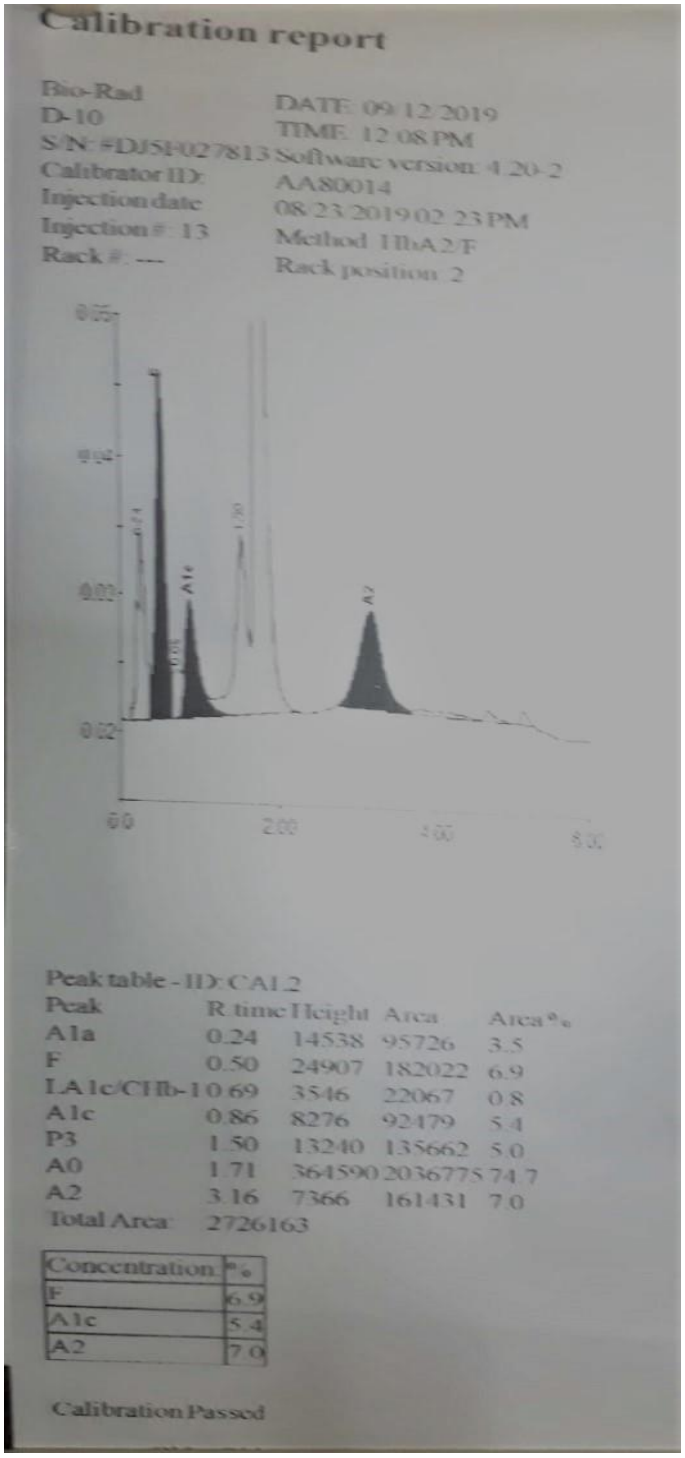
Peak table - ID: CAL1

Peak	R time	Height	Area	Area %
A1a	0.20	7478	19562	0.7
A1b	0.28	9735	56634	2.1
F	0.45	7116	50117	1.7
LA1c/C1b	0.69	3733	21502	0.8
A1c	0.83	19560	219918	10.2
P3	1.52	16511	148156	5.5
A0	1.71	371935	2125101	78.9
A2	3.16	2787	54019	2.5
Total Area:			2695007	

Concentration:	%
F	1.7
A1c	10.2
A2	2.5

FIGURE 13 CALIBRATION REPORT OF CALIBRATOR 2

Calibrator 2 shows HbA_2 retention time at 3.16 time and area under curve - 7%.



Calibration report

Bio-Rad
D-10
S/N: #DJSH027813
Calibrator ID:
Injection date
Injection #: 13
Rack #: ---

DATE: 09/12/2019
TIME: 12:08 PM
Software version: 4.20-2
AA80014
08/23/2019 02:23 PM
Method: HbA2/F
Rack position: 2

Peak table - ID: CAL2

Peak	R time	Height	Area	Area %
A1a	0.24	14538	95726	3.5
F	0.50	24907	182022	6.9
LA1c/CHb-1	0.69	3546	22067	0.8
A1c	0.86	8276	92479	5.4
P3	1.50	13240	135662	5.0
A0	1.71	364590	2036775	74.7
A2	3.16	7366	161431	7.0
Total Area:		2726163		

Concentration:	%
F	6.9
A1c	5.4
A2	7.0

Calibration Passed

Figure 14 Low level of control

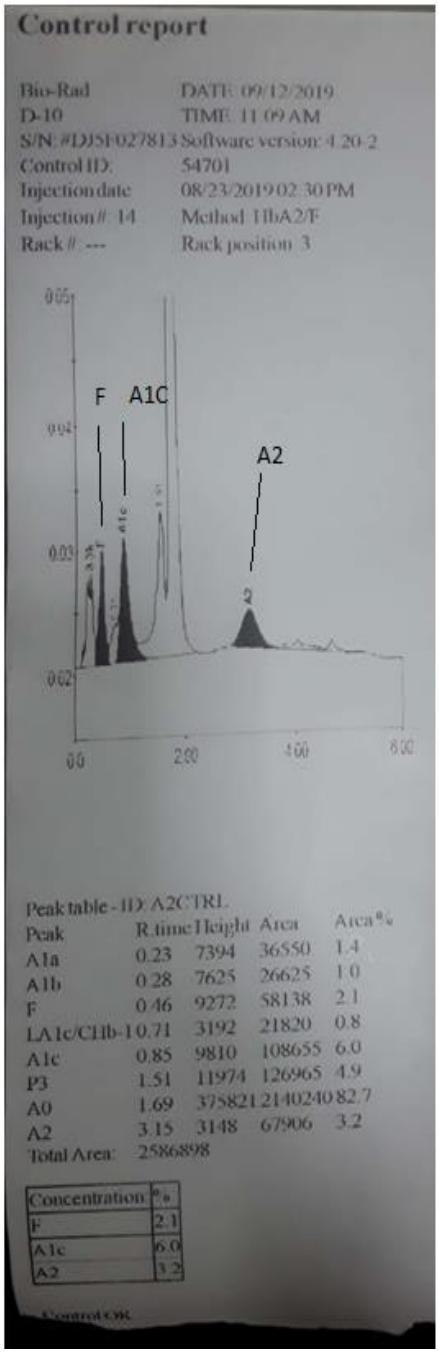
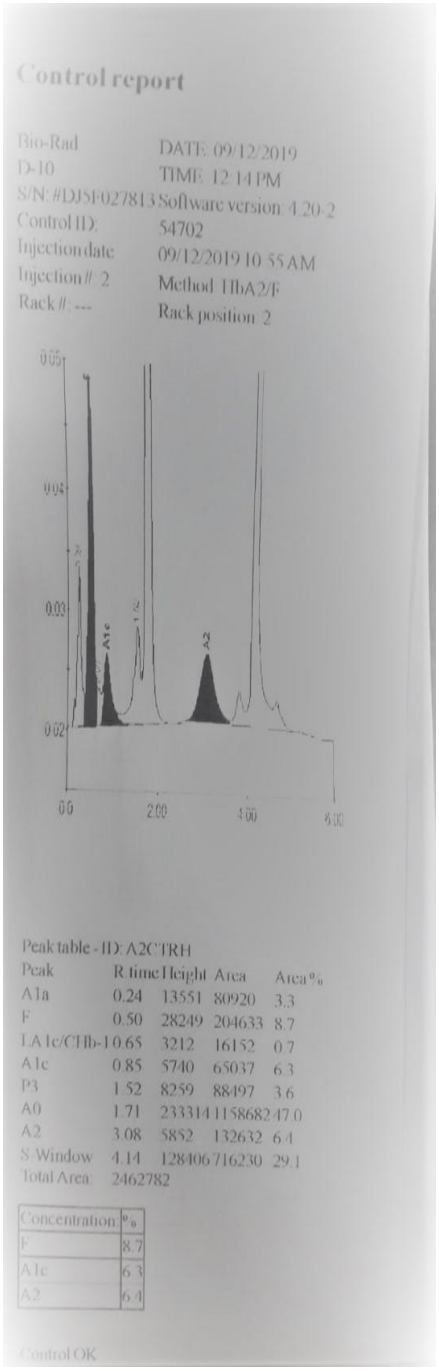


Figure 15 Control high



Control low shows HbA_2 value of 3.2%. Control high level shows HbA_2 of 6.4%.

STATISTICS AND RESULTS

RESULTS:

DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS OF THE STUDY

TABLE 3 DISTRIBUTION ACCORDING TO AGE OF PARTICIPANTS

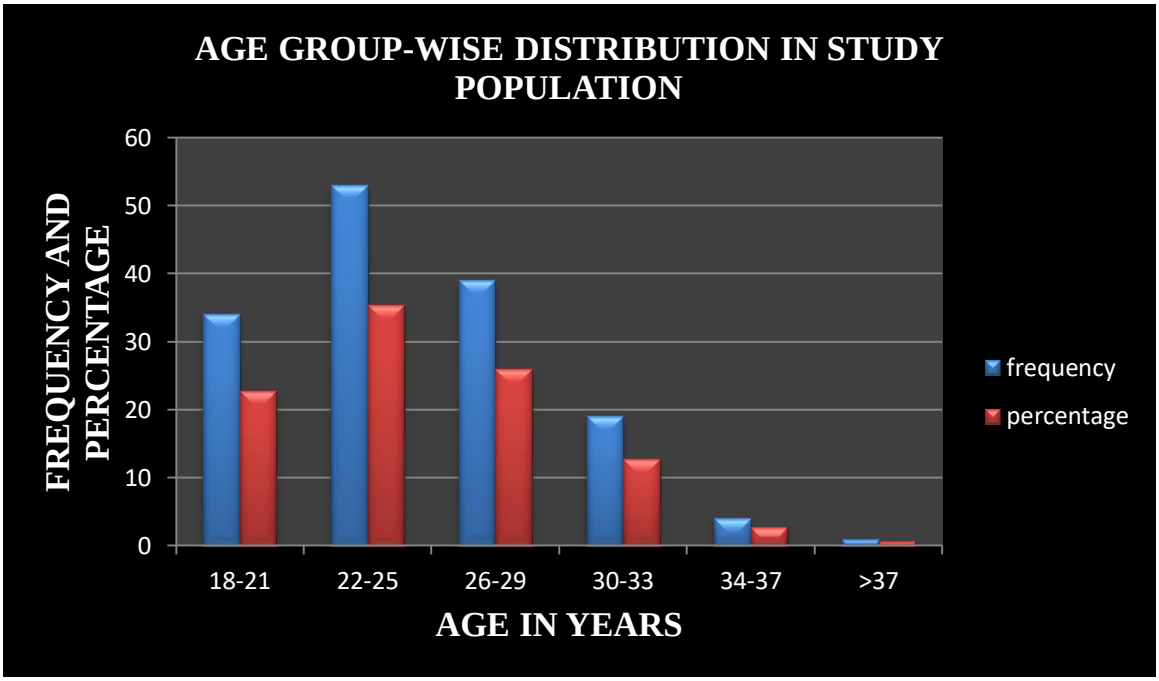
Age characteristics	value
Minimum	18
Maximum	38
Mean	25
Standard deviation	4.27

The distribution of age in the study participants ranges from 18 to 38 years. The mean age of study participants were 25 (95% CI is 24.30-25.67) with a SD of 4.27. Majority of the study participants were within 22-25(35.3%), followed by 26-29(26%).

TABLE 4 AGE GROUP-WISE DISTRIBUTION IN STUDY POPULATION

Age	Frequency	Percentage
18-21	34	22.7
22-25	53	35.3
26-29	39	26
30-33	19	12.7
34-37	4	2.7
>37	1	0.7

FIGURE 16 AGE GROUP-WISE DISTRIBUTION IN STUDY POPULATION



53 persons (35.3%) out of 150 persons were within 22-25 age .

TABLE 5 SOCIOECONOMIC STATUS OF STUDY POPULATION

Modified BG Prasad's Social classification for 2019	Per capita monthly income	Low Hb (<10g/dL)	Normal Hb (>10)	Total
I	≥7008	34	68	102
II	3504-7007	20	26	46
III	2102-3503	1	1	2
IV	1051-2101	-	-	-
V	≤ 1050	-	-	-

FIGURE17 SOCIOECONOMIC STATUS OF STUDY POPULATION

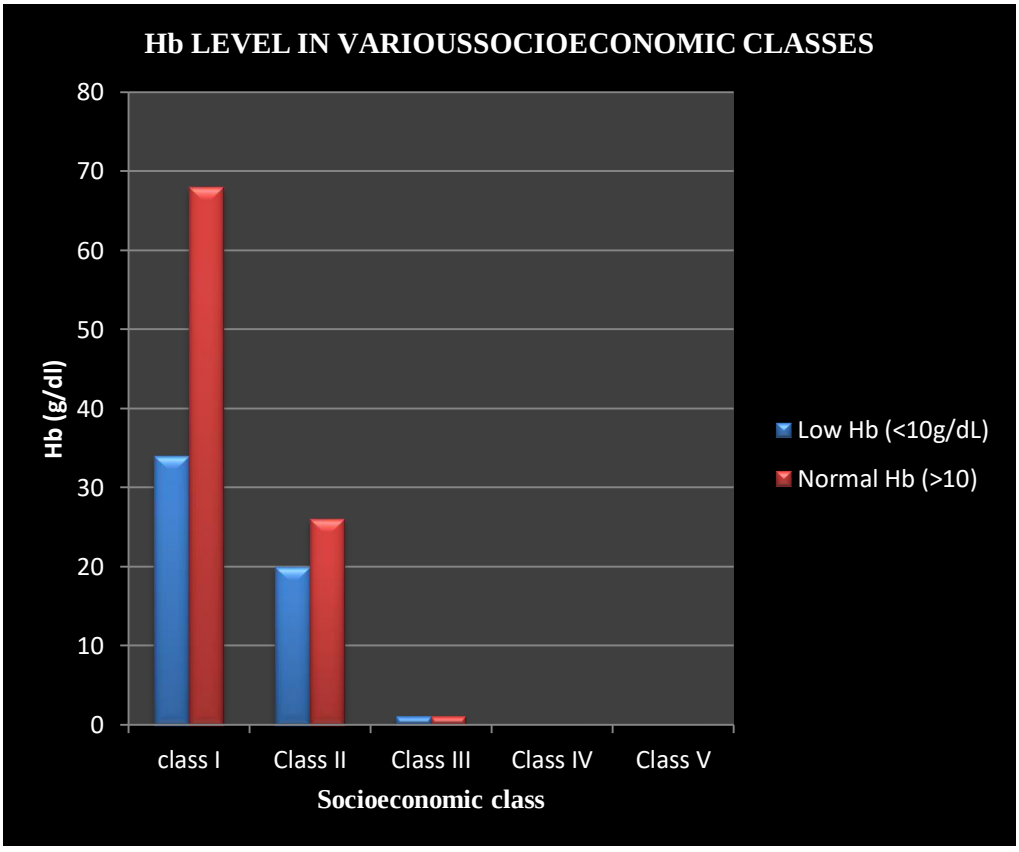


TABLE 6 NO OF PERSON IN EACH RELIGION

Religion	Hindus	Muslims	Christians
Number	105	33	12

FIGURE 18 NO OF PERSONS IN EACH RELIGION

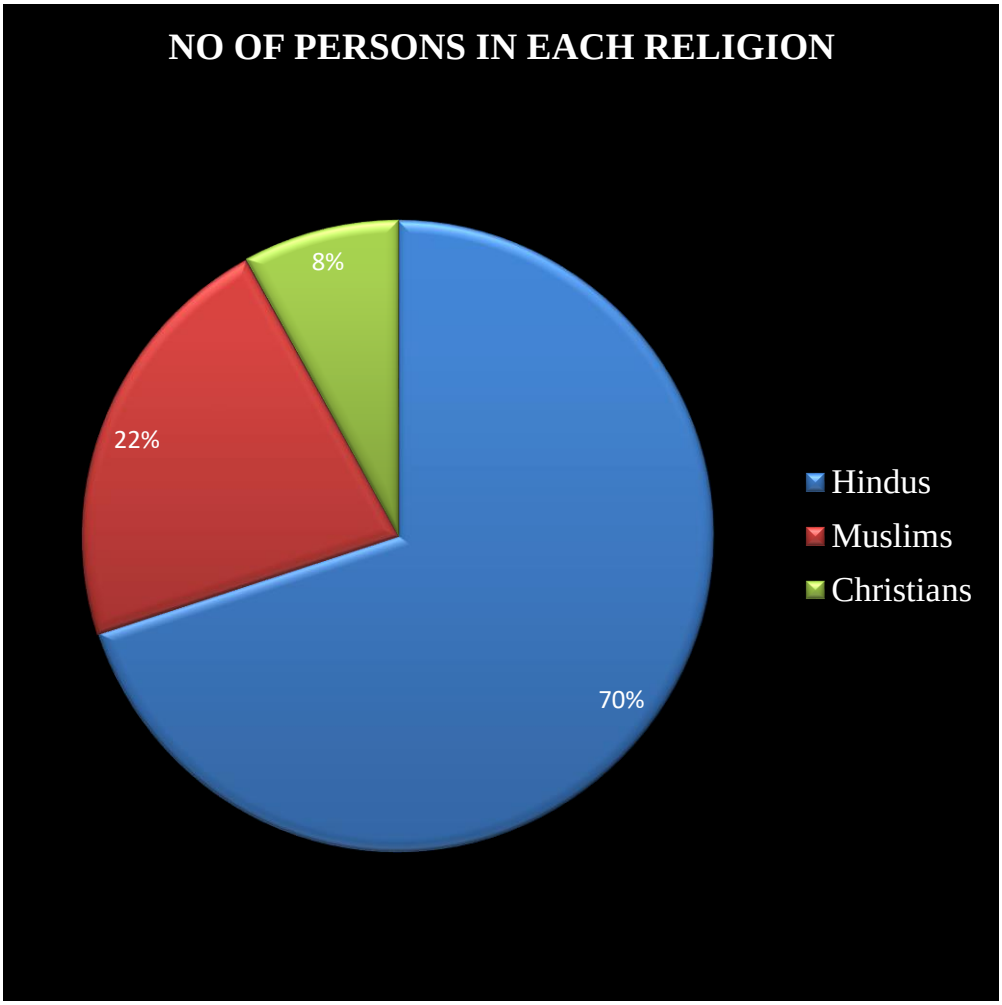


TABLE 7 NO OF GRAVIDA IN STUDY POPULATION

S NO	Gravida	No	Percentage (%)
1	Primi	64	42.7
2	Multi (2-3)	81	54
3	Grandmulti (≥4)	5	3.3

FIGURE 19 NO OF GRAVIDA IN STUDY POPULATION

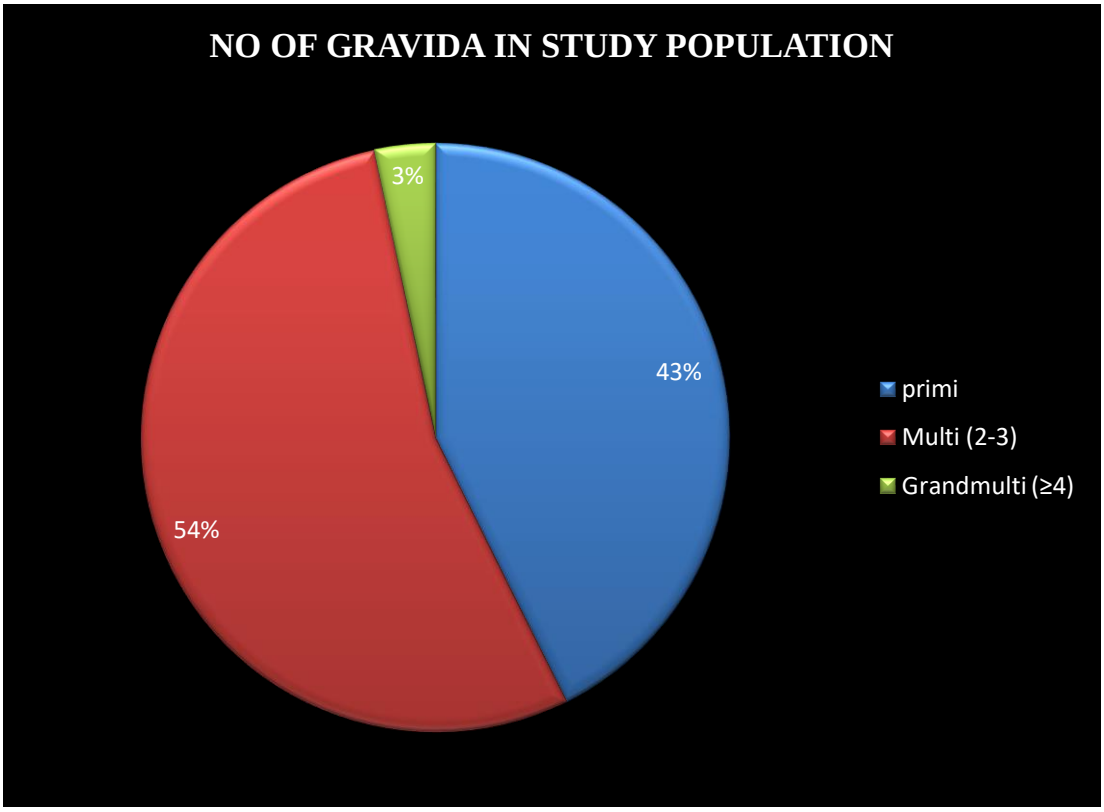


TABLE 8 NUMBER OF ANTENATAL WOMEN IN EACH TRIMESTER

Trimester	No	Percentage (%)
First	16	10.7
Second	27	18
Third	107	71.3

FIGURE 20 NUMBER OF ANTENATAL WOMEN IN EACH TRIMESTER

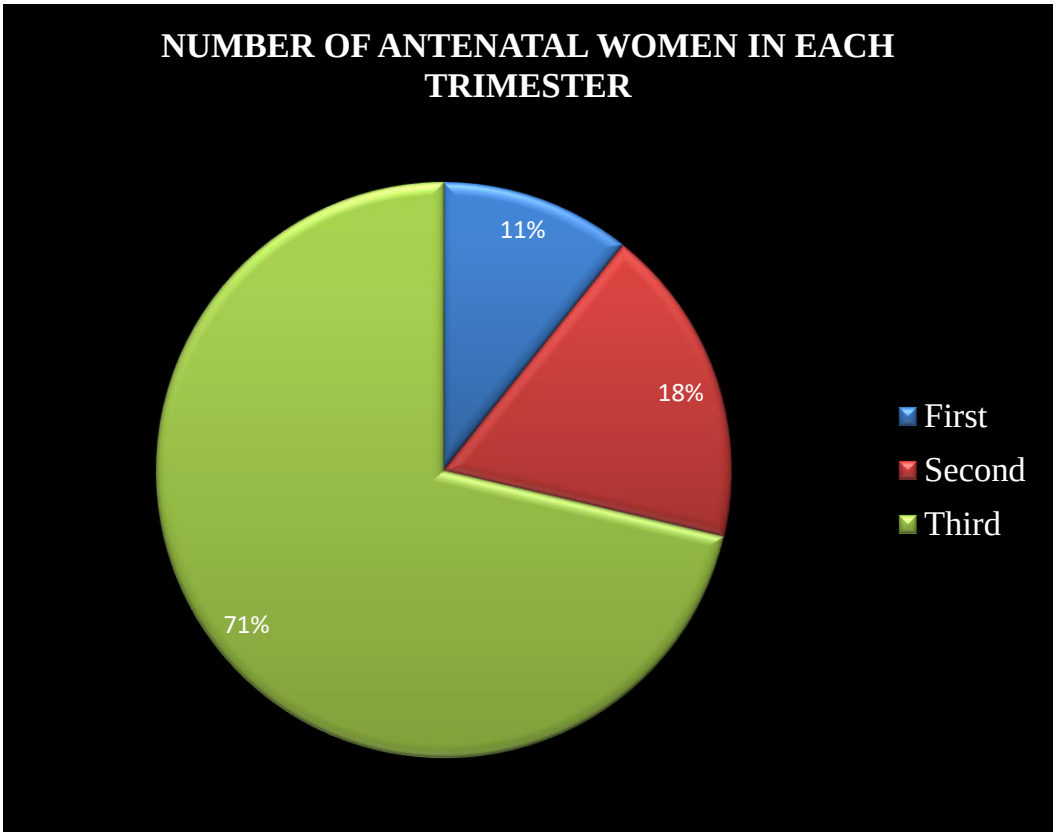
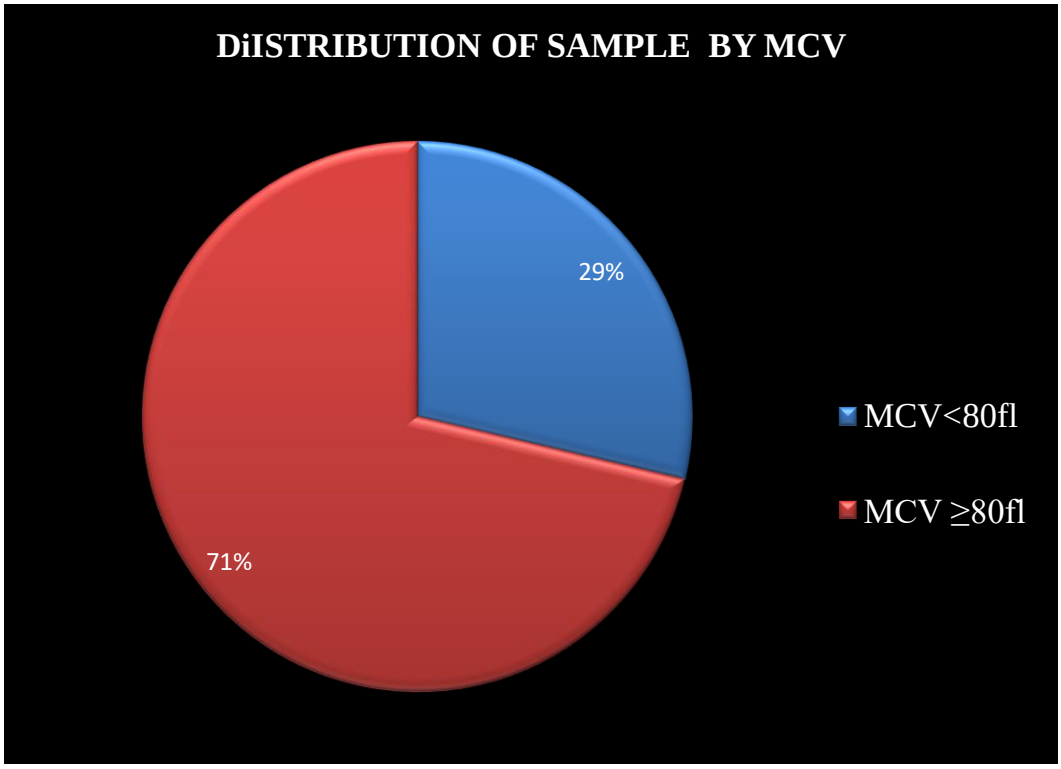


TABLE 9 DISTRIBUTION OF SAMPLE BY MCV

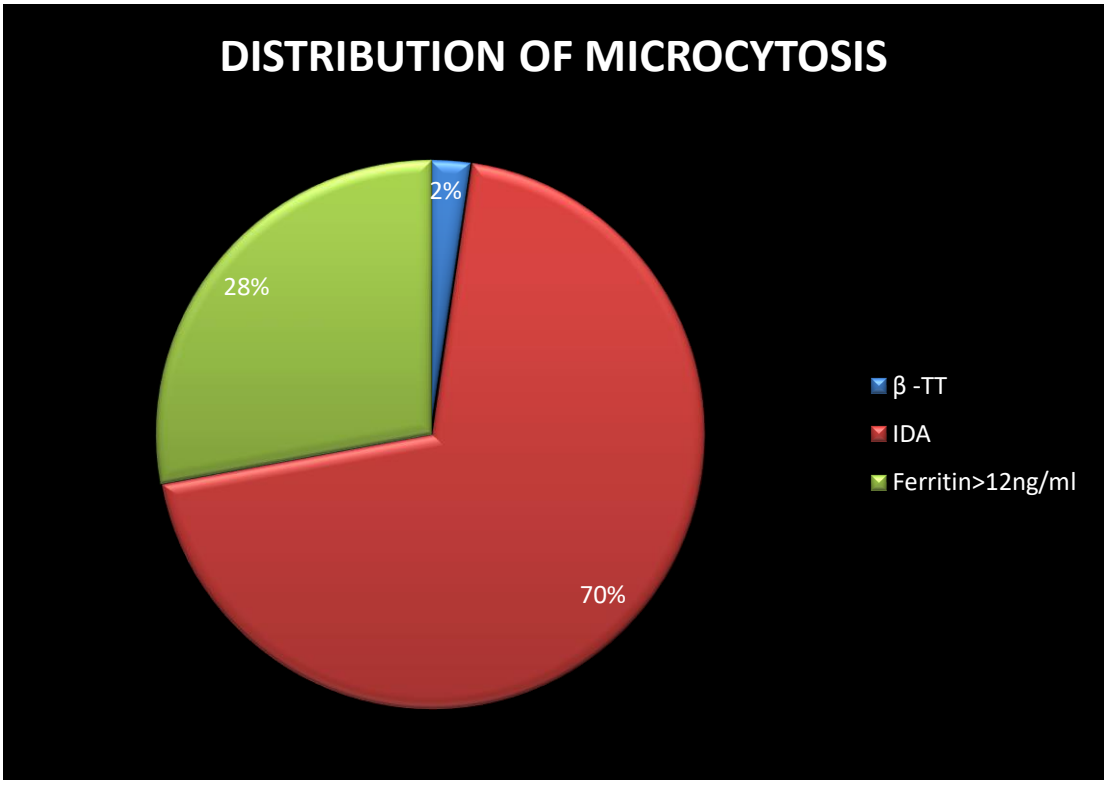
Parameters	MCV <80fl	MCV ≥ 80 fl
Number and percentage	43 (28.7%)	107 (71.3%)

FIGURE 21 DISTRIBUTION OF SAMPLE BY MCV



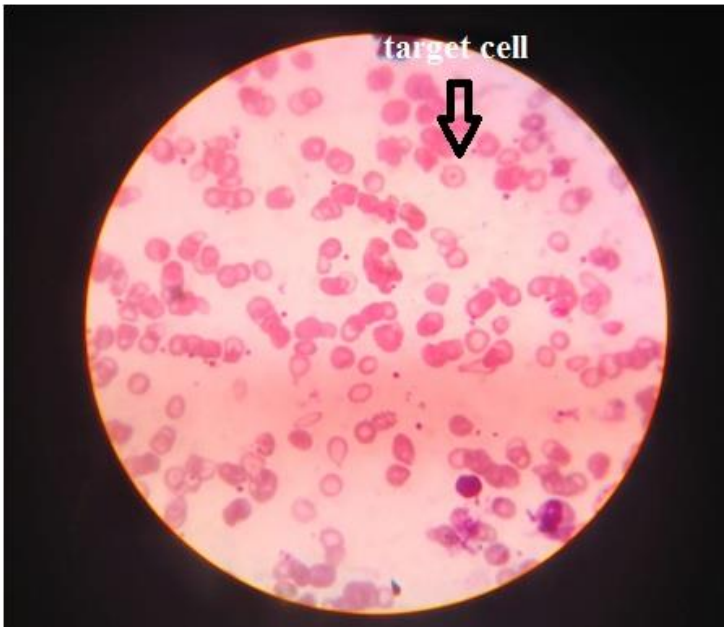
43 persons (28.7%) had microcytosis in study population.

FIGURE 22 DISTRIBUTIONS OF PERSONS WITH MICROCYTOSIS



Out of 150 persons, 43 persons (28.7%) showed microcytosis. In this group **12 (27.9)** persons had ferritin >12ng/ml. **1** person (2.3%) had β-TT. **29** persons (69.7%) had IDA. **1** person had β-TT with coexistent IDA.

FIGURE 23 TARGET CELLS IN β-THALASSEMIA TRAIT

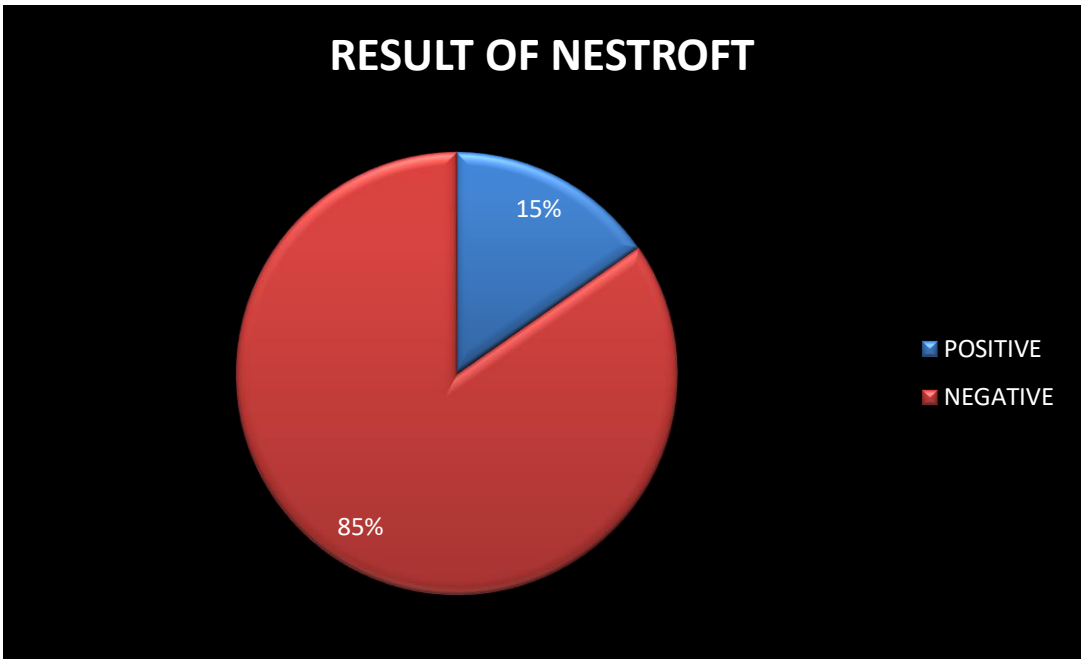


43 persons showed microcytosis in peripheral smear. Many of them had target cells.

TABLE 10 RESULT OF NESTROFT

NESTROFT	POSITIVE	NEGATIVE
NUMBER	23	127

FIGURE 24 PERCENTAGE OF NESTROFT POSITIVE AND NEGATIVE RESULT



23 persons (15%) of sample showed positive reaction to NESTROFT.11 persons (7.3%) even though they had normal size RBCs, they also showed positive reaction.

TABLE 11 CHARACTERISTICS OF NESTROFT POSITIVE RESULT

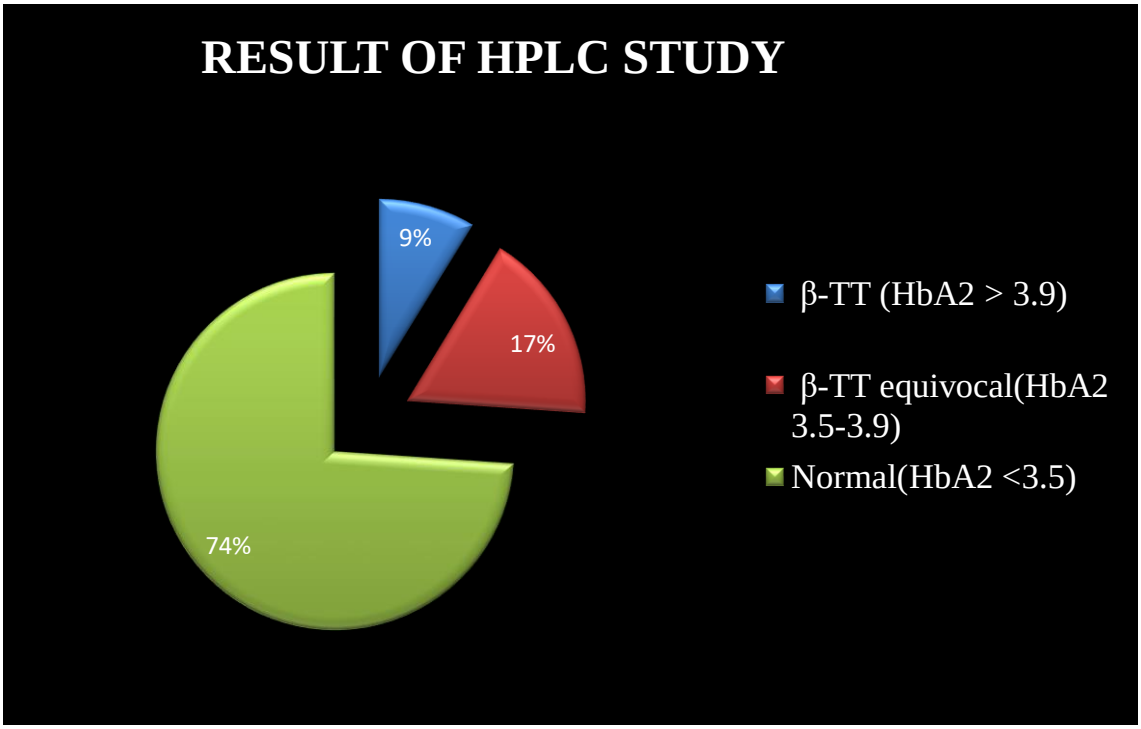
Parameter	NESTROFT POSITIVE	NESTROFT POSITIVE
MCV	12(<80)	11(≥ 80)
Ferritin	8(<12ng/ml)	15(≥ 12ng/ml)

Among NESTROFT POSITIVE Persons, 12 persons (52.17%) with microcytosis showed positive result to NESTROFT. In this group, 8 persons had iron deficiency anaemia. 11 persons even though they are not iron depleted and microcytic, they showed positive reaction.

Table 12 RESULTS OF HPLC STUDY (IN NESTROFT POSITIVE PERSONS)

HPLC result	β-TT (HbA_2 >3.9%)	β-TT equivocal (HbA_2 3.5-3.9)	Normal (HbA_2 <3.5)
Number	2	4	17

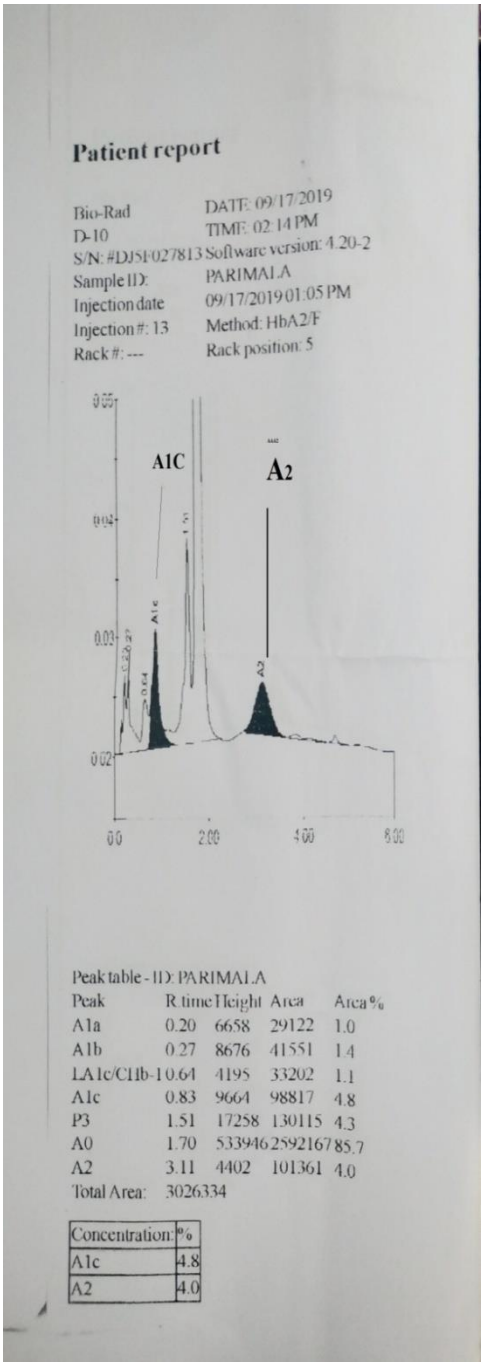
FIGURE 25 RESULTS OF HPLC STUDY



2 persons showed HbA_2 >3.9 % (beta thalassemia trait). 4 persons showed HbA_2 between 3.5 -3.9%.17 persons showed HbA2 below 3.5%

FIGURE 23 AND FIGURE 24 RESULT OF BETA THALASSEMIA TRAIT

FIGURE 23



Patient report

Bio-Rad
D-10
S/N: #DJSI-027813
Sample ID:
Injection date
Injection #: 13
Rack #: ---

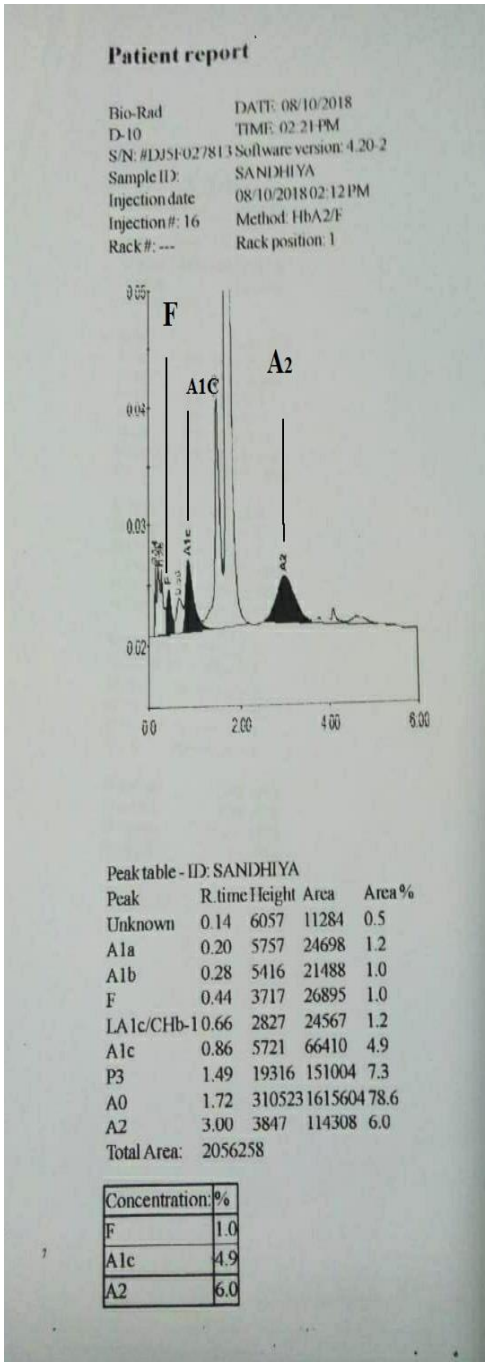
DATE: 09/17/2019
TIME: 02:14 PM
Software version: 4.20-2
PARIMALA
09/17/2019 01:05 PM
Method: HbA2/F
Rack position: 5

Peak table - ID: PARIMALA

Peak	R.time	Height	Area	Area %
A1a	0.20	6658	29122	1.0
A1b	0.27	8676	41551	1.4
LA1c/C1b-1	0.64	4195	33202	1.1
A1c	0.83	9664	98817	4.8
P3	1.51	17258	130115	4.3
A0	1.70	533946	2592167	85.7
A2	3.11	4402	101361	4.0
Total Area:			3026334	

Concentration:	%
A1c	4.8
A2	4.0

FIGURE 24



Patient report

Bio-Rad
D-10
S/N: #DJSI-027813
Sample ID:
Injection date
Injection #: 16
Rack #: ---

DATE: 08/10/2018
TIME: 02:21 PM
Software version: 4.20-2
SANDHIYA
08/10/2018 02:12 PM
Method: HbA2/F
Rack position: 1

Peak table - ID: SANDHIYA

Peak	R.time	Height	Area	Area %
Unknown	0.14	6057	11284	0.5
A1a	0.20	5757	24698	1.2
A1b	0.28	5416	21488	1.0
F	0.44	3717	26895	1.0
LA1c/CHb-1	0.66	2827	24567	1.2
A1c	0.86	5721	66410	4.9
P3	1.49	19316	151004	7.3
A0	1.72	310523	1615604	78.6
A2	3.00	3847	114308	6.0
Total Area:			2056258	

Concentration:	%
F	1.0
A1c	4.9
A2	6.0

Firure 23 showed HbA_2 of 4%. She also had iron deficiency anaemia.Her husband showed equivocal result.Figure 24 showed HbA2 of 6%. Her husband was not willing for investigation.

TABLE 13 PREVALENCE OF BETA THALASSEMIA TRAIT, IRON DEFICIENCY ANAEMIA IN STUDY POPULATION

β-TT (No and %)	**Coexistent β-TT with IDA (No and %)**	**β-TTequivocal HbA_2 (3.5-3.9) (No and %)**	**IDA (No and %)**	**Microcytosis (others)**
1(0.7%)	1 (0.7%)	3(2%)	30(20%)	12(8%)

Out of 150 persons, 2 persons (1.3%) had beta thalassemia trait. (In this 2 person, 1 person had coexistent iron deficiency anaemia).5persons (3%) showed value of HbA2 between 3.5%-3.9% (1 persons had coexistent IDA). 29 persons (19.3%) had Iron deficiency anaemia.

TABLE 14 LAB PARAMETERS IN PERSONS WITH β- TT

Lab parameters	Person 1	Person2	Mean
Hb (g/dl)	9.8	5.1	7.45
RBC count(X 10^6/µl)	4.12	3.3	3.71
MCV (fl)	82	64	73
MCH(pg)	26	15	20.5
RDW-CV%	14	20.3	17.15
NESTROFT	Positive	Positive	-
Ferritin (ng/ml)	13.2	6.01	9.6
$HbA_{2\ (\%)}$	6	4	5

TABLE 15 LAB PARAMETERS IN PERSONS WITH β -TT EQUIVOCAL RESULT ON HPLC (HBA_2 BETWEEN 3.5-3.9)

Lab parameters	Person 1	Person 2	Person 3	Person 4	mean
Hb (g/dl)	10.5	8.9	12.4	12.3	11
RBC count (x10^6/µl)	4.52	3.63	4.6	4.31	4.3
MCV (fl)	77	79	78	79	78.3
MCH (pg)	23	25	26	29	25.8
RDW-CV%	17.5	13	15.3	15	15.2
NESTROFT	Positive	Positive	positive	Positive	-
FERRITIN (ng/ml)	7.34	12.2	23.81	14.07	14.4
HbA_2 (%)	3.8	3.6	3.7	3.6	3.7

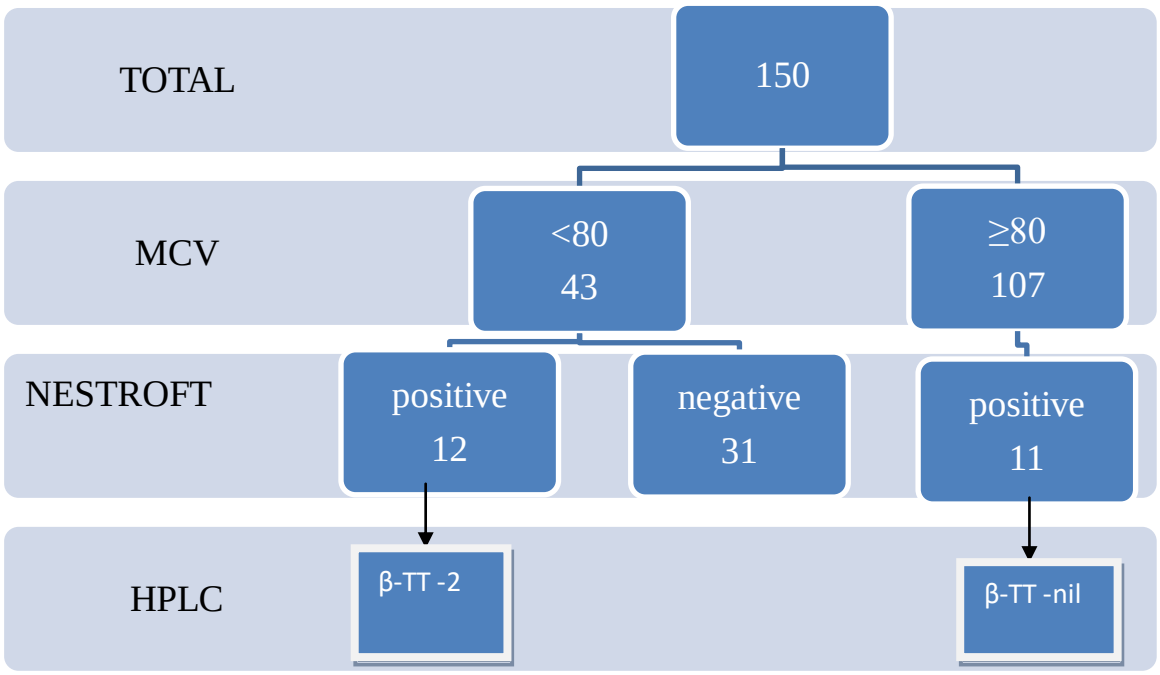
TABLE 16 COMPARISON OF LAB PARAMETERS IN IDA AND B-THALASSEMIA TRAIT

Lab parameters	β-TT	IDA
Hb (g/dl)	7.5	8.8
RBC count (x 106/µl)	3.71	4.02
MCV (fl)	71	73.45
MCH (pg)	19.5	21.61
RDW (%)	17.15	17.53
Ferritin (ng/ml)	9.6	8.83

TABLE 17 COMPARISON OF HEMATOLOGICAL PARAMETERS OF β-TT WITH NORMAL SUBJECTS

Hematological parameters	β –thalassemia trait mean (no-2)	Normal subjects Mean (no-107)
Hb (g/dl)	7.5	10.9
RBC count ($X/10^6$/µl)	3.7	4.0
MCV (fl)	73	87
MCH (pg)	20.5	27
RDW –CV (%)	17.2	14.7

FIGURE 24 PROCEDURES AND RESULT OF THE STUDY



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Serum Ferritin measurement was used to rule out iron deficiency anaemia.

DISCUSSION

The first multicentre study for β-thalassemia trait was done by the Indian Council of Medical Research in the mid 1980s where high school children from Mumbai t, Delhi and Kolkata were studied. In this study the prevalence of β-thalassemia trait was 2.7 % in Mumbai, 5.5 % in Delhi and 10.2 % in Kolkata. However, a follow-up of carriers done about 20 years after screening in Mumbai found that counselling children at the school going age did not have the desired impact. They forgot about their carrier status when they reached their adulthood (65).Antenatal period is the most receptive period for counselling and they will listen to the wellbeing of their child.. If the carrier state is identified, it is possible to do prenatal test and followed by termination of pregnancy.

There are various screening programmes available in India -mass screening, cascade screening, antenatal screening. The type of screening programme depends on prevalence in the region.

To our knowledge there is limited study available to know the prevalence of β-TT in antenatal women in Tamilnadu. This hospital based study was conducted with the aim to determine the prevalence in them.

Iron deficiency anaemia(IDA) and β thalassemia trait (β-TT) represent the most common causes of microcytic hypochromic anaemia. As suggested by Ferrara et al, a full clinical history, determination of red blood cell (RBC) indices by electronic cell counter, serum ferritin measurement in screened

subjects with microcytosis and/or hypochromia was carried out. The next step was the diagnosis of β-TT by quantitation of HbA_2 (36).

In the present study, hundred and fifty antenatal women were selected for β-tt over a period of 1 year and their complete hemogram was checked immediately and NESTROFT was done in all samples. The samples which showed positive for NESTROFT and MCV<80fl and MCH< 27pg were further checked for HbA_2 using CE-HPLC to confirm β thalassemia trait.

A total of 150 antenatal women were screened.43 persons (28.7%) had microcytic hypochromic anaemia. In this group 2 persons (4.7% of microcytic hypochromia) had beta thalassemia trait .30 persons (69.8%) had iron deficiency anaemia.

Study conducted by Raghavan et al showed that NESTROFT had sensitivity (95.5%) and specificity (87%). In the present study NESTROFT was positive for 23persons, of which only 2 antenatal women showed HbA_2 >3.9% (beta thalassemia trait), 7 persons with iron deficiency anaemia also showed positive reaction, but their HbA_2 levels were within normal limits. Hence it had false positive value of 91.3%.But NESTROFT was superior to MCV and MCH. Mamtani et al also observed similar findings in their study. They mentioned that even though NESTROFT missed 7% of beta thalassemia trait cases; it was superior to MCV and HbA_2. Study by Singh and Gupta et al showed that NESTROFT (0.36% buffered saline) with a very high negative

predictive value (90.9%).Taking this into account, HPLC analysis was done for NESTROFT positive samples.

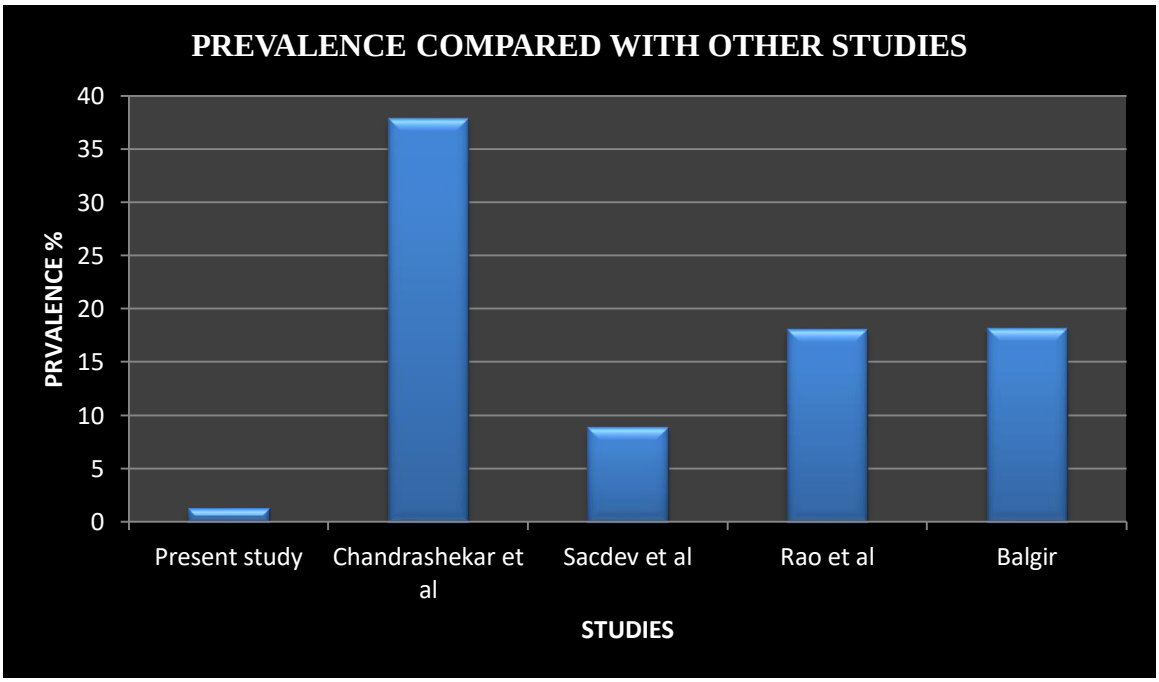
Table 15 shows β-TT equivocal results of 4 persons (HbA_2 3.5-3.9).Since the persons with β-TT with equivocal result also showed positive reaction to NESTROFT, they can be considered as having beta thalassemia trait as many studies suggesting the HbA_2 level of >3.5%.However guidelines on the prevention and control of hemoglobinopathies in India - thalassemias, sickle cell disease and other variant hemoglobins by Ghosh et al clearly stating that before declaring the equivocal result as beta thalassemia trait, detailed evaluation is needed. (53,66). Husbands of women with HbA_2 between 3.5-3.9 were not willing for HPLC and molecular analysis.The patients with borderline HbA_2 levels that could not be explained by iron status, family history require molecular analysis. Molecular analysis is the final confirmatory diagnosis and this was not carried out in our study as the antenatal women were not willing.

Iron deficiency anemia reduces HbA_2level .In the present study, one person with beta thalassemia trait equivocal had ferritin value of 7.34.This person might had β-TT as there was a chance of reduced HbA_2 level due to presence of iron deficiency anaemia. Iron therapy influencing HbA2 level was observed by Usman et al in their study by doing HbA_2 analysis before and after iron therapy.(7)

The present study showed prevalence of 1.3% in antenatal women. Guidelines on prevention and control of hemoglobinopathies (India) -2016 showed overall prevalence of β thalassemia in Tamilnadu is 1-3%.

Comparison with other studies

Figure 25 Prevalence of beta thalassemia trait reported by different authors



Balgir conducted a study in anaemic patients referred from peripheral hospitals, Chandrasekhar et al, Sacdev et al and Rao et al conducted hospital based studies on samples referred for Hb variant analysis in HPLC .This present study was done on antenatal women attending Stanley medical college, Chennai for their routine check up.

We found out RBC parameters and NESTROFT test are not reliable parameters to detect carrier status in this present study. It is best to screen all antenatal women using HPLC.

Thalassemia prevention program is the need of the hour in India. The approach to deal with the thalassemic problem is to prevent and control the birth of the new cases

For example in Cyprus, Italy, USA, and recently UK and other parts of Europe and Africa, proper implementation of measures regarding thalassemia caused a marked reduction in the incidence and prevalence of the disease. A notable example is Cyprus where the incidence has dropped by 96%. Also premarital screening to identify carrier couples and subsequently provision of counselling in Iran has resulted in a 70% reduction in the annual birth rate of affected infants and a large amount of medical expenses.

Implementation of mandatory national premarital screening programme, and screening young and unmarried women for detection of carriers have strikingly reduced the incidence of infants born with thalassemia major in several countries worldwide.

Thalassemia trait Carrier screening by antenatal screening followed by prenatal diagnosis and medical termination of pregnancy is acceptable to our communities. This approach is safe without significant maternal, fetal and social adverse events. Screening program can be made successful in India by increasing awareness not only among population but also among health care

professionals so that they can offer screening test on correct time and can improve detection rates.

CONCLUSION

The prevalence of beta thalassemia trait in antenatal women 1.3% and it is a significant problem when considering the possibility of birth of child with thalassemia major child and the cost needed for the treatment of that child. We suggest that universal screening of all antenatal women by HPLC is mandatory to prevent the birth of thalassemia major child. Awareness creation, education of health workers, antenatal women and women of reproductive age groups should be implemented to prevent the birth of thalassemia child.

LIMITATION OF THE STUDY

1. The study included small sample size
2. Ferritin analysis was done for microcytic hypochromic anaemic cases and HPLC analysis was done for NESTROFT positive cases.
3. DNA analysis was not done for confirmed cases and persons with β-TT equivocal results.

FUTURE SCOPE

1. Study on HPLC analysis in all antenatal women.
2. Study on Total Negative predictive (TNP) value, positive predictive (TPP) value of NESTROFT based on HbA_2, iron studies, molecular analysis.
3. DNA analysis in thalassemia trait and with β-TT with equivocal result (HbA_2 between 3.5-3.9)
4. Study before and after iron therapy to know the change in HbA_2 level

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ANNEXURE

Department of Biochemistry

Govt Stanley Medical College, Chennai

Prevalence of Beta Thalassemia trait among Antenatal women attending

A Tertiary care center

PROFORMA Ref No: /

1. NAME: 2.AGE 1. <15 2.15-30 3 .31-45

3. SEX 1-M/ 2-F/0-child 4.a.OP No:

5. OCCUPATION: 1. working/ 2. not working

6. EDUCATION: 1.educated 2.illetrate

7. INCOME:1.4860 and above 2.2406-4859 3.1424-2405 4.737-1423 5.<736

8. RELIGION:1. H/2.M/3.C/4.others

8. RESIDENCE & LANDMARK

9. PHONE NO: 1. 2.

10.a. LMP 10b.EDD

11. a.Gravida

b.Para

c. Live birth

d. abortion

PRESENT HISTORY

12. H/O palpitation 1.YES2.NO

13. H/O fatigue 1.YES2.NO

14. H/O giddiness 1.YES2.NO

15. H/O chest pain 1.YES2.NO

16. H/O dyspnoea 1.YES2.NO

17. H/O headache 1.YES2.NO

18. H/O loss of appetite 1.YES2.NO

19. H/O pallor 1.YES2.NO

20. H/O abdominal swelling 1.YES2.NO

PAST HISTORY

21. H/O blood transfusion

22. H/O blood loss-hemorrhoids

23. DM-duration

Drugs

24. HT-duration

Drugs

25. Epilepsy-Duration

Drugs

PERSONAL HISTORY

26. Diet- 1. Veg / 2. Non-veg

If 1 : Veg-meals-rice/chapatti

If 2 : Nonveg/week/ per Head Consumption

27. Smoking 1.Yes/ 2. No. if a details:

28. Alcoholism1.Yes/ 2.No if a details:

MENSTRUAL HISTORY

29. a Cycle, b. Duration.c.Passing clots

MARIETAL HISTORY

30. 1.**Consanguineous** marriage/ nonconsanguinous 2. degree of consanguinity

DRUG HISTROY:

31. Antiepileptic-type/drugs/duration/compliance

32. ART Drugs/duration

FAMILY HISTORY

33. H/O hemoglobinopathy: a. anaemia

b.frequent blood transfusion

c. Jaundice

d.Organomegaly

Pedigree chart

GENERAL EXAMINATION:

34. Nourishment: a.well-nourished b.moderate c.under nourishment

35. Pallor : 1. +/ 2.-

Conjunctiva/nails/ tongue- papillae

36. Jaundice: 1.+/2.-

Conjunctiva

37. Generalised lymphadenopathy:1.+/2.-

38. Pedal oedema:1.+/2.-

Unilateral /bilateral

Pitting/non pitting

39. Facial features:

40. Height:

41. Weight:

42. BMI:

<u>VITALS:</u>

43. PULSE

Rate:
Rhythm:
Volume:

44. BLOOD PRESSURE : mm/Hg

45. TEMPERATURE:

46. Oral:

47.<u>SYSTEMIC EXAMINATION:</u>

a.CARDIOVASCULAR	S1: S2:
	Murmur
b.Respiratory	NVBS
	Added sounds
c.abdomen	Fundal height
	FHS +/-
	HEPATOMEGALY-size
	SPLENOMEGALY, size

d.**CENTRAL NERVOUS SYSTEM:**

MOTOR SYSTEM:

SENSORY SYSTEM:

CRANIAL NERVE EXAMINATION:

REFLEXES

48.PROVISIONAL DIAGNOSIS:

49. USG FINDINGS

50. Laboratory Tests

a.CBC-WBC		MCV	RDW-SD	NRBC≠
RBC		MCH	PDW	NRBC%
Hb		MCHC	MPV	
Hematocrit		PLT	PCT	
N≠	E≠	N%	E%	
L≠	B≠	L%	B%	
M≠		M%	IG%	

Flags

RBC

WBC

PLT

b.peripheral Smear

c. NESTROFT test: a. Negative / b.Positive

52. HPLC - a.Conc b.area c.retention time

HbA1c

HbA0

HbA2

HbF

HbS

Others

53. Serum - Ferritin, urea, creatinine, bilirubin

54. Urine - Porphobilinogen

பங்குதாரரின் ஒப்புதல்

ஆய்வு : மரபு வழியாக வரும் ரத்த சிவப்பு அணுக்கள் குறைபாடு (ஹீமோகுளோபினோபதி) தொடர்பான ஆய்வு

பெயர் : வயது / பாலினம் O.P. No.

நான் இதன் மூலம் உறுதியளிப்பது என்னவெனில் இந்த பரிசோதனை பற்றி அனைத்தும் எனக்கு தமிழில் விளக்கப்பட்டது. இந்த ஆய்வு பற்றி எழுந்த சந்தேகங்களுக்கு எனக்கு விளக்கம் அளிக்கப்பட்டது. நான் இந்த ஆய்வில் எனது சுயவிருப்பத்துடன் கலந்து கொள்கிறேன். மற்றும் நான் இந்த ஆய்வில் இருந்து எப்பொழுது வேண்டுமானாலும் எந்தவித முன் அறிவிப்பும் இன்றி விலகிக்கொள்ளவும் எனக்கு முழு சுதந்தரம் கொடுக்கப்பட்டுள்ளது. மற்றும் இதில் எந்தவிதமான சட்டச்சிக்கலும் இல்லை என்பதும் எடுத்துரைக்கப்பட்டது.

இந்த ஆய்வுக்காக எனக்கு ரத்த பரிசோதனை செய்து கொள்ள சமதிகிறேன் இந்த பரிசோதனை மூலம் வரும் முடிவுகளை கல்வி சம்பந்தப்பட்ட ஆராய்ச்சிக்காகவும், முதுகலை படிப்பிற்கான ஆராய்ச்சிக்காகவும் இவர்கள் பயன்படுத்திக்கொள்ள நான் முழுமனதுடன் சம்மதிக்கிறேன்.

எனவே நான் எனது மனப்பூர்வமான சம்மதத்துடன் இந்த பரிசோதனையில் பங்கு பெறவும், பரிசோதனை பற்றிய முடிவுகளை இவர்கள் பயன்படுத்திக் கொள்ளவும் சம்மதிக்கிறேன்.

பங்குபெறுபவரின் கையெப்பம் Investigator ‘s கையெப்பம்

தேதி

தகவல் படிவம்

ஆய்வு : மரபு வழியாக வரும் ரத்த சிவப்பு அணுக்கள் குறைபாடு (ஹீமோகுளோபினோபதி) தொடர்பான ஆய்வு

மதிப்பிற்குரிய ஐயா/ அம்மையீர்,

உங்கள் விருப்பத்தின் பேரில் மகப்பேறு காலத்தில் ஹீமோகுளோபினோபதி (மரபு வழியாக வரும் இரத்த சிவப்பு அணுக்கள் குறைபாடு தொடர்பானது) நோய்க்கான மரபணுக்களை கொண்டு இருப்பவர் தொடர்பான ஆய்வு பற்றிய ஆய்வில் பங்கேற்கும்படி அன்புடன் கேட்டுக்கொள்கிறோம். இந்த ஆய்வில் ஆராய்ச்சி நோக்கத்திற்காக தாங்கள் பரிசோதனைக்கு உட்படுத்தப்படுவீர்கள். ஆய்வின் முடிவில் தங்களுக்கு ஏதேனும் மேற்குறிய குறைபாடு இருப்பது உறுதிசெய்யப்பட்டால், தங்களுக்கு அதற்குரிய அறிவுரையும், வழிகாட்டுதலும், வழங்கப்படும். தங்களுக்கு இந்த ஆய்வில் பங்கேற்க விருப்பம் இருந்தால் தங்கள் அருள்கூர்ந்து ஒப்புதல் படிவத்தை படித்துப்பார்த்து கையொப்பம் இடும்படி கேட்டுக்கொள்கிறேன்.

சாட்சிகள் கையொப்பம்

1)

2)

GOVT. STANLEY MEDICAL COLLEGE, CHENNAI - 600001

INFORMED CONSENT

PREVALENCE OF THALASSEMIA TRAIT AMONG ANTENATAL WOMEN ATTENDING A TERTIARY CARE CENTRE

Place of Study: Govt. Stanley Medical College, Chennai

I.....have been informed about the details of the study in my own language.

· I have completely understood the details of the study

· I am aware of the possible risks and benefits, while taking part in the study.

· I can understand that I can withdraw from the study at any points of time and even then, I can receive the medical treatment as usual.

· I understand that I will not get any money for taking part in the study.

· I will not object if the results of this study are getting published in any medical journal, provided my personal identity is not revealed.

· I know what I am supposed to do by taking part in this study and I assure that would extend my full cooperation for this study.

Volunteer :

Name and Address :

Signature :

Date :

Investigator :

Signature and Date :

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
1	222068	21	2	1	1	3	4.24	10.3	77	24	15.2	1	7.35	2.9	0.8
2	509	22	2	1	2	3	4.73	13.6	86	29	14.4	2			
3	224145	30	2	3	3	3	4.98	13.5	84	27	16.9	2			
4	223678	24	1	2	2	1	4.11	9.8	78	24	14.8	1	6.61	2.8	0.8
5	224175	24	2	1	2	2	3.77	10.3	85	27	14.4	1	12.42	2.7	0.9
6	220725	22	1	1	2	2	4.17	11.3	82	27	13.1	1	13.42	2.6	0.7
7	220722	25	1	1	2	2	4.52	10.5	77	23	17.5	1	7.34	3.8	0.8
8	224135	30	2	1	1	2	3.83	7.4	69	23	17	2	6.5		
9	223343	26	2	1	1	3	3.5	9.8	90	28	15.3	2			
10	224107	38	2	1	2	3	3.24	10.3	90	31	13.8	1	30.5	3.4	
11	213919	30	2	2	1	3	4.62	13.4	85	29	14.4	1	27.65	2.7	
12	27656	20	1	2	1	2	3.55	10	88	28	14.1	1	28.43	2.9	
13	214402	20	2	2	4	3	4.04	12.7	73	23	15.9	2	7.12		
14	223944	34	2	2	3	3	3.94	9	73	22	15.6	2	7.23		
15	225932	26	2	3	3	3	4.14	11.7	86	28	14.8	2			
16	209791	28	2	3	2	3	3.86	10.4	87	26	17	2			
17	221732	23	2	2	2	3	4	10.7	84	27	15.2	2			
18	213504	27	2	1	3	3	3.83	11	87	29	13	2			
19	201246	27	2	1	2	3	3.96	10.7	84	24	13	2			
20	226046	22	2	2	1	3	3.98	9.6	78	24	18.4	2	11.34		
21	225430	18	2	1	1	3	3.98	9	80	27	14	2			
22	206325	23	2	1	2	3	4.12	9.8	80	27	15	2			
23	219511	23	1	2	1	3	4.12	11	80	26	14.2	2			
24	222038	24	2	1	1	3	4.12	9.8	78	24	14	1	13.2	6	
25	212905	30	1	1	2	3	3.72	9.6	84	26	13	2	19.07		

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
26	202541	26	1	2	4	2	3.7	9.9	84	27	13	2	17.92		
27	226070	20	2	1	2	3	3.59	8.4	74	23	16	2	10.2		
28	225497	22	2	2	2	3	3.56	8.1	78	23	15	2	10.3		
29	28842	23	2	1	2	1	4.07	8	66	19	18	2	7.23		
30	225018	23	2	1	2	3	4	10.5	84	26	17	2			
31	28228	26	2	1	1	3	3.39	10.2	95	30	18	2			
32	225103	28	2	1	2	3	4.45	11.9	88	26	18	2			
33	226504	20	1	2	1	3	4.27	8.4	74	19	22	2	9		
34	226484	20	2	1	3	3	4.2	8.3	73	19	18	2	8.23		
35	226550	23	1	2	2	3	4.17	10.7	86	26	16.8	2			
36	6649	22	2	2	1	3	5.1	13	90	26	16	2			
37	231370	24	2	3	1	3	4.3	8.5	74	20	22.3	2	7.34		
38	231447	25	2	1	2	3	3.4	7	75	21	16.3	2	8.54		
39	6671	26	2	1	1	3	3.7	8.7	80	24	16.3	2			
40	228192	18	2	2	1	3	3.82	10.98	92	29	14.4	2			
41	6673	26	2	1	2	3	3.63	8.9	79	25	13	1	12.2	3.6	
42	220560	22	2	1	2	3	3.65	10.4	89	29	14.4	2			
43	231457	27	2	1	2	3	4.4	11.9	90	27	13.1	2			
44	210932	23	2	1	2	3	4.02	8.7	76	22	16.7	1	7.27	2.9	
45	230924	25	2	1	3	3	3.66	11.1	97	30	14.9	2			
46	230896	22	2	2	1	3	4.46	10.6	83	24	17.9	2			
47	228429	25	2	1	4	2	3.58	9.8	88	27	15.6	2			
48	205863	29	2	1	2	3	5.53	13.5	80	24	13.2	2			
49	230537	27	2	1	1	3	4.03	11.6	93	29	14.3	2			
50	230540	23	2	2	2	3	4.13	9.1	76	22	15.4	2	9.01		

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
51	228127	27	1	3	3	3	4.3	9.8	72	23	16	1	8.78	2.8	
52	242661	31	1	3	3	2	4.7	10.5	69	22	16.6	1	10.26	2.7	
53	243133	24	1	1	3	1	4.5	14.1	87	30	13.3	1	13.78	3.2	
54	242768	28	1	1	2	3	4.1	12.4	86	30	12.8	1	14.89	3.1	
55	241900	23	1	2	1	3	4	12.7	88	30	15	2			
56	240666	31	1	2	2	3	3.4	9.8	94	29	14	2			
57	187195	21	1	1	2	3	3.35	9.1	87	27	14	2			
58	243303	22	1	1	2	3	3.28	9.2	92	28	13	2			
59	243408	21	2	1	2	2	3.68	8.6	81	23	15	2			
60	238114	19	1	1	1	2	3.79	10	86	26	16	2			
61	243210	22	1	3	1	2	4.2	11.2	88	26	16	2			
62	243209	20	1	1	1	3	3.77	11.7	97	31	13	2			
63	227881	24	1	1	2	3	4.32	10.7	81	35	14	2			
64	247190	23	1	1	1	1	3.9	11.5	91	29	14.5	2			
65	243461	24	1	1	1	3	4.3	10.6	79	24	14.3	2	9.71		
66	247337	21	1	1	1	3	4.5	8.8	72	19	17	2	7.94		
67	247256	20	1	1	1	2	4.1	11.3	90	28	19	2			
68	245485	34	2	2	3	3	4.11	11.6	89	28	13.5	2			
69	247208	23	1	1	1	1	4.5	11.9	84	26	16.2	2			
70	243952	20	2	1	1	3	3.9	10.9	95	27	14.5	2			
71	239063	26	1	1	2	3	4.3	13.2	98	30	15	2			
72	247752	29	2	1	3	1	4.5	12.9	91	28	13.5	2			
73	243153	22	1	1	1	3	3.2	12.1	94	28	13.3	2			
74	244392	32	1	1	4	1	3.3	5.1	64	15	20.3	1	6.01	4	
75	248053	21	1	2	1	3	4.2	9.8	81	23	16.9	1	110.7	2.9	

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
76	248059	19	1	1	1	3	3.7	11	95	29	14	1	37.97	3.2	
77	248061	24	1	1	2	2	3.9	11	90	30	24	2			
78	242041	25	1	3	3	3	4.5	10.6	80	23	17.7	1	17.82	2.9	
79	237016	28	1	1	2	3	4.3	11.5	86	26	17.2	2			
80	248071	21	1	1	1	3	4.5	11.5	83	25	18.6	2			
81	255951	32	1	1	2	1	4.6	13.3	89	29	12.9	2			
82	255107	28	1	1	1	2	4.2	11.2	79	24	12.4	2	18.12		
83	253359	20	1	2	1	2	4.01	9.1	73	23	15.9	2	72.95		
84	256061	25	1	2	1	2	4.21	11.9	88	28	15.1	2			
85	256021	20	1	1	2	1	4.2	10.6	78	25	14.4	2	44.23		
86	256081	27	1	1	2	1	3.89	10.4	83	27	15.4	2			
87	245107	36	1	1	7	2	4.12	12.1	86	29	13.4	2			
88	247663	29	1	2	3	2	3.89	10.4	83	27	15.4	2			
89	253531	30	1	1	1	2	3.49	10.3	90	30	17.3	2			
90	254057	21	1	1	2	2	4.04	10.6	80	26	13.6	2			
91	256143	25	1	1	2	1	4.57	9	66	20	22.3	2	96.74		
92	256588	26	1	3	2	2	4.12	12.1	86	29	13.4	2			
93	256600	29	1	1	2	1	4.6	12.4	78	26	15.3	1	23.81	3.7	<0.8
94	244047	22	1	1	2	2	3.7	11.1	79	25	13.6	2	13.54		
95	256587	29	1	1	2	1	3.9	10.7	80	25	14.5	2			
96	256490	29	1	1	1	1	3.9	10.5	86	28	13.9	2			
97	255978	23	1	1	1	1	3.54	10.5	85	28	13.5	2			
98	257188	30	1	1	2	1	3.43	8.4	78	25	14.1	2	12.06		
99	250573	22	1	1	1	3	3.95	10.5	85	27	14.9	2			
100	255106	25	2	1	2	2	3.23	8.7	83	27	13.9	2			

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
101	255864	30	1	1	2	3	4.4	5.7	85	13	13	2			
102	256951	18	1	1	1	2	4.9	5.2	91	11	14	2			
103	245279	19	1	1	1	3	4.8	16	97	34	14	2			
104	256949	28	1	1	1	3	3.21	10.2	96	32	18	2			
105	242721	20	1	1	1	3	4.8	11.3	79	24	16	2	10.06		
106	251641	20	1	1	1	3	4.8	14.2	87	29	15	2			
107	257203	32	1	1	3	3	3.6	5.9	66	16	18.9	2	5.43		
108	257261	20	1	1	1	3	4.07	11.4	89	28	14	2			
109	257299	27	1	2	2	3	3.93	7.2	83	18	18.2	2			
110	257394	22	1	1	1	2	4.3	9.5	75	22	21	2	19.48		
111	255773	29	1	1	3	3	3.46	9.3	85	27	16	2			
112	256842	18	1	2	1	3	4.53	8.1	64	18	27	2	9.45		
113	257451	25	1	1	3	3	3.74	9.3	82	25	14	2			
114	19934	27	1	1	2	3	3.98	11.6	89	29	13.8	2			
115	243289	32	1	1	2	3	3.6	9	79	25	14.6	2	43.41		
116	257375	22	1	1	2	3	4.21	10.8	82	26	14	2			
117	257437	31	1	1	2	3	4.24	11.6	85	27	13.7	2	131.6		
118	254848	25	1	1	1	3	3.36	10.2	92	30	14.1	2			
119	257450	29	1	1	2	3	3.9	10.4	84	27	13	2			
120	257391	22	1	1	1	3	3.81	10.6	86	27.8	13.7	2			
121	257534	23	1	2	1	3	4.31	12.3	79	29	15	1	14.07	3.6	0.8
122	4312	28	1	1	3	3	3.18	9.9	94	31	12.7	2			
123	257369	27	1	1	3	3	3.66	11	89	30	14.3	2			
124	257537	26	1	1	2	3	3.71	7.8	69	21	18.1	1	11.86	2.5	<0.8
125	257538	26	1	2	2	3	3.97	11.2	87	28	13.7	2			

s.no	pin /op no	age	income	religion	gravida	trimester	(x106/µL)	hb	MCV fL	MCH pg	RDW	NESTROFT	FERRITIN ng/ml	HbA2 %	HbF%
126	257510	29	1	3	2	3	4.03	10.3	80	26	17.3	1	12.79	2.5	-
127	254689	18	1	1	1	2	4.03	9.6	88	30	17.3	2			
128	243120	25	1	1	3	3	3.67	8.5	77	23	14.5	2	10.93		
129	254711	21	1	1	1	3	3.9	11.6	90	30	13.2	2			
130	252757	18	1	1	1	3	3.9	11.6	90	30	13.2	2			
131	255541	27	1	2	1	3	3.8	11.4	89	28	13.2	2			
132	251243	35	1	1	3	3	3.7	11.4	85	25	12.7	2			
133	253343	24	1	1	2	3	3.91	8.2	71	21	17	2	9.17		
134	246362	23	1	1	1	3	3.93	11.7	89	30	12.8	2			
135	243508	20	1	2	1	3	3.8	11	81	29	15.3	2			
136	258135	28	1	1	1	3	3.82	9.2	76	24	15.1	2	11.23		
137	256795	32	1	1	1	3	3.99	11.9	90	30	12.3	2			
138	253981	20	1	2	1	3	3.58	9.9	84	28	13.8	2			
139	22346	22	1	1	1	3	3.69	8.8	78	24	18.5	2	11.51		
140	255599	20	1	2	1	3	4.07	9.2	74	23	20.6	2	9.34		
141	257010	25	1	3	3	3	3.73	10.5	88	28	12.2	2			
142	258426	27	3	3	1	3	3.7	10.4	90	28	14.7	2			
143	3658	32	1	1	3	3	4.01	11.7	91	29	13.5	2			
144	6415	30	1	1	2	3	4.07	10.4	84	26	15.9	2			
145	256222	33	1	1	2	3	4.31	11.1	80	26	15	2			
146	258432	21	1	1	1	2	3.64	10.4	84	29	14.4	2			
147	251225	32	1	1	3	3	3.19	9	88	28	13.6	2			
148	258417	23	1	2	1	3	3.38	7.5	76	22	19.6	2	11.43		
149	258440	29	1	1	1	3	3.82	11.3	92	30	13.7	2			
150	15729	23	1	2	3	3	4	11.2	85	28	14.6	2			

KEY TO MASTER CHART:

Socioeconomic class I- ≥7008, II- 3504 – 7007,III- 2102-3503,IV-10512-2101,V-≤1050.

Hindu-1,Muslim-2,Christian-3

Primi -1,multi (2-3)-2,grand multi (≥ 4)-3

NESTROF positive -1, negative -2.