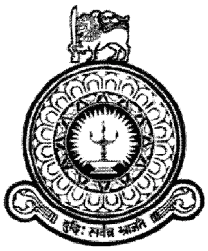

PHENOTYPIC CHARACTERISTICS OF A COHORT OF PATIENTS WITH $-\alpha^{3.7}$ AND $-\alpha^{4.2}$ DELETIONS

BY

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MASTER OF SCIENCE IN CLINICAL GENETICS

AUGUST 2014

CERTIFICATION

I certify that the contents of this dissertation are my own work and that I have acknowledged the sources where relevant.

……

Signature of the candidate

This is to certify that the contents of this dissertation were supervised by the following supervisors:

…….

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ABSTRACT

Introduction

Alpha thalassaemia is caused by defective alpha globin synthesis. Carriers have either one or two defective alpha genes. Deletion / non-deletion mutation that inactivates one of the two α globin genes (i.e., *HBA1* or *HBA2*) on one chromosome (α^+thalassaemia) results in a silent carrier state, of which the phenotype is either haematologically normal(silent) or may have a mildly reduced MCV and MCH, but normal HbA2 and HbF levels. Deletion /inactivation of two α globin genes (--/αα in *cis* configuration or -α/-α in *trans* configuration) results in alpha thalassaemia carrier state (α^0 thalassaemia) haematologically microcytosis [low mean corpuscular volume(MCV)] and hypochromia [low mean corpuscular haemoglobin (MCH)] is seen with normal percentages of HbA_2 and HbF, and RBC inclusion bodies. Three defective genes results in HbH disease which is associated with haemolytic anaemia, hepatosplenomegaly, mild jaundice, and sometimes thalassaemia-like bone changes. The most severe defect is deletion / inactivation of the all four α-globin genes which results in the condition Hb Bart's hydrops fetalis; it clinically manifests as severe anaemia and death in utero / death in the neonatal period. Deletional mutations are commoner but non-deletional mutations have more severe phenotype. The $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions are very common deletions worldwide as well as in Sri Lanka. In this study we describe phenotypes of individuals with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions and looked for an association between the genotype and the phenotype.

Methodology

This descriptive study was carried out at the Human Genetics Unit, Faculty of Medicine, Colombo using patients referred for alpha thalassaemia genetic studies. Clinical details, red

blood parameters haemoglobin (Hb),blood cell count (RBC), MCV, MCH, mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), high performance liquid chromatography(HPLC) haemoglobin quantification data and red cell morphology data were obtained from the clinic records. Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions were done using gap-PCR methodology. Data was analyzed using software SPSS 17.0 and p value < 0.05 was considered as statistically significant

Results

Thirty one individuals carrying $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions were included. Twenty three (74%) were asymptomatic and initially recognized incidentally based on red blood cell parameters. Five (16%)had presented with anaemic symptoms or hepatosplenomegaly. Three (10%) individuals were recruited for family screening. Fifteen (48.4 %) had the $-\alpha^{3.7}/-\alpha^{3.7}$ genotype while the $\alpha\alpha/-\alpha^{3.7}$,$\alpha\alpha/-\alpha^{4.2}$and $-\alpha^{3.7}/-\alpha^{4.2}$ genotypes were observed in 9(29%),5 (16.1%) and 2(6.5%) respectively. Majority (n =26 ,84 %) showed a phenotype compatible with the genotype. Five (16%) showed a phenotype incompatible with the genotype and needs further genetic study.

The MCH was consistently low in all the study subjects(14-25.9 pg, mean 21.65±2.98) Haemoglobin levels had statistically significant difference between different genotypes(p-0.047**). No** statistically significant difference of the red cell parameters or the HPLC data were seen between the different genotypes [RBC (p- 0.161), MCV (p- 0.382), MCH (p- 0.295), MCHC (p- 0.865), RDW (p- 0.956), HbA(p- 0.936), HbA_2 (p- 0.745), HbF (p- 0.799)]. Similarly the presence of different morphological cell types in the blood picture does not vary significantly between genotypes($p<0.05$).

This cohort was further analyzed according to number of defective genes[One defective gene ($\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$) and Two defective genes($-\alpha^{3.7}/-\alpha^{4.2}$ and $\alpha^{3.7}/-\alpha^{3.7}$)]. There was no statistically significant difference in either in RBC parameters and HPLC parameters .The presence of target cells was significantly higher (p- 0.045) in individuals with two defective genes but the presence of other different morphological cell types did not show statistically significant difference between two groups.

Conclusions

Phenotype and genotype correlation of the studied group was similar to that of reported data in the literature. The MCH can be used as an indicator to suspect alpha thalassaemia. Further molecular genetic studies are needed to explain some atypical phenotypes.

1. INTRODUCTION

1.1 General introduction

Thalassaemias are a group of inherited disorders which occur due to defective synthesis of globin chains. The word “thalassaemia” is derived from Greek word”Thalassaa”, which means the sea[1]. It is generally used to describe the conditions causing significant reduction in the rate of globin chain synthesis. It is the commonest single gene disorder in the world and possesses a major public health burden to many countries. Thalassaemia has been identified as a growing global health problem and incidence is rising in some parts of the world due to changing demographical patterns [2]. Management of thalassaemia major, which include long term blood transfusion and iron chelation, remains a challenge. Although haematopoietic stem cell transplant is curative, its use has been limited owing to high cost and lack of HLA matched donors [3].

Thalassaemia is considered a major public health problem in Sri Lanka. Depending on gene frequencies, it is estimated that more than 2000 thalassaemia patients are in Sri Lanka at a given time and management of these patients may consume 5% national health budget[4]. Thalassaemia patients are unevenly distributed in different regions of Sri Lanka. Most of the patients are from Wayamba, North-Central and Uva provinces [4,5]. Despite the availability of almost all management options including regular blood transfusions and expensive iron chelation therapy free of charge, the level of management is sub optimal in a significant proportion of patients [6].

1.2 History of thalassaemia

Thalassaemia was first described in 1925 by Dr. Thomas Cooley, a paediatrician from Detroit and by Dr Pearl Lee. They described a case series of children with Mediterranean origin who presented with severe anaemia, splenomegaly and characteristic bone changes. Around the same time, an Italian physician, Fernando Rietti described a condition similar to Cooley's anaemia but with less severe symptoms. Today it is considered as the first documentation of thalassaemia intermedia. The term "thalassaemia" was first used by George Whipple and Bradford in 1932. In1937 ,Angelini from Italy and in 1938 Caminopetros from Greece described the genetically determined nature of this condition. In 1943 two Italian haematologists, Ezio Silverstoni and Ida Bianco described the pattern of inheritance of this autosomal recessive condition [7].

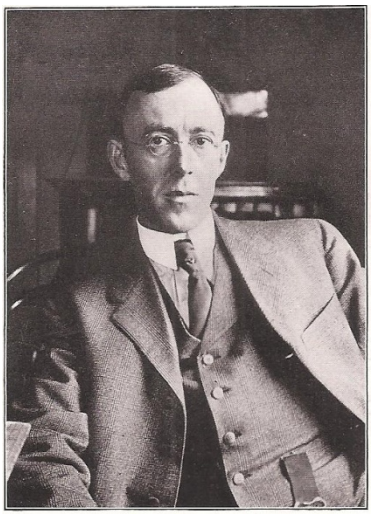

Figure 1: Dr. Thomas Cooley

(Abstracted from Encyclopedia Wikipedia)**[8]**

Figure 2: Dr.Ezio Silverstoni , Dr. Ida Bianco and the team

(Abstracted from WWW.blod.info)[9]

The first report of thalassaemia in Sri Lanka was published in 1951. De Silva and Weeratunga described four Sinhalese children with clinical features suggestive of Cooley's anaemia. Out of four patients described, two of them survived longer and most probably had Thalassaemia intermedia phenotype [10]. Presence of HbE variant was first reported in Sri Lanka several years after the initial report [11]. However HbH disease was an unknown entity in Sri Lanka until 1967 when it was described by Nagarathnam and Sukumaran[12].

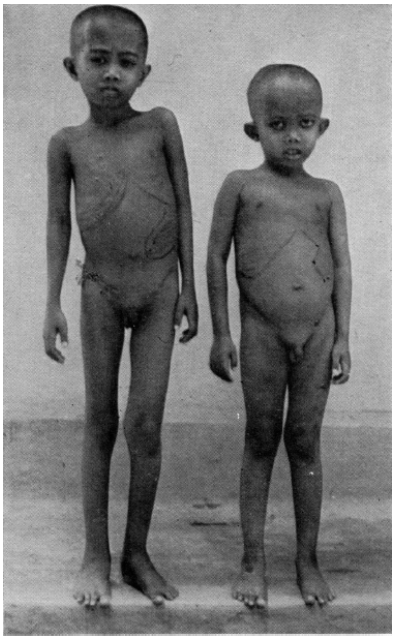

Figure 3: Two of the patients described in the initial report on thalassaemia in Sri Lanka

Abstracted from De Silva CC, Weeratunga CES. Cooley's Anaemia in Sinhalese Children. *Arch Dis Child* 1951;. **26(127):**224–230 [10]

1.3 Erythropoiesis

Erythropoiesis is the process in which multipotent stem cells differentiate in to mature red blood cells [13]. Normal human red blood cell (RBC) has a lifespan about 120 days. About one percent of our RBC is produced each day but this can be increased in response to acute stresses such as acute haemorrhage or to chronic stresses such as chronic haemolysis[14,15].As for any mamal, erythropoiesis in humans occurs successively in the yolk sac, the fetal liver and in the bone marrow[14].

Erythropoiesis is a tightly regulated process by many growth factors and micro-environmental factors[13]. Definitive haematopoietic stem cells (HCS) emerge from the ventral wall of dorsal aorta migrate to the liver by about 60^{th} day of gestation and start fetal blood cell production[16]. These cells are responsible for maintaining and replenishing all blood cell types and they give rise to multipotent , oligopotent and unipotent haematopoietic progenitor cells [17]. With further fetal development, HCS migrate to the bone marrow which is the site for haematopoiesis throughout the postnatal life. Cells derived from HCS, differentiate to form mature red blood cells through erythroid precursors and progenitors[16]**.** Burst forming unit-erythroid (BFU-E) is the earliest progenitors in the erythroid cell line. These slowly dividing cells divide and differentiate in to rapidly dividing colony forming unit-erythroid (CFU-E). This CFU-E divides 3-5 times and differentiates to become erythroblasts. Erythropoietin, secreted primarily by kidneys in response to hypoxia stimulates terminal proliferation and differentiation of CFU-Es [14]. CFU-E will further differentiate through several stages of erythroblasts. Pro-erythroblast stage is the first identifiable stage of the erythroid lineage and then differentiates in to basophilic erythroblasts, polychromatophilic erythroblasts and acidophilic erythroblasts in successive stages. Reticulocytes are formed following the enucleation of acidophilic erythroblasts [13]. Both haem synthesis and globin chain synthesis occur from pro-erythroblast stage to reticulocyte stage [18,19].

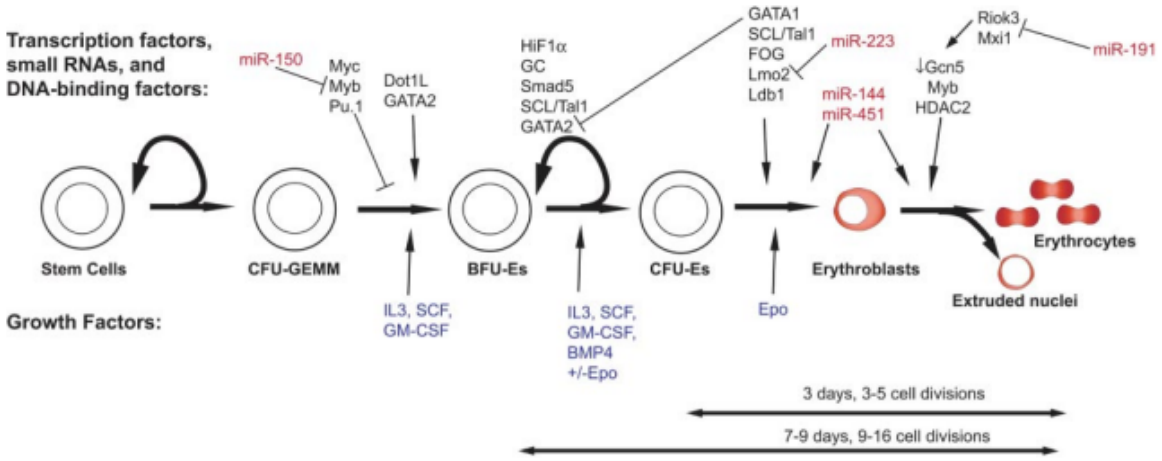

Figure 4: Factors affecting erythropoiesis

Abstracted from Hattangadi SM, *et al* . From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. *Blood.* 2011 Dec 8;118(24):6258-68 [14].

1.4 Structure of the Haemoglobin

The structure of the Haemoglobin molecule was first described in 1960 by Max Perutz and the team[20]. Haemoglobin molecule has four subunits. Each subunit is composed of one peptide and a haem molecule. All the types of haemoglobins share the same haem molecule. Adult haemoglobin(HbA) , is a heterotetramer of two alpha and two beta chains[21].

The structure of Hb molecule can be described at four levels. The primary structure is the sequence of amino acids in the globin chain. Alpha chain has 141 amino acid residues while beta chain has 146 residues. The secondary structure is the arrangement of alpha helixes and non helical parts. Beta globin has eight alpha helical segments designated from A to H and alpha globin lacks the D helix residue. The tertiary three dimensional structure has a haem containing pocket between E and F helices. The ferrous molecule of the heam is linked to a N of a histidine

molecule. The quartenary structure describes the relationship between four globin chains which forms the functioning heterotetramer [15,21].

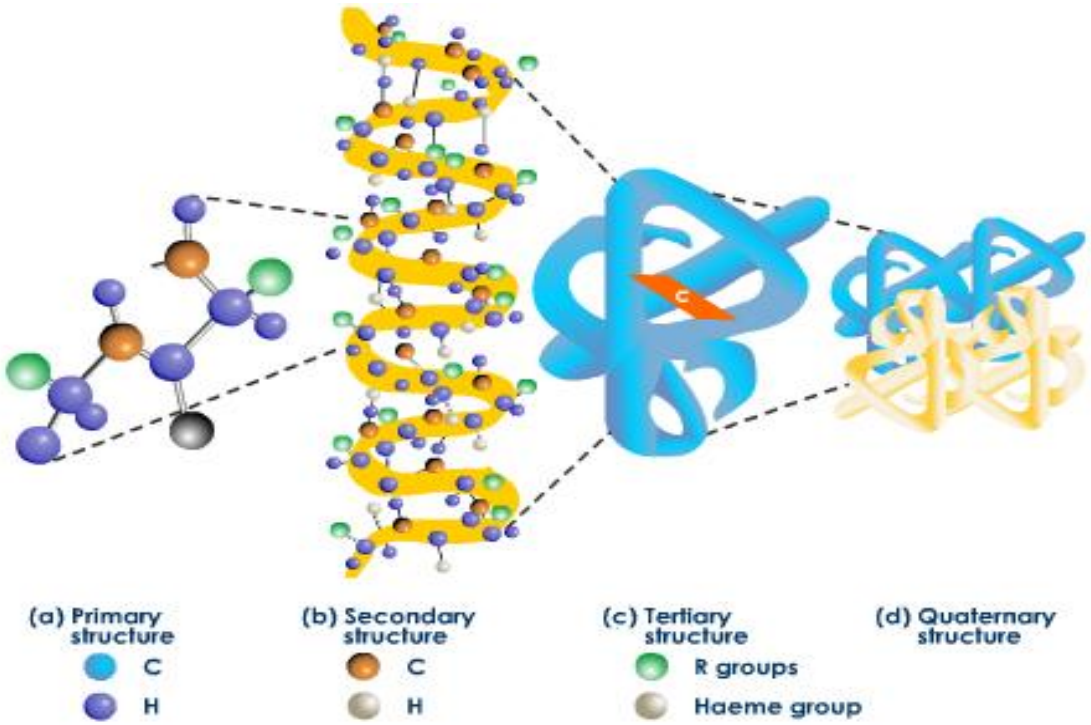

Figure 5: Primary, secondary, tertiary and quaternary structure of the haemoglobin molecule

(Abstracted from kopmanps.wikispaces.com)[22]

1.5 Haemoglobin genes

Human haemoglobin genes and related genes which have originated from a common ancestral gene are scattered over five different chromosomal locations. These genes include alpha and beta erythroid haemoglobins(alpha and beta gene clusters), myoglobin, cytoglobin and neuroglobin[23].

Erythroid haemoglobins are encoded by two different gene clusters. The alpha cluster is mapped to chromosomal location 16p13.3 while beta gene cluster is located to 11p15.4[23]. When evolutionary history is concerned it is believed that alpha and beta genes separated about 500 million years ago and since then they evolved in to two clusters containing several functioning and pseudogenes [24].

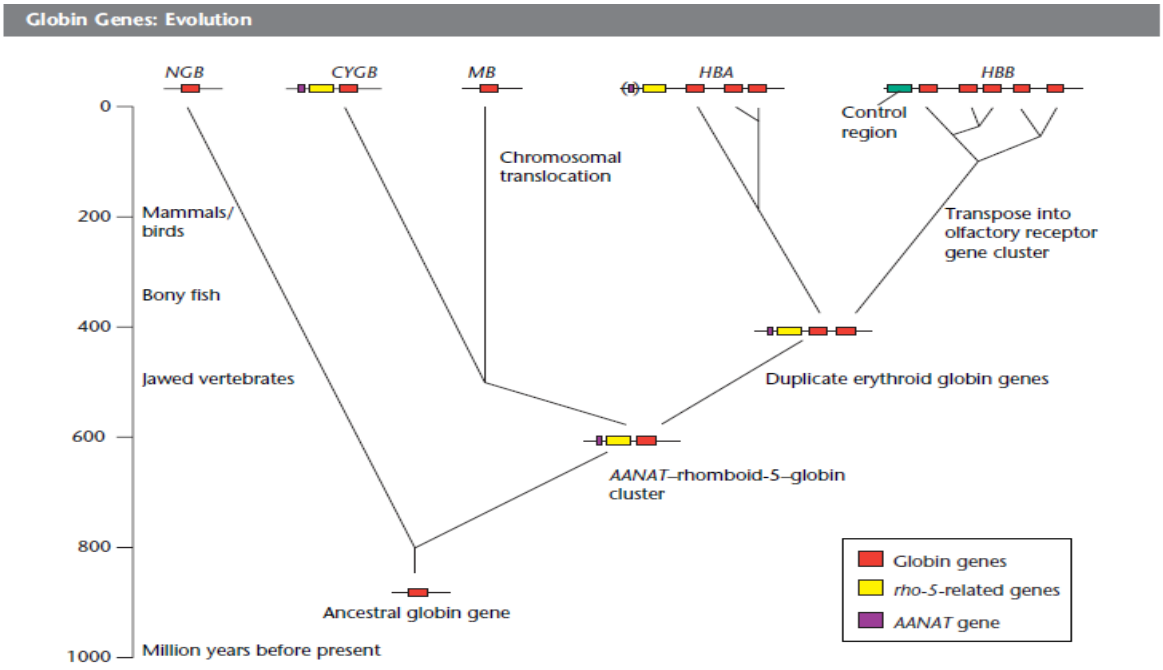

Figure 6: Evolution of the Haemoglobin molecule

Abstracted from Hardisom RC.. Globin gene: Evolution. Encyclopedia of Life Sciences John Wiley & Sons, Ltd 2005 doi: 10.1038/npg.els.0005134[23]

All globin genes in both clusters are arranged in 5' to 3' direction and also in the order of expression during different developmental stages[25]. Alpha gene cluster is close to the telomere of the short arm of the chromosome 16. This cluster is around 28 Kb in size and contains four

pseudogenes and three functional genes. They are arranged in the following order from5' to 3' ; aζ(ζ2) gene, a pseudoζ gene(ψζ1), two pseudo alpha gene (ψα2 and ψα1) ,two α genes(α2 and α1) and θ. Number of α genes per chromosome 16 is usually 2 , but that number ranges from 0 to 4 due to unequal crossing over[26]. The beta gene cluster on chromosome 11 spans 60 kb and contains one pseudogene and five active genes. They are arranged in following order from 5' t0 3'; ε,Gγ,Aγ,ψβ,δ and β. Bets genes has two intervening sequences (IVS) or introns between 30^{th} and 31^{st} codons (IVS-1) and 104^{th} and 105^{th} codons(IVS-2) and these IVSs have 122-130 and 850-900 base pairs respectively. Alpha genes also have the similar arrangement with smaller introns. The two introns, IVS-1 and IVS-2 are situated between 31^{st} to 32^{nd} and 99^{th} to 100^{th} codons respectively[25,26].

In beta cluster , Locus Control Region (LCR) is situated several kilobases upstream the ε gene, constitute of five DNase 1 hypersensitive sites designated from HS1 to HS5. Large deletions affecting this LCR results in DNase 1 resistant and transcriptionaly inactive gene cluster[27]. In the corresponding region of alpha gene cluster four multi species conserved sequence (MCS) regions exists. They are designated from MCS-R1 to MCS-R4. Only MCS-R2 which is also known as HS-40 since it lies 40 kb upstream , has a DNase 1 hypersensitive region and it is the only MCS-R essential for the gene expression[28]. These two regions are essential for the expression of genes of respective clusters. Furthermore several other erythroid specific and non specific transcription factor binding enhancer regions have been identified in both gene clusters. Tissue specific expression can be attributed to presence of binding sites for erythroid specific transcription factors like GATA_1, NF-E2 and EKLF[29].

Three types of embryonal haemoglobins (Hb Gower1, Hb Portland and HbGower2) , one type of foetal haemoglobin type(HbF) and two types of adult haemoglobins (HbA and HbA_2) are produced during different developmental stages as hetero tetramers of two types of globin chains[29,30].

- Hb Gower1- $\zeta_2\varepsilon_2$
- Hb Portland - $\zeta_2\gamma_2$
- HbGower2- $\alpha_2\varepsilon_2$
- HbF – $\alpha_2\gamma_2$
- HbA- $\alpha_2\beta_2$
- HbA2-$\alpha_2\delta_2$

These upstream regulatory elements are believed to be involved in switching between different Hb types in different developmental stage. A dual mechanism of autonomous gene silencing and competition for the direct interaction in upstream regulatory region has been suggested to explain the developmental regulation of globin genes. Activation and suppression of genes is controlled by the process of methylation and unmethylation[29].

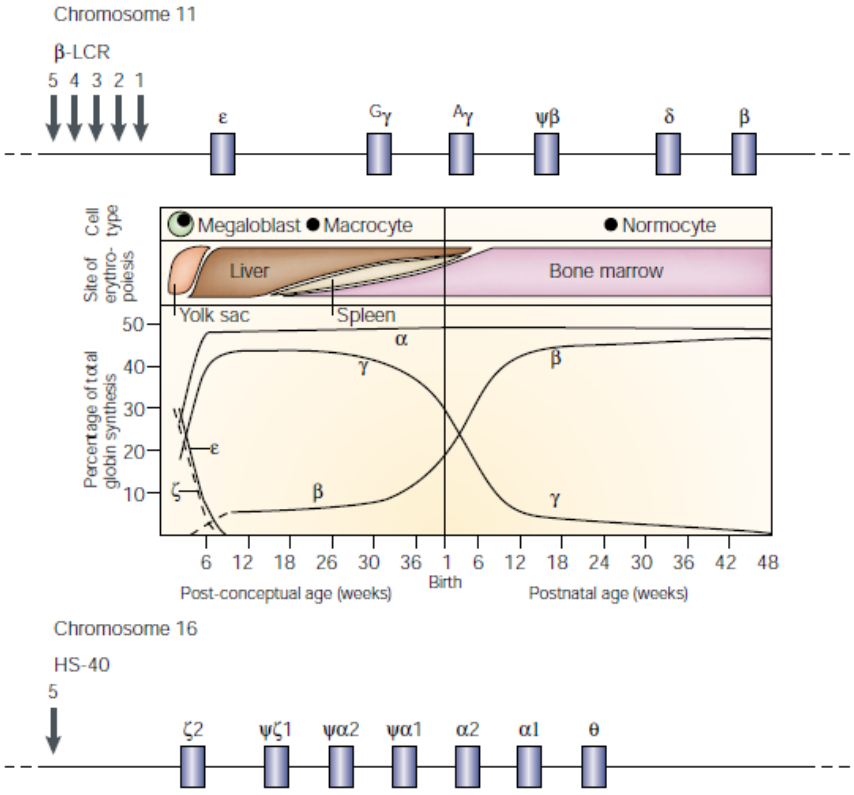

Figure 7: Sequential expression of globin genes

Abstracted from Weatherall DJ. Phenotype–genotype relationships in monogenic disease: lessons from the Thalassaemias. Nature reviews/ genetics **2001;2:** 245-256 **[**30]

1.6 Haemoglobinopathies

Haemoglobinopathies are caused by conditions causing synthesis of structurally abnormal Hb. Mutations in globin genes giving rise to abnormal proteins produce variant haemoglobins while mutations causing altered protein output produce thalassaemia syndromes[7,31]. Conditions causing both reduced rate of synthesis and abnormality in the globin chain structure itself are

known as thalassaemic haemoglobinopathies . Conditions like HbCS and HbE are two examples for thalassaemic haemoglobinopathies affecting alpha and beta chains respectively[15]. Thalassaemic and non thalassaemic haemoglobinpathies collectively constitute the commonest genetic defect worldwide[31]. According to HbVAr database a total of 1609 haemoglobinopathies have been reported and 1190 of them are classified as thalassaemic syndromes while variant haemoglobins accounts for 467. A total of 51 entries have been classified as both thalassaemic and varint Hbs[32].

1.7 Thalassaemias

Thalassaemia is classified according to the globin chain or chains affected. Alpha (α) and Beta (β) thalassaemia are the two commonest forms of thalssaemia caused by reduced or loss of production of α and β globin chains respectively. Several other rare types of thalassaemia such as δβ and εγδβ have also been described [7,15].

1.7.1 Alpha thalassaemia

At phenotypic level, four clinical conditions with increased severity is described. First two conditions, silent carriers and α thalassaemia trait are carrier states. Silent carrier state is caused by the deletion or dysfunction of one α gene (-α/αα). In α thalassaemia trait , only two functional α genes are spared. When α globin chain synthesis is reduced to 25% or less, excess β globin chains leads to formation of β4 tetramers known as HbH. This condition is known as HbH disease. In HbH disease only one α gene remains functional. If α globin chain synthesis is further reduced or completely abolished, it leads to severe intrauterine anaemia and formation of

γ4 tetramers due to excess γ chains. This condition is known as Hb Bart's hydrops fetalis [33].

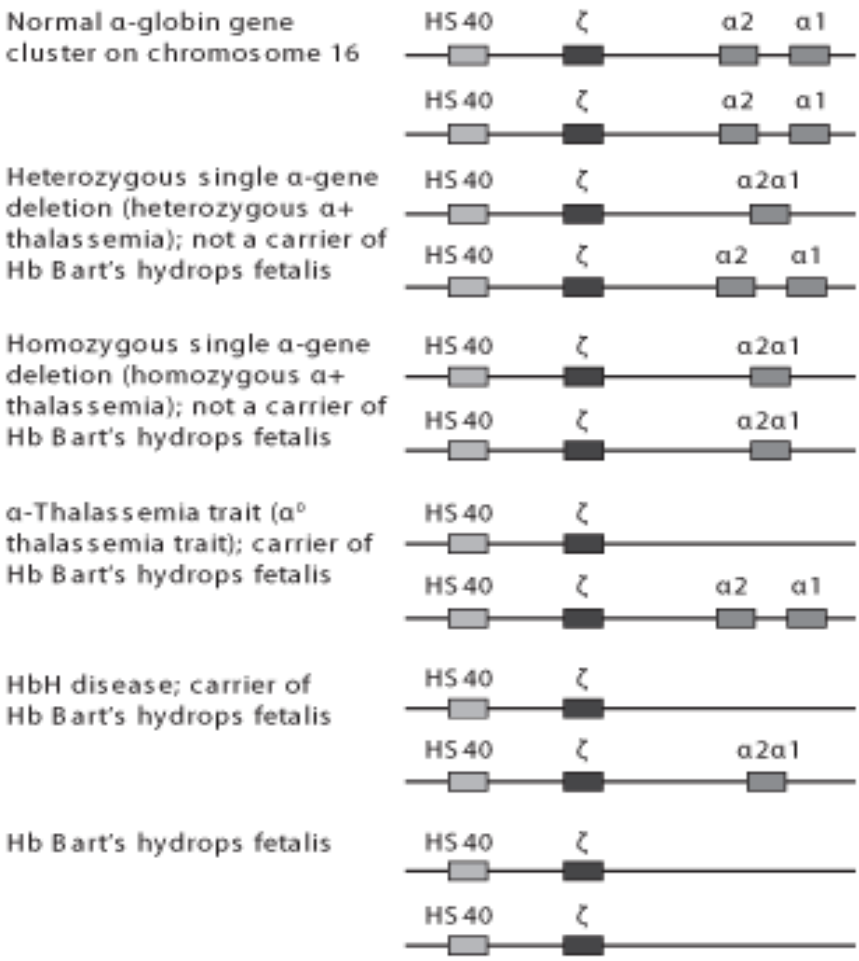

Figure 8: Defective genes in alpha thalassaemia

Abstracted from Lafferty JD, Barth DS, Sheridan BL, McFarlane AG, Halchuk LM, Raby A, Crowther MA,2008. A Multicenter Trial of the Effectiveness of Zeta-Globin Enzyme-Linked Immunosorbent Assay and Hemoglobin H Inclusion Body Screening for the Detection of Alpha0-Thalassemia Trait. Am J Clin Pathol. 129(2):309-315.[34]

- **Silent carriers and alpha thalassaemia trait**

Vast majority of alpha thalassaemia carriers are clinically asymptomatic. Heterozogotes for α^+ thalassaemia (-α/αα) may have either a completely normal blood indices or slightly reduced MCV and MCH with normal HbA2 and HbF levels [33,35]. Their mean Hb level is 1g less than that of individuals with all four α genes. In newborns with single α chain deletion have usually have 1-2% of Hb Bart. However absence of Hb Bart in cord blood does not exclude the possibility of α thalassaemia carrier state [15,33] . In individuals with two functional α genes (alpha thalassaemia trait) either in cis (--/α α) or trans (-α/- α) configuration shows more marked haematological changes similar to those seen in β thalassaemia carriers. Their MCV and MCH levels are lower than that of silent carriers[15,33,35].

- **HbH disease**

In HbH disease , there is only one functioning α gene. It is usually caused by a combination of α^0 mutation and a α^+ mutation [35]. Significantly reduced α chain production and relative excess β chains leads to the formation of highly unstable β tetramers(β4). These tetramers have higher oxygen affinity and tend to precipitate in the RBC when oxidized. They mainly precipitate in older red blood cells and leads to premature destruction in the spleen causing moderate to severe haemolysis [36]. In some nondeletional HbH diseases like Hb Constant Spring and Hb Quang Sze which are more unstable than β tetramers cause further membrane dysfunction and haemolysis[35].

HbH disease is characterized by anaemia with variable amount of HbH which ranges from 0.8% to 40%. Splenomegaly is a usual finding in these patients and some of them have hypersplenism. Jaundice, growth retardation and other complications such as leg ulcers and gallstones may be

present in children and features of iron overload in older patients. Severity of the disease is related to the molecular pathology. Generally nondeletional mutations cause more severe phenotype than deletional mutations [28]. In HbH disease the Hb level usually ranges from 7-10 g/dl , MCV is around 58 fl in childhood and 64 fl in adulthood, and MCH is around 18 pg irrespective of the age[36]. Blood film shows hypochromic microcytosis ,anisocytosis and poikilocytosis with target cells, fragments and teardrop cells. Basophilic stippling is may be there and nucleated red cells are seen rarely[15].

HbH is also considered as the most common form of thalassaemia intermedia. Haemoglobin levels of HbH patients are usually maintained around 9-10 g/dl and they don't need regular transfusions. Iron chelation is also not generally required in HbH disease. However they need blood transfusions in acute haemolytic crisis frequently precipitated by high fever or by infections. There may be rapid drop in Hb levels since cells with HbH are rapidly destroyed. [37]. Aplastic crisis due to parvovirus B 19 infections is another rare but well recognized complication associated with HbH disease[38]. However HbH due to severe non deletional mutations may require regular blood transfusions and iron chelation as for β thalassaemia major patients[39].

- **Hb Bart hydrops fetalis**

Absent function of all four α genes causes this most severe form of alpha thalassaemia. HbF and HbA is not produced. Hb Bart comprise the most of the blood in fetal blood. Small amount of haemoglobin Portland 1 and Portland 2 can also be there. This condition is characterized by very severe intrauterine anaemia causing hepatosplenomegaly , cardiac failure and hydrops fetalis.

HBHF is not compatible with postnatal life and most affected fetuses are stillborn and very few live births die soon after birth. Sometimes congenital abnormalities of cardiac, urogenital and skeletal systems can be associated and maternal complications such as pre-eclampsia, polyhydroamnios, oligohydroamnios and haemorrhage can also be associated [33]. Sometimes when congenital malformations are present, the possibility of Hb Bart's hydrops fetalis may not be considered[40].

Haemoglobin concentration is generally between 3-8 g/dl with markedly reduced MCH and MCHC. MCV in this condition can be normal. Haemoglobin electrophoresis and HPLC usually shows 70-100% of Hb Bart. In blood picture large but markedly hypochromic RBC , anisocytosis and poikilocytosis including target cells and elongated cells can be seen[15].

1.7.2 Beta thalassaemia

Beta thalassaemia (β thalassaemia) is caused by defective β chain production. It can be subdivided in to β^0 thalassaemia in which there is no β chain production and β^+ thalassaemia or β^{++} thalassaemia in which there is severe or mild reduction in β chain production respectively[41]. Beta Thalassaemia major (TM), thalassaemia intermedia (TI) and thalassaemia trait (TT) are the three major clinical phenotypes of β thalassaemia. More than 200 mutations causing β thalassaemia have been described and majority of them are point mutations[1].

- **Beta thalassaemia trait**

In β thalassaemia trait (also known as β thalassaemia minor) which has only one defective gene, is usually clinically asymptomatic. They have slightly reduced or normal Hb levels with

elevated red cell count, reduced Mean corpuscular volume (MCV) and reduced mean corpuscular haemoglobin(MCH). Red cell distribution width (RDW) m and Mean corpuscular haemoglobin concentration (MCHC) is usually within normal range[15]. Although there is a significant difference in haemoglobin concentration, when MCV and MCH values between β^0 and β^+ thalassaemia trait are concerned, there is an overlap[42]. If there is a coexisting α thalassaemia , these changes tend to be minimal[43]. Beta thalassaemia trait can be diagnosed by detecting increased haemolobinA_2 (HbA_2) levels but other conditions causing elevated HbA2 levels should also be considered in some situation[15].

- **Thalassaemia intermedia**

Thalassaemia intermedia is a clinical entity that describes wide spectrum of clinical phenotypes. Individuals with TI may have significant anaemia, splenomegaly, leg ulcers and bone disease. They are not transfusion dependant however they may need occasional transfusions. They may develop some of the complications such as gallstones and complications due to iron overload[15,44].

The genetic basis of TI is extremely diverse. Inheritance of mild or silent β chain mutations, Co-inheritance of determinants associated with increased γ chain production and co-inheritance of α thalassaemia are the three mechanisms are responsible for the intermediate phenotype seen in TI [44]. TI due to two β thalassaemia alleles may arise due to different combinations of homozygosity or compound heterozygosity for mild β^+ or β^0 thalassaemia alleles. Compound Heterozygosity for one β thalassaemia allele and variant Hb such as HbE , HbD-Punjab, HbC and HbO-Arab can also give rise to TI [15]. Heterozygous for β-thalassaemia mutations with abnormally severe thalassaemia intermedia phenotype can arise due to the homozygosity for

quadruplicated and triplicated α genes[45]. Dominantly inherited β thalassaemia variants have also been described and these conditions are primarily due to hyperunstable β globins[46] or initiator codon mutations[47].

- **Beta thalassaemia major**

Homozygosity or compound heterozygosity for β thalassaemia causes βTM. Compound heterozygosity for β thalassaemia allele and a thalassaemic haemoglobinopathy such as HbE also causes TM phenotype[1]. This condition is transfusion dependant since early childhood. Usually they present between age 6 months to 24 months with severe anaemia, hepatosplenomegaly and failure to thrive. Red cell indices show markedly reduced MCV(50-70 fl) and MCH (12-20 pg). Blood picture shows hypochromic microcytosis with marked anisocytosis, poikilocytosis(especially elongated cells and speculated target cells) and nucleated red cells which is related to the degree of anaemia [1,3,15].

These patients will develop complications of iron overload due to repeated transfusions. In children it results in growth retardation and lack of development of secondary sexual characteristics and in adults it leads to dilated cardiomyopathy ,endocrinopathies and cirrhosis [48]. This group of patients are at a higher risk of developing other complications of repeated blood transfusions such as infections (HIV,HCV and HBV), haemolytic and non haemolytic reactions[1,3]. Severity of the clinical phenotype depends on β thalassaemia allele itself and other modifier genes[49].

1.7.3 HbE

HbE is a variant Hb caused by a point mutation at codon 26 (CD 26). This mutation results in substitution of glutamic acid by lysine and also activation of a cryptic mRNA splice site which leads to reduced production of β-E globin chains[50]. It is considered as thalassaemic haemoglobinopathy[15] and one of the world's commonest mutations. Compound heterozygosity with β-thalassaemic alleles results in HbE/β thalassaemia and it is the commonest cause for thalassaemia major phenotype. HbE is particularly common in certain Asian countries including Sri Lanka[5,51].

1.7.4 Other types of thalassaemia

The δβ thalassaemia and εγδβ thalassaemia are very rare types of thalassaemias. They are caused by large deletions in the β gene cluster those remove either δ and β genes or all four genes[15,30].

Deletions in the β gene cluster and point mutations in the upstream of HBG gene causes many forms of Hereditary Persistence of Fetal Haemoglobin(HPFH) which is a heterogeneous group of inherited conditions characterized by presence of significantly increased levels of fetal haemoglobin in an adult [30]. Usually HbF level in a normal adult is <1% but it may range between 10%-40% in heterozygotes for mutations causing HPFH. These individuals are otherwise normal and it may be advantageous in a homozygous status for other major haemoglobinopathy such as thalassaemia major . Sometimes they can compensate for the complete lack of HbA [52].

1.8 Epidemiology

Tthalassaemia is one of the top five disorders that account for 25% of 7000000 children with birth defects born annually[53]. Alpha thalassaemia is prevalent in tropical and sub tropical regions and carrier frequency of >1% is seen in all areas. In some areas carrier frequency up to 80-90% can be seen. It is also considered as the most widely distributed haemoglobinopathy[28]. Although alpha thalassaemia is very common, severe forms are largely restricted to South East Asia and China[54].

In global scale ,the geographical regions with high thalassaemia frequency are either endemic areas or historically had been endemic areas for *plasmodium falciparum* . Haemoglobinopathy carrier states are thought be protective in this potentially fatal parasitic disease. High frequency of carriers can be explained by heterozygote advantage offered against *plasmodium falciparum* malaria. This hypothesis was first described by J.B.S. Haldane in 1948. However this hypothesis is yet to be proven[7]. This observation remains true even at a micro epidemiological scale[55]. Even in Sri Lanka , thalassaemia, HbE disease and G6PD deficiency is prevalent in areas where malaria had been endemic or hyperendemic over a long period of time [4,11,56,57]. However the protective effect against *plasmodium falciparum* offered by alpha thalassaemia have been questioned. Some studies have shown that, the incidence of uncomplicated malaria is equal in alpha thalassaemia and sometimes it is higher. However studies focused on severe malaria provides evidence for the protection against severe and fatal malaria. This protective effect is more marked for homozygotes than for heterozygotes[58-61].

Figure 9: Distribution of Alpha thalassaemia

Abstracted from Singer ST. 2008. Variable clinical phenotypes of α-thalassemia syndromes. TheScientific World Journal **9:** 615–625. **[**54]

Mass scale migration in today's world has led thalassaemias to become an emerging global health problem. With the changing demographic patterns of the world thalassaemias including alpha thalassaemia is becoming a frequent condition in some countries situated outside the thalassaemia belt[53.62].

1.9 Genetic basis of alpha thalassaemia

Alpha thalassaemia is caused by down regulation of α globin chain synthesis. This leads to reduced production of both fetal (HbF) and adult(HbA/ HbA_2) haemoglobins[63]. Deletions involving one or both α genes are responsible for the majority of the α thalassaemia cases while non deletional mutations accounts for the minority. If a deletion or a non deletional mutation leads to transcriptionally completely inactive α genes on a single chromosome such a condition is designated as α^0 thalassaemia. Conditions where there is some residual function and some α globin chain production are designated as α^+ thalassaemia[15,33]. Generally deletions and non deletion mutations causing α thalassaemia can be classified in to seven broad categories [15].

1. Deletions of all or part of one or both α genes
2. Deletion of upstream regulatory elements
3. Mutations affecting RNA splicing
4. Mutations affecting polyadenylation
5. Mutations affecting RNA translation
6. Mutations causing post translational instability
7. ATRX gene mutation

- **Deletions**

Common deletions causing α thalassaemia usually remove a single α gene and result in α^+ thalassamia. However there are some extended deletions ranges from 100 kb - >250 kb that may remove the entire α cluster or a large proportion of it together with both α genes result in α^0 thalassaemias[33]. A thalassaemia causing deletions can be further subdivided in to several categories[15].

- Deletion of an α_2 (*HBA2*) gene
- Deletion of an α_1(*HBA1*) gene
- Deletion of parts of both α_2 and α_1 genes with a formation of a fusion gene.
- Deletion of both α_2 and α_1 genes
- Deletion of an α_1 gene together with more than 18 kb region downstream with inactivation of remaining structurally normal α_2 gene

Table 1: Classification of deletional mutations causing alpha thalassaemia

Type of deletion	Phenotype	Examples
All or part of one α gene	α^+ thalassaemia	$-\alpha^{3.7I}$, $-\alpha^{3.7II}$ $-\alpha^{3.7III}$, $\alpha^{4.2}$, $-\alpha^{3.5}$,- $\alpha^{2.7}$, $\alpha(\alpha)^{5.3}$
All or part of both α genes, without deletion of HS-40	α^0 thalassaemia	$--^{SEA}$, $--^{THAI}$, $--^{MED}$, $--^{FIL}$ $--^{BRIT}$, $--^{SPAN}$,- $(\alpha)^{20.5}$, $-(\alpha)^{5.2}$
Deletion of both α genes and HS-40	α^0 thalassaemia	$--^{DUTCH}$
Extensive loss of 16p13.3 including both α genes and HS-40	α^0 thalassaemia	$--^{BO}$
Deletion of α1 gene and 18-20 Kb downstream of α1 gene	α^0 thalassaemia	$(\alpha)-^{ZF}$

Adopted from Bain BJ. Haemoglobinopathy Diagnosis. 2nd ed. Malden: Blackwell Publishing limited; 2006 [15]

- **$-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions**

Reciprocal recombination between homologous regions is considered to be the mechanism for some of the commonest deletions. Both α genes(*HBA1* and *HBA2*) are embedded within two highly homologous 4 kb duplication units. These units are again subdivided in to three homologous segments(X,Y and Z) by non homologous segments (I,II and III)[64].

Rightward reciprocal recombination between highly homologous Z boxes results in formation of $-\alpha^{3.7}$ deletion and $\alpha\alpha\alpha^{anti\ 3.7}$. These Z boxes are situated 3.7kb apart. This $-\alpha^{3.7}$ containing chromosome has lost a 3.7 Kb region including 3' end part of α_2 (*HBA2*) gene and5' end part of α_1(*HBA1*) gene. This results in the formation of an α_2-α_1 fusion gene on one chromosome and triplication of the α gene on the other chromosome($\alpha\alpha\alpha^{anti\ 3.7}$). This $\alpha\alpha\alpha^{anti\ 3.7}$ containing chromosome has a normal α_2 gene, an $\alpha_1\alpha_2$ fusion gene and a normal α_1 gene[15,33,64].

Although $-\alpha^{3.7}$ is the commonest deletion throughout the world it did not originated from a single event in early human evolution. Further studies has shown that at least three variants of $-\alpha^{3.7}$ mutation exists in different ethnic groups. These variants are designated as $-\alpha^{3.7I}$, $-\alpha^{3.7II}$ and $-\alpha^{3.7III}$. Out of these three variants $-\alpha^{3.7I}$ is the commonest type worldwide while $-\alpha^{3.7II}$ is seen in India and Nepal and $-\alpha^{3.7III}$ is seen only in Oceania[65].

Leftward recombinations between homologous X boxes which are situated 4.2kb apart results in $-\alpha^{4.2}$ deletion on one chromosome and $\alpha\alpha\alpha^{anti4.2}$ on the other chromosome. This deleted 4.2Kb segment encompass the entire α_2 gene and as result of this deletion the chromosome with $-\alpha^{4.2}$ has only an α_1 gene . The $\alpha\alpha\alpha^{anti\ 4.2}$ on the other chromosome has two α_2 genes and one α_1 gene [15,33,64].

Both $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions result in reduction of α chain production from the affected chromosome. Although generally the α_2 is responsible for about 70% of total α chain production, when the α_2 gene is deleted α_1 gene is up regulated. In $-\alpha^{3.7}$ deletion α_2 α_1 fusion gene is down regulated when compared to α_2 function[15].

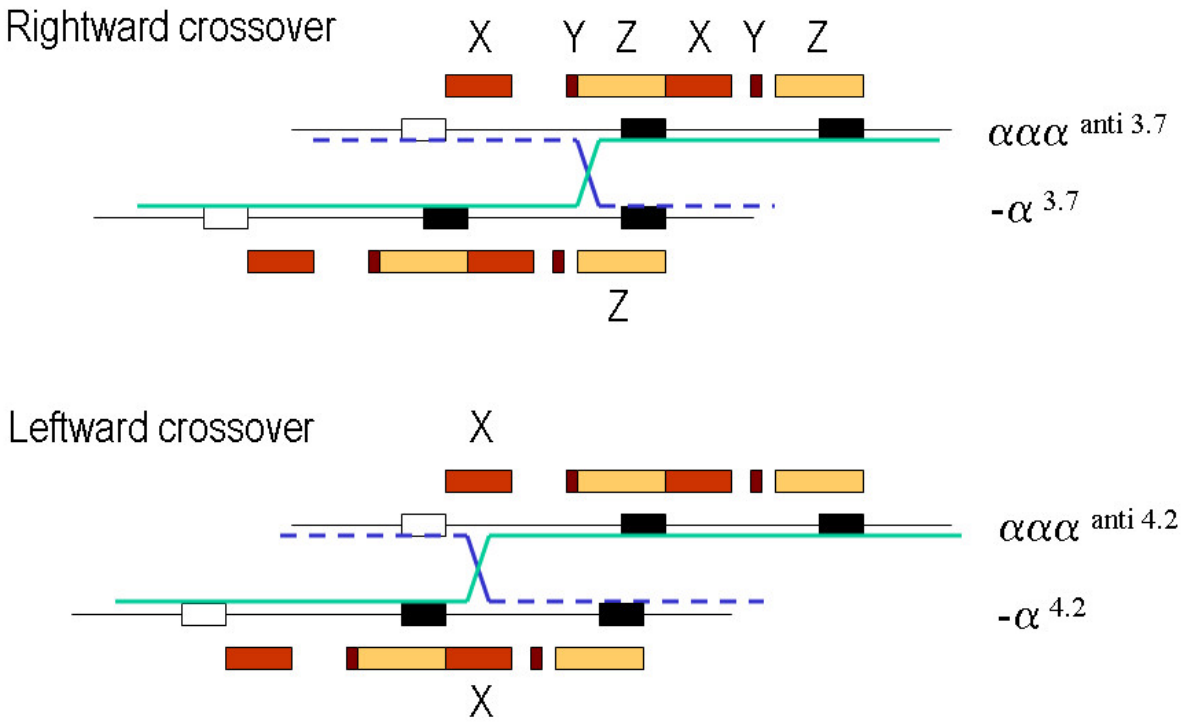

Figure 10: Mechanism causing -α 3.7 and –α 4.2 deletions

Abstracted from Hartveld CL, Higgs DR, α-thalassaemia: review Orphanet Journal of Rare Diseases. 2010; 5:13 [28]

- **Other common deletions**

South East Asia ($--^{SEA}$) mutation is a 20.5 kb size deletion encompassing both α globin genes but sparing ζ-$_2$globin gene. This is a common mutation in South East Asia and results in α^0

thalassaemia. Homozygosity for this deletion (--$^{SEA/}$--^{SEA}) results in Hb-Bart Hydrops fetalis. Mediterranean(--^{MED}) deletion is similar to –^{SEA} deletion and spares ζ-$_2$globin gene while –^{FIL} and –^{THAI} removes all genes. The –(α)$^{20.5}$ deletion spares ζ-$_2$globin gene as well as 5' region of the α_1 gene. The –^{HW} is a large deletion over 300 kb which removes entire α gene cluster[66]. The --^{SEA} and --^{FIL} deletions are particularly important they carry higher risk of producing HbH when inherited along with single defective gene and causing Hb Bart's hydrops fetalis in the homozygous state[35].

- **Deletional mutations affecting both α_1 and α_2 genes**

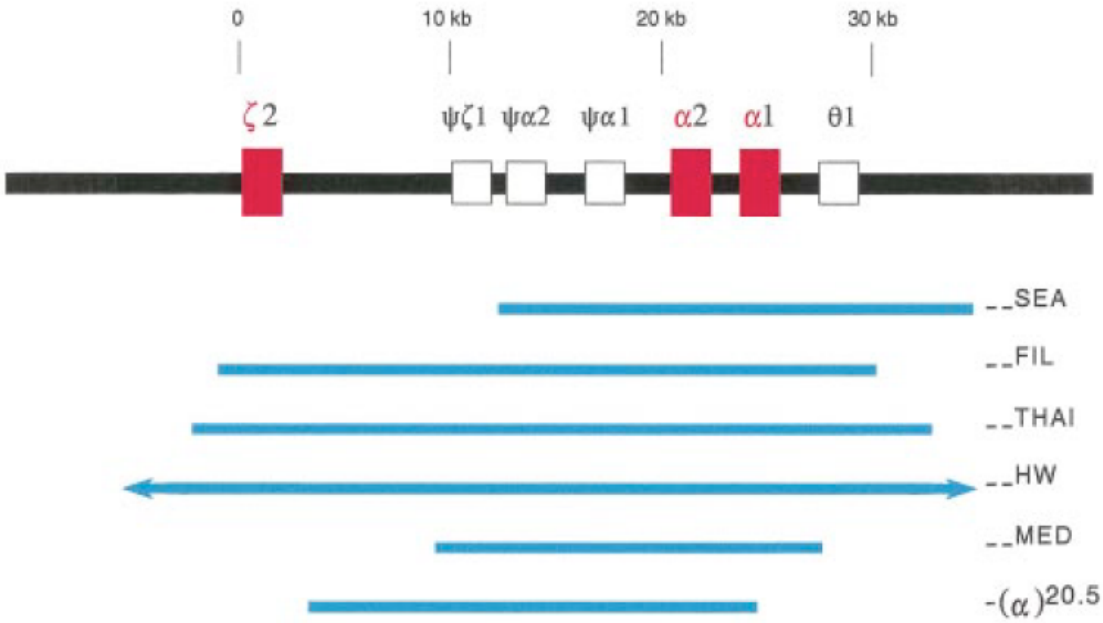

Figure 11: Common α^0 deletions

Abstracted from Chui DH, Waye JS.1998. Hydrops fetalis caused by alpha-thalassemia: an emerging health care problem. *Blood.* **91(7):**2213-22.[66]

- **Non deletional mutations**

About 70 non deletional α^+ thalassaemia have been reported. Most of them are extremely rare and limited to only one or few families[37]. Table 2 illutrates a classification of non deletional mutations.

Table 2: Classification of non deletional mutations causing alpha thalassaemia

Type of mutation	Phenotype	Example
RNA splice site mutation in α_1 or α_2 genes	α^+ thalassaemia	α_2 IVS1 (–5nt)
RNA polyadenylation signal mutation	Severe α^+ or α^+ thalassaemia	α_2 AATAAA→AATAAG (αPA6A→Gα, αTSaudi)
Impaired RNA translation consequent on initiation codon or initiation consensus sequence mutation	Severe α^+ or α^+ thalassaemia or α^0 thalassaemia	α_2 ATG→ACG, GTG or A–G; –$\alpha^{3.7}$ ATG→GTG (mutation in association with deletion gives α^0 phenotype)
frameshift or nonsense mutation	α^+ or α^0 thalassaemia	Codon 30/31 (–4nt) frame shift α_2 CD116 GAG→TAG nonsense
Impaired RNA translation consequent on a termination codon mutation leading to an elongated mRNA and α globin chain	α^+ thalassaemia	Hb Constant Spring, Hb Icaria , Hb KoyaDora, Hb Pakse, Hb Seal Rock
Production of highly unstable α chain as a result of point mutation or a small deletion	α^+ thalassaemia	Hb Arginia, Hb Peta Tikvah, HbQuong Sze, Hb Suan Dok, Hb Evaston, Hb Taybe
Lack of transactvating factor encoded by the ATRX gene	α^+ thalassaemia	ATR-X syndrome

Adopted from Bain BJ. Haemoglobinopathy Diagnosis. 2nd ed. Malden: Blackwell Publishing limited; 2006 [15].

Hb Constant Spring(HbCS) is a variant Hb caused by T>C transition mutation at codon 142 of α_2 gene. HbCS is characterized by abnormal elongation of the α chain. Combined heterozygosity with α^0 mutation results in HbH disease and generally it is more severe than HbH disease caused by deletional mutations alone [67]. Haemoglobin level is about 2g/dl lower but MCV is higher due to overhydration of RB[28]. This mutation is particularly common in South-East Asia and Southern China. It 's frequency ranges from 1% to 8% [68]. It is also rarely found in Mediterraneans and it is believed to have originated independently[67].

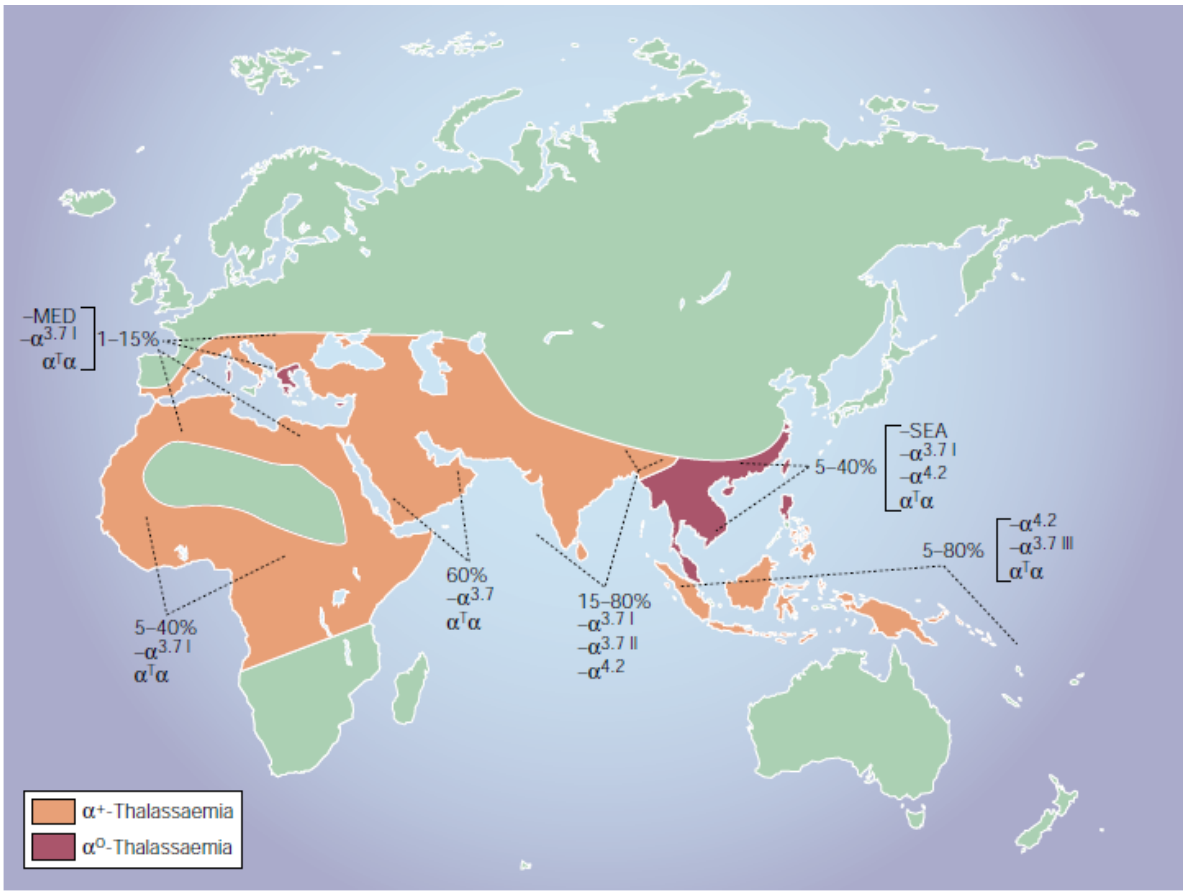

Figure 12: Global distribution of common mutations

Abstracted from Weatherall DJ. Phenotype–genotype relationships in monogenic disease: lessons from the Thalassaemias. Nature reviews/ genetics **2001;2:** 245-256 [30]

- **X-linked alpha thalassaemia mental retardation syndrome**

X-linked alpha thalassaemia mental retardation syndrome is an X –linked condition present predominantly in males . Patients have developmental delay , learning difficulties , facial dysmorphic features and genital abnormalities. Alpha thalassaemia is present in 90% of patients despite having intact alpha globin gene cluster. This is caused by mutations of ATRX gene at Xq13.3. The protein coded by this gene belongs to SNF2 family of helicase/ATPase. Exact functions of this protein is still unknown but it is believed that it plays a role in regulation of transcription as a trans-activating factor [69].

1.9.1 Mutational basis of α thalassaemia in Sri Lanka

De Silva *et al* screened 472 individuals both thalassaemia patients and carriers for - $\alpha^{3.7}$ and -$\alpha^{4.2}$ mutations and found 13.6% of the population was heterozygous for -$\alpha^{3.7}$ mutation and 1.1% was heterozygous for -$\alpha^{4.2}$ mutation[4]. Another study on Sri Lankan thalassaemia patients carried out by Fisher *et al* reported the -$\alpha^{3.7}$ deletion as the commonest α mutation accounting for 7.6% of 908 tested individuals . The - $\alpha^{4.2}$ deletion was present in only 1.1% of tested individuals. Non deletional mutations of α gene has not been described in Sri Lanka so far. Triplicated and quadruplicated α genes were described in 1.5% of patients. They also screened 188 patients from one center for common non deletional mutation in Indian population[Hb Evanston, IVS1-117(A>G),IVS1(5bp del), Hb Sun Prairie, Hb Koya Dora, Hb CS and Poly A tail (ATAAA>ATA)] but no mutations were found [5].

Another resent study using nine families with unexplained hypochromic microcytic anaemia has found a novel deletion causing α^0 thalassaemia, previously known --^{THAI} deletion, and five non deletional mutations. This novel deletion --^{SL} , is a 12809 bp size segment encompassing both

HBA1 and *HBA2* genes. It was found in three unrelated Sri Lankan Muslim families and the propositus of each family was positive for HbH inclusions. Two of them had the - $\alpha^{3.7}$ /--^{SL} genotype and one had - $\alpha^{4.2}$/ --^{SL} genotype[70].

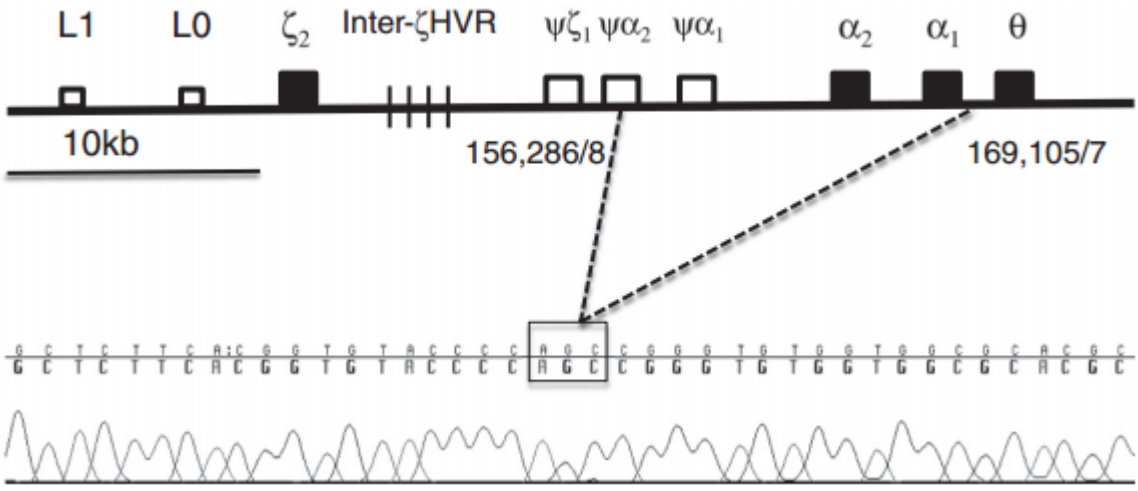

Figure 13 : The extent of the --^{SL} deletion

Abstracted from Suresh S, Fisher C, Ayyub H, Premawardhena A, Allen A, Perera A, Bandara D, Olivieri N, Weatherall D. Alpha thalassaemia and extended alpha globin genes in Sri Lanka. Blood Cells Mol Dis.2013; **50(2):**93-8[70]

1.10 Pathophysiology

The complex nature of the pathophysiology of thalassaemia is not fully understood yet. Absent or reduced production of one or more globin chain is the initial point of pathophysiological mechanisms. As result of reduced production of globin chains, functional tetramers of haemoglobin are impaired. This lead to hypochromic microcytic anaemia. Impaired production of one type of globin chain causes imbalance in overall globin chain production and excess of other globin chains. This free, unpaired globin chains are insoluble and tend to polymerize to

form homotetramers. In α thalassaemia this lead to formation of Hb Bart and HbH due to polymerization of γ and β globin chains respectively. These homotetramers have higher affinity for oxygen and tend to precipitate as the cell matures. Accumulation of unpaired globin chains has more profound effect in pathophysiology of the disease rather than impaired production of functioning haemoglobin [71] .

In β thalassaemia major , the imbalance between α and β chains is severe lead to excess α chain to be precipitated. These precipitated inclusion bodies causes oxidative damage to the red cell membrane and lead to destruction of immature erythroblasts . Destruction of developing erythroblasts within bone marrow results in ineffective erythropoiesis[72]. Ineffective erythropoiesis caused by accelerated apoptosis is one of the major pathophysiological mechanisms in thalassaemia where only very few cells complete erythroid maturation and are released in to peripheral circulation. Furthermore circulating cells may have inclusion bodies and tend to be removed from circulation in the reticular endothelial system producing haemolytic anaemia. Quantative studies have shown that there is fourfold increase in apoptosis of erythroid precursors in β thalassaemia major. There is a correlation between ineffective erythropoiesis and apoptosis in the marrow in severe forms of both α and β thalassaemias [72-74]).

Grossly reduced oxygen carrying capacity results in increased production of erythropoietin and which in turn leads to compensatory erythroid hyperplasia. Despite massive bone marrow expansion erythropoiesis remains ineffective and marrows are packed with premature erythroblasts. Further expansion of haematopoietic tissues lead to extramedullary haematopoiesis [71].

In HbH disease , unpaired β globins are somewhat soluble and β4 tetramer(HbH) are formed. However HbH results in relatively less inclusion body formation in erythroblasts. They usually precipitate in mature red blood cells . Therefore in HbH disease there is a moderately severe haemolytic anaemia with little ineffective erythropoiesis[66,71] .

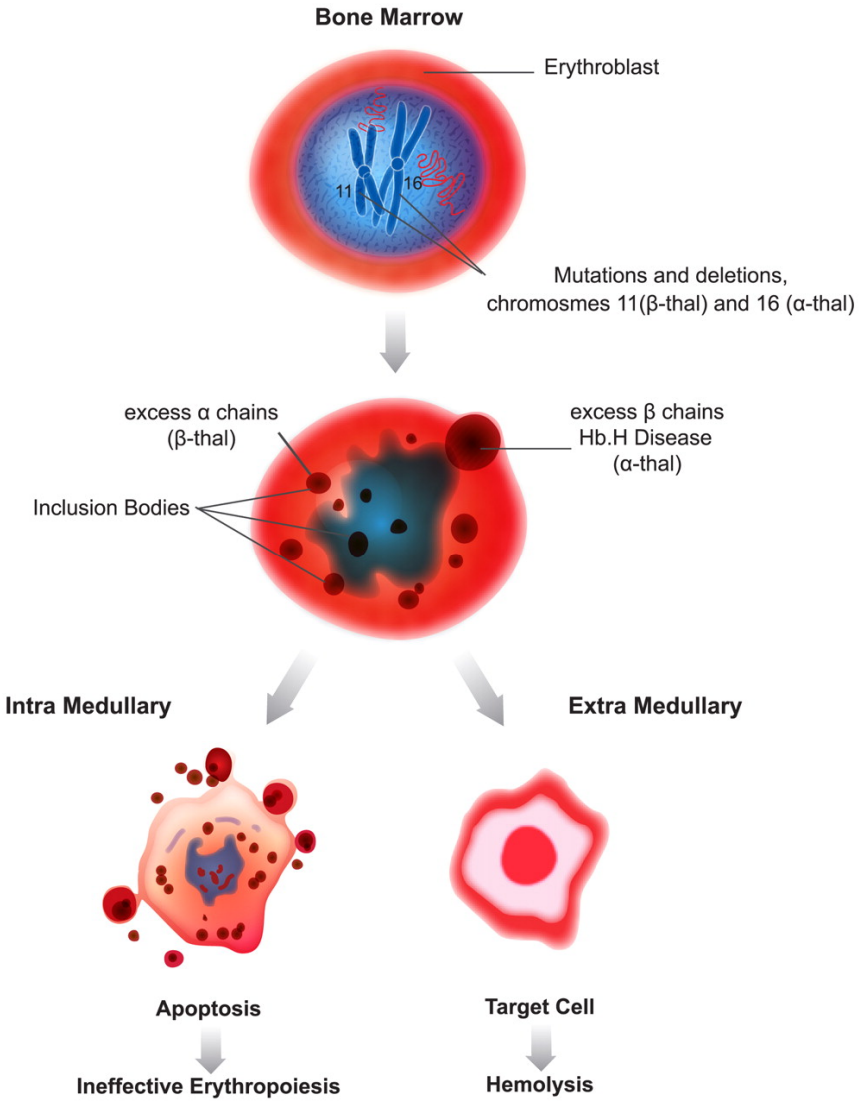

Figure1 4:Mechanism of ineffective erythropoiesis and haemolysis in thalassaemia

Abstracted from Rachmilewitz EA, Giardina PJ .2011. How I treat thalassemia, Blood **118:** 3479-3488[75]

In Hb Bart's hydrops fetalis, absence of all α genes results in profound anaemia. Most of the Hb in the circulation being Hb Bart, which is not effective in oxygen delivery there is severe tissue hypoxia. Anaemia results in fetal heart failure causing hydrops and tissue hypoxia leads to massive placental enlargement causing maternal complications like pre-eclampsia and haemorrhage. Extramedullary erythropoiesis results in hepatosplenomegaly and together with hypoxia result in congenital abnormalities. However the fetus is kept alive by Hb Portland which comprise 10-20% of circulating Hb[66].

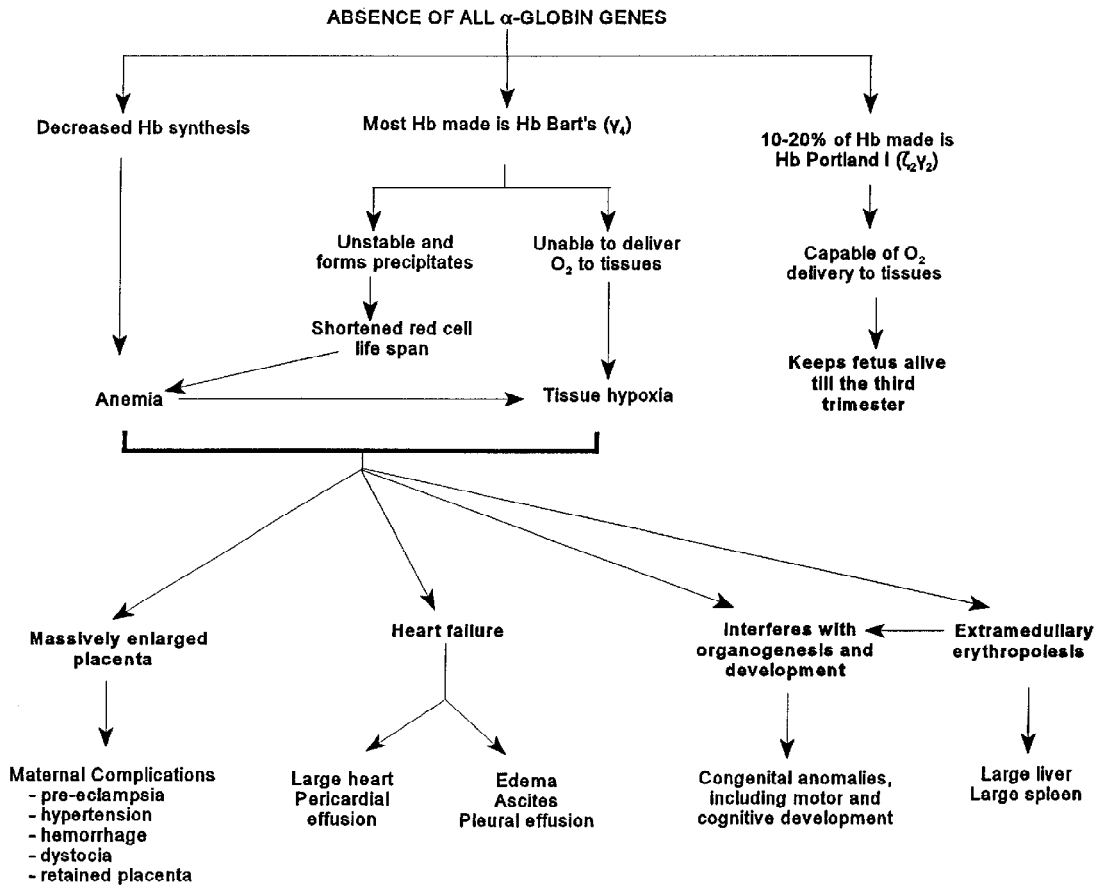

Figure 15: Pathophysiology of Hb Bart's hydrops fetalis

Abstracted from Chui DH, Waye JS.1998. Hydrops fetalis caused by alpha-thalassemia: an emerging health care problem. Blood. **91(7):**2213-22.[66]

1.11 Complications of thalassaemia

Silent carrier state and alpha thalassaemia trait are usually asymptomatic and microcytosis and erthrocytosis may be the only identifiable features. In alpha thalassaemia trait Hb level may be 1-1.5 g/dl lower than normal. Sometimes these conditions may be treated as iron deficiency anaemia unnecessarily[35].

Most of the complications of thalassaemia are due to iron overload. Repeated blood transfusion is the main contributory factor in transfusion dependant patients while excessive iron absorption triggered by ineffective erythropoiesis may also contribute. Deposition of iron is toxic and damages many organs including the liver, heart, endocrine glands and joints[76]. In HbH disease iron overload is present in 70% of adult patients. Increased intestinal absorption of iron stimulated by increased haemolysis and erythropoiesis is the primary cause for iron overload in HbH disease[35,77]. Men with HbH disease have higher serum ferritin levels [36] but there is no difference in patients with deletional and non-deletional mutations[78]. However endocrinopathies due to iron overload is rarely reported in HbH disease[35].

Splenomegaly with or without hypersplenism is a common finding in HbH disease. Hepatomegaly is also associated but less common than splenomegaly[35,37]. Splenectomy is known to increase Hb levels but it is not generally recommended since Hb levels in HbH disease is well tolerated. Severe phenotype especially in non deletional HbH disease, abdominal discomfort and need for frequent transfusion are the indications for splenectomy. Post splenectomy complications include overwhelming bacterial infections and venous thrombosis[37,79]. Gallstones are frequently found in HbH disease. About 30 % of asymptomatic patients may have gall stone disease. It can cause abdominal discomfort and hepatobilliary infection[80].

Acute haemolytic crisis due to infections is a common complication in HbH disease. The drop of Hb level is brisk and may be as high as 3 g/dl overnight. This can lead to the development of shock and acute renal failure[37]. Aplastic crisis due to parvovirus B 19 infection is a well recognized complication in HbH disease[38].

Bone disease which is a common complication in β thalassaemia is not generally associated with α thalassaemia. Complications during pregnancy such as fall of Hb to 7g/dl or less, higher rate of premature labour, pre-eclampsia and congestive cardiac failure in third trimester has been reported[35].

1.12 Modifier genes

Very little attention has been offered towards the identification of disease modifying genetic components in alpha thalassaemia**.** However genetic modifiers of beta thalassaemia have been studied extensively and most of the studies on genetic modifiers of alpha thalassaemia follows that of beta thalassaemia [37].

Three types of modifiers affecting severity of β thalassaemia are been described. Heterogeneity and variable severity of β thalassaemia alleles are considered as primary modifiers. Alleles causing complete absence of β globin production (β^{o}) are more severe than alleles causing reduced production (β^{+}). Variation of the amount of α globin levels and fetal Hb levels can affect the severity. These kinds of factors are known as secondary modifiers[49,81]. An increased level of foetal Hb is known to ameliorate the effects of severe β thalassaemia major. Several

mutations causing hereditary persistence of fetal haemoglobin have been described . A polymorphism in BCL11A gene (rs 11886868) and Xmn-1(Gγ 158 C>T) polymorphism in Gγ gene are associated with elevated HbF and less severe phenotype[82,83,84]. Co existence of deletions of α genes in β- TM and β- TI has shown to be associated with less severe phenotype[83].

Genetic variants contributing to the severity of complications such as hyperbilirubinaemia, iron overload and osteoporosis are considered as tertiary modifiers. All three categories of modifiers are known to vary in different populations[49,81].

Iron absorption in humans is regulated by Hepcidin[85]. Studies on animal models have suggested that low hepcidin levels are responsible for abnormal iron absorption in thalassaemia. HFE gene may play a role in hepcidin expression in thalassaemia[86]. Another study has described the contribution of Growth Differentiation Factor 15(GDF15) over expression arising from expanded erythroid compartment to iron overload in thalassaemia. GDF15 suppresses the iron regulatory protein hepcidin[87].

Hyperbillirubunaemia is a well known complication in thalassaemia. Modifier genes are believed to play a key role in jaundice and gall stones in thalassaemic patients[49]. Many polymorphisms of UGT1A1 gene are known to associated with Gilbert syndrome, which is characterized by hyperbillirubinaemia. Similarly polymorphic variants of G6PD and SLOCO1B1 genes are also associated with hyperbillirubunaemia. These three genes may interact with each other and with environmental factors in clinically significantly hyperbillirubinaemia[88]. An Italian study to find the prevalence of cholelithiasis and co-inherited Gilbert syndrome genotype of 261 TM and 35 TI patients revealed 20.3% of TM and 57.1% of TI patients found to have cholelithiasis and individuals homozygous for (TA_7) in promoter region of UGT1-A1 gene to have higher

incidence of cholelithiasis[89]. A study done in Sri Lanka also revealed homozygosity for (TA_7) genotype to be associated with a higher risk for gallstone disease compared to (TA_7) /(TA_6) and (TA_6) / (TA_6) genotypes in patients with HbE/ β thalassaemia[90]. A study carried out in Chinese population has shown that thalassaemia genetic profile itself is also a contributory factor for hyperbillirubunaemia[91].

Risk factors associated with thrombosis include increasing age, transfusion independence and splenectomy Coagulation abnormalities are well documented in β- TM , TI and HbH patients. Thrombotic events are also common, particularly in β-TI patients[92]. Modifier genes also play a role in progressive osteoporosis and osteopenia which is commonly found in young adults with β thalassaemia. Polymorphisms in VDR gene and COL1A1 is known to associated with osteopenia in Thalassaemia[93,94]. Frequency of apolipoprotein E4 allele which lead to decreased antioxidant activity of apolipoprotein E4, is known to be associated with left ventricular failure in thalassaemic patients[95].

1.13 Diagnosis of Alpha thalassaemia

Laboratory investigations of alpha thalassaemia include full blood count, blood picture, quantification of Hb and mutational analysis of alpha genes. FBC shows low red cell indices such as MCV and MCH and wide red cell distribution width(RDW). Blood picture shows features similar to other thalassaemias. In HbH disease red cell inclusions can be seen when stained with dyes such as methylene blue and brilliant cresyl blue. Quantification of Hb is

another essential investigation which helps to exclude β carrier state and to identify Hb variants[96].

- **Clinical suspicion**

HbH disease is suspected in the presence of microcytic anaemia with splenomegaly and increased reticulocyte count. Some patients present either with symptoms of anaemia or with splenic discomfort. Diagnosis of HbH can also be incidental [15,40]. Haemoglobin Bart's Hydrops fetalis is suspected whenever there is an intrauterine death of a hydropic fetus or neonatal death of a hydropic baby to parents of an appropriate ethnic group. Investigation for alpha thalassaemia is also indicated in unexplained microcytosis and in unexplained haemolytic anaemias[40].

- **Haematological investigations**

Red cell indices

Although DNA based diagnostic methods are used increasingly they do not replace haematological investigations. Furthermore haematological phenotype especially in the carrier state helps to guide DNA diagnostics[96,97].

Thalassaemias are classified as hypochromic microcytic anaemias and other main differential diagnosis of this type of anaemia are Iron deficiency anaemia(IDA), anaemia of chronic disease, sideroblastic anaemia and lead poisoning[40,98]. Anaemia is classified as mild , moderate and

severe depending on Hb concentration. Table 3 gives the xlassification of severity of anaemia [99]

Table 3: WHO classification of the severity of anaemia

Population	Normal	Mild	Moderate	Severe
Children 6-59 months	11 or higher	10-10.9	7-7.9	<7
Children 5-11 years	11.5 or higher	11-11.4	8-10.9	<8
Children 12-14 years	12 or higher	11-11.9	8-10.9	<8
Non- pregnant women(15 years of age and above)	12 or higher	11-11.9	8-10.9	<8
Pregnant women	11 or higher	10-10.9	7-9.9	<7
Men(15 years of age and above)	13 or higher	11-12.9	8-10.9	<8

Adopted from Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001[99].

Sometimes it is difficult to differentiate IDA and thalassaemia clinically and by laboratory investigations[100]. However full blood count with all the indices will be the first investigation to suspect any type of thalassaemia and MCV is the most important indicator. MCV of 72 fl has been found as the most sensitive and specific indicator for presumptive diagnosis of Thalassaemia[101]. Red cell distribution width (RDW) is an indicator of variation of red cell size. It is increased in iron deficiency anaemia(IDA) but generally within normal range in thalassaemia which produces small red cells with uniform size. However in HbH disease and in δβ thalassaemia trait there is an increase in RDW . RBC count is generally increased in

thalassaemia while in other conditions causing hypochromic microcytic anaemia such as IDA and anaemia of chronic disease usually reduces the RBC count[102].

Several cell counter based formulas using red cell indices such as MCV, MCH ,Haemoglobin level, RBC count and RDW have been described to differentiate IDA from β thalassaemia trait. Shine and Lal Index (MCV*MCV*MCH/100)[103], Mentzer Index (MCV/RBC count) [104], Srivastava Index (MCH/RBC count) [105], England and Fraser Index (MCV-5(Hb)-RBC) [106], Green Index (MCV*MCV*RDW/Hb*100) [107] and Ricerca Index(RDW/RBC count) [108] are some of those. Study conducted by Rathod *et al*(2007) has shown MCV and MCH based formulas have better function compared to RBC count and RDW based formulas in discriminating IDA from thalassaemia trait[109]. Red cell distribution index(RDWI) is also considered a good method to differentiate thalassaemia trait from IDA[110]. However the usefulness of these formulas is limited in children, in pregnancy and β thalassaemia trait in individuals with IDA [15].

Several attempts have been taken to correlate the thalassaemia genotype and red cell indices. A study conducted by Mehdi and Dahmash (2011) on Arab populations did not find a correlation between the red cell indices (MCV, RBC counts) and the α and β thalassaemia genotype[111]. Gorakshakar and Colah (2011) showed some correlation between genotype and red cell indices but they concluded that RBC indices alone cannot differentiate two major types of thalassaemia[112]. Rund *et al*(1991) showed that alleles causing β^{o}- thalassaemia is associated with a narrow range for MCV(63.1 fl, SD=3.4) while β^{+}-thalassaemia alleles are associated with wider range of MCV(69.3 fl, SD=5.6)[113].

A study done in Vanuatu comparing haematological parameters of normal, heterozygous and homozygous individuals for α^{+} thalassaemia found that MCV and MCH differ significantly

among groups throughout the entire age range. They concluded that MCH as the most sensitive discriminator since none of the homozygous individuals had MCH <27 pg[114]. Akhavan-Niaki *et al* (2012) studied haematological parameters of 722 Iranians with different alpha gene mutations and found statistically significant differences in MCV and MCH between different genotypes. They also found both MCV and MCH were lower in patients with two functional genes when compared to individuals with one mutated alpha gene [115].

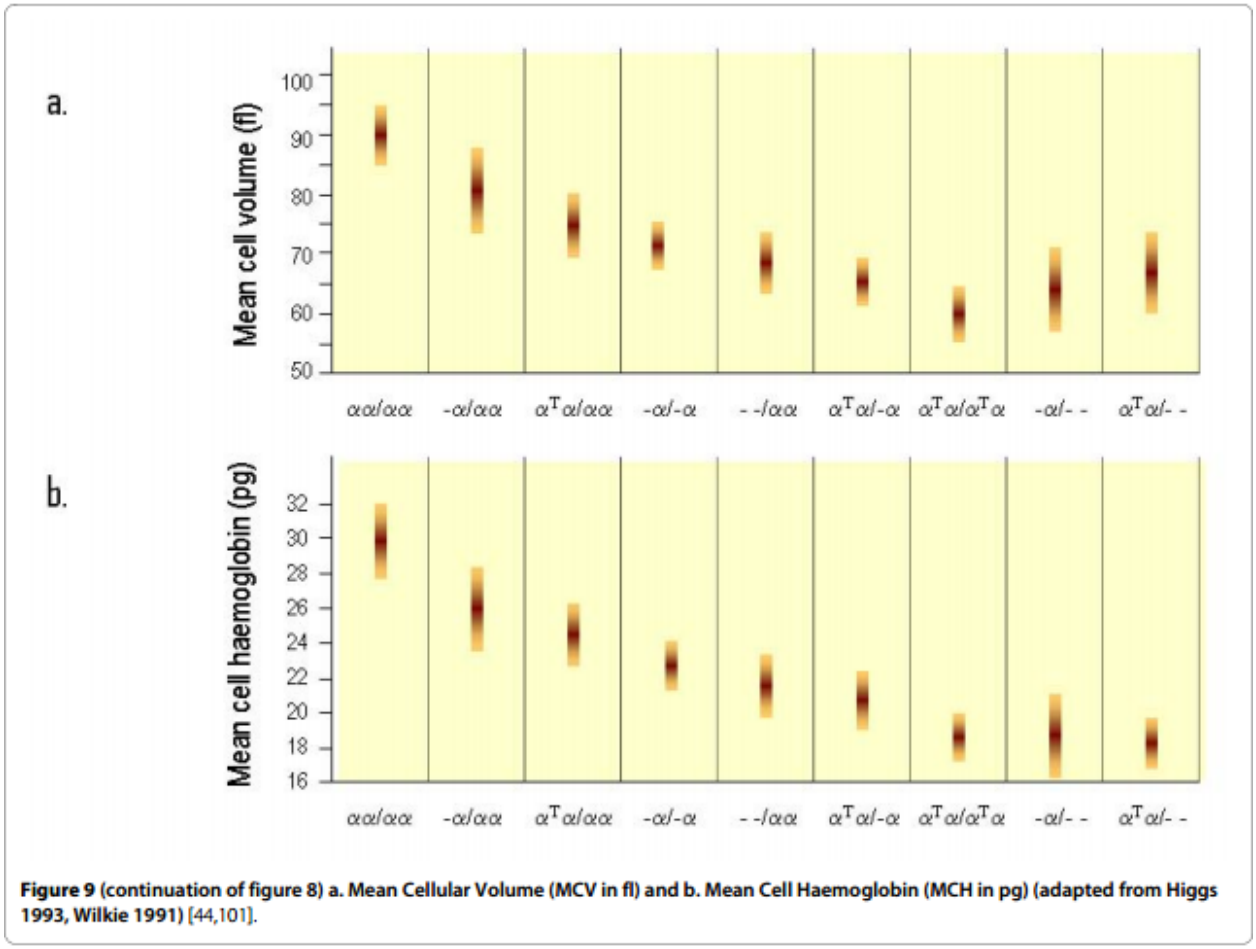

Figure 9 (continuation of figure 8) a. Mean Cellular Volume (MCV in fl) and b. Mean Cell Haemoglobin (MCH in pg) (adapted from Higgs 1993, Wilkie 1991) [44,101].

Figure 12: MCV and MCH of different genotypes

Abstracted from Hartveld CL, Higgs DR, α-thalassaemia: review Orphanet Journal of Rare Diseases. 2010; 5:13[28]

Red cell morphology

Due to ineffective erythorpoiesis, red cells morphology is affected and can be detected In a blood film of a thalassaemic patient and is seen as increased central pallor (hypochromasia) and changes in size and shape (anisocytosis and poikilocytosis). Poikilocytes is seen as target cells, tear drop cells and cell fragments. Other features are presence of basophilic stippling and premature red cells (nucleated red cells) in the circulation[15].

In α^+ heterozygotes blood film may be completely normal. Homozygosity for α^+ mutations expected to cause hypochromic microcytosis.The digree of hypochromic microcytosis correlates wih the number of defective genes[15,28]. In α^0 thalassaemia trait also, blood film shows microcytosis with variable degree of hypochromasia. Basophillic stipping is particularly prominent in HbCS homozygotes. Reticulocyte count can be increased in the presence of ongoing haemolysis[15,40]. In HbH disease there is a marked hypochromic microcytosis with anisocytosis and poikilocytosis such as target cells, fragmented cells and tear drop cells. Nucleated red cells are seen rarely[15].

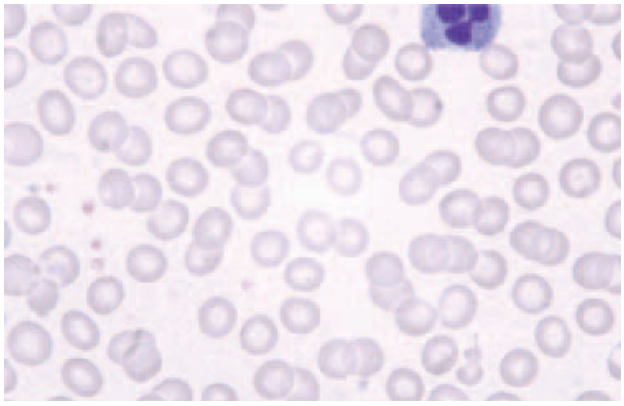

Figure 13:Blood picture of an adult with -α 3.7 /-α 3.7 genotype

Abstracted from Bain BJ. Haemoglobinopathy Diagnosis. 2nd ed. Malden: Blackwell Publishing limited; 2006 [15]

Iron deficiency anaemia also gives rise to a blood picture similar to thalassaemia trait. Early iron deficiency anaemia is characterized by normochromic normocytic anaemia with anisocytosis and later progress to hypochromic microcytic anaemia with anisochromasia and poikilocytosis. Poikilocytes include eliptocytes, pencil cells and occasional target cells[98].

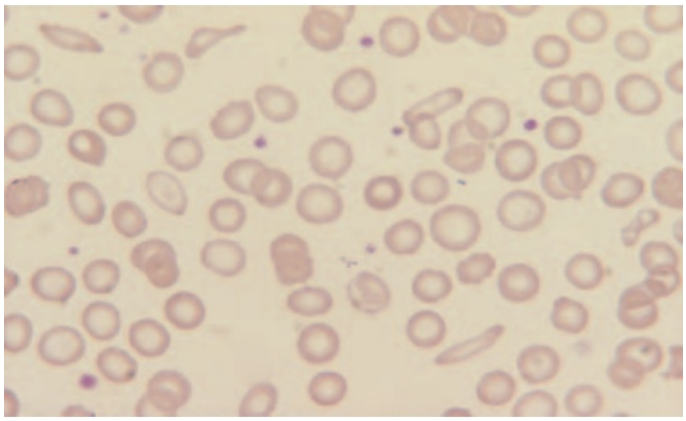

Figure 14: The blood picture of a patient with iron deficiency anaemia showing anisocytosis, poikilocytosis (including elliptocytes),hypochromia and microcytosis

Abstracted from Bain BJ. Blood cells : A practical guide 4th ed. Malden. BlackwellPublishing limited;2006 [98]

- **Haemoglobin quantification**

Alpha thalassaemia carrier states usually give normal HPLC pattern except for tendency to have low HbA_2 percentage. However it is an useful investigation in excluding β thalassaemia trait**[15].**

In the past Hb electrophoresis was used for identification and quantification of Hb variants but at present Cation-exchange high performance liquid chromatography(CE-HPLC) is considered as the method of choice for Hb quantification [102,116,117]. Haemoglobin electrophoresis

depends on separation of proteins applied to a membrane when exposed to a charge gradient. Separated variants can be visualized separately when stained for protein or haem. Several methods of haemoglobin electrophoresis such as cellulose acetate electrophoresis, citrate agar/ agarose gel electrophoresis and capillary electrophoresis are used to quantify haemoglobin[15]. CE- HPLC is a separation process which has a stationary and a mobile phase as for any chromatographic process. In this process a mixture of molecules with net positive charge is adsorbed in to a negatively charged stationary phase followed by elution in the mobile phase [15,118] Quantification of Hb following elution is by computing the area under the corresponding peak in the elution profile [15]. Most widely used CE-HPLC system for the quantification of haemoglobin is the Variant Hemoglobin Testing System developed by BioRad Laboratories, Hercules, CA[116].

Presence of HbH can also be identified by HPLC. It produces a characteristic early double peak [40]. Carrier state of β and α thalassaemia can also be differentiated by HPLC. In β thalassaemia carrier state , HbA_2 level is generally elevated above 3.5%. However co-inherited δ globin abnormalities result in low HbA_2 levels in β thalassaemia traits [119]. Quantification of HbA_2 becomes difficult in the presence Hb variants such as HbE, Hb Osu Christiansburg, and HbG Copenhagen due to co-elution with HbA_2 in HPLC[102]. Co-existing iron deficiency(ID) anaemia also interfere with HbA_2 levels[15]. Harthoorn-Lasthuisen , Lindemans & Langenhuijsen (1999) have shown that ID lowers the HbA_2 percentage and makes the diagnosis more complicated[120]. Akhavan-Niaki *et al* (2012) showed that there are no significant differences in HbA_2 and HbF levels in individuals with one and two defective alpha genes[115].

- **Demonstration of inclusion bodies**

Presence of HbH inclusions, produced by β globin tetramers (β4) can be identified by special staining techniques. HbH precipitates when exposed to a mild oxidant such as brilliant cresyl blue. It produces small blue staining inclusions evenly distributed through the cell giving rise to "golf ball" appearance in non splenectomized patients. In splenectomized patients it give rise to preformed Heinz bodies[15]. Pan *et.al* has shown that BCB staining as a reliable method to detect α-thalassaemia trait. They have also emphasized the possibility of using brilliant cresyl blue staining as an auxiliary method in α-thalassaemia screening[121]. Occasionally cells with HbH inclusions may be present in alpha thalassaemia trait also[15].

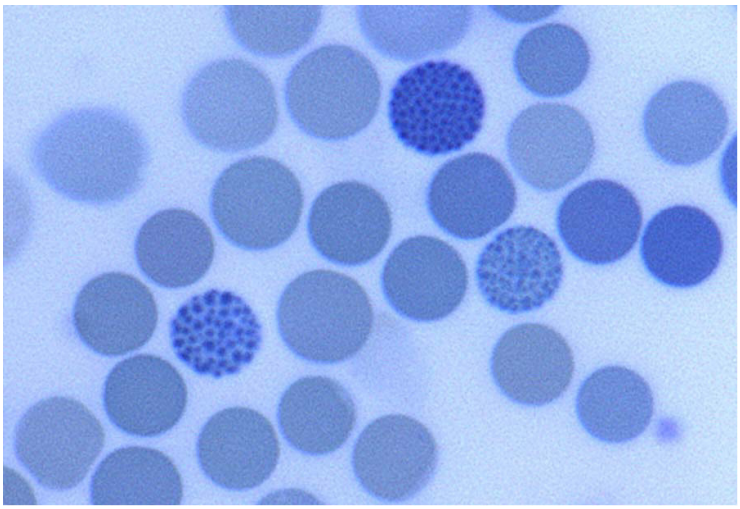

Figure 15: Golf ball appearance caused by HbH inclusions

Abstracted from Gibbons R. 2006. Alpha thalassaemia-mental retardation, X linked. Orphanet Journal of Rare Diseases 1:15[69]

- **Genetic studies**

Although HbH disease can be identified bu demonstrating HbH inclusions, genetic studies are essential for the definitive diagnosis of carrier states in alpha thalassaemia [15, 40].

Polymerase chain reaction(PCR) based methods are used to obtain a genetic diagnosis in most of the haemoglobinopathies including thalassaemia. These techniques include allele specific oliginucleotide (ASO) hybridization, amplification refractory mutation system (ARMS) , restriction enzyme analysis, amplification created restriction analysis, mutagenically separated PCR and gap-PCR . They are useful in identifying known mutations[96]. Most of the sequence variants in alpha gene cluster are neutral single nucleotide polymorphisms (SNPs). However there are as many as 69 point mutations and oligonucleotide variants alter gene expression and result in non deletional alpha thalassaemia[64]. Sequence analysis of alpha gene cluster is also helpful in the diagnosis of non deletional alpha thalassaemia which accounts for 10% of the cases[122]. Being small genes HBA1 and HBA2 (~1.2 kb) are suitable for sequencing when compared to larger genes like DMD (~2.3 Mb). However due to high GC content and high homology between two α genes limit the choice of primers and specific reaction conditions such as using betain and DMSO are needed. Multiplex Ligation-dependant Probe Amplification (MLPA) and Southern blotting are also used to identify suspected but currently unknown rearrangements[28].

- **Gap-PCR**

Inability of primers to generate a PCR product unless there is a deletion that brings the flanking regions together is used as the principle for gap-PCR. In this method specific forward and reverse primers are designed to flank the known deletion which produces a amplicon smaller

than the wild type[123]. When the deletion is large (>2kb) a product for wild type is not generated. In this case a primer that anneals within the deleted sequence is used to generate a control band which helps to monitor false negatives and to identify heterozygotes[96,124].

In alpha thalassaemia gapPCR method is used to identify most of the common deletions including $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions. $--^{MED}$, $--^{SEA}$, $--^{FIL}$, $--^{THAI}$ and $-(\alpha)^{20.5}$ are the α^0 thalassaemia deletions detected using gap-PCR method [28,96,125]. Multiplex gap-PCR methodology can be used to detect multiple deletions simultaneously [125,126]. The recently described $--^{SL}$ deletion can also be detected by using gap-PCR method[70].

2 JUSTIFICATION

Thalassaemia is a big burden to the public health system of Sri Lanka. Long-term management of thalassaemia patients includes regular blood transfusions and iron chelation. The overall management of thalassaemia patients consumes about 5% of national health budget. With improved treatment options , increased life expectancy of patients adding further burden to the health services with newer challenges such as management of complications in the aging thalassaemia populations. Although less common than β thalassaemia α thalassaemia is also present in Sri Lanka. It possesses a diagnostic challenge since the diagnosis of alpha thalassaemia carrier state is not as straightforward as in β thalassaemia.

The basic diagnostic investigations in thalassaemia includes full blood count (FBC), blood picture (BP), High Performance Liquid Chromatography (HPLC) and sometimes testing for HbH inclusions. These methods enable the diagnosis of beta-thalassaemia after 6 months of age, however they would not enable prenatal diagnosis and neonatal screening of thalassaemia or diagnosis of alpha-thalassaemia and rare forms of thalassaemias which requires genetic testing; thus alpha- thalassaemia carriers are not identified in the current population screening programme in Sri Lanka. Difficulties also arise in diagnosis of individuals with co-existing iron deficiency anaemia.

Experiences in other countries have shown that effective preventive programmes are can reduce thalassaemia birth rates by over >90% and the accurate identification of carriers is a key to success of such programmes. Genetic screening for identification of carriers and pre natal diagnosis was used in these programmes [127-129]. Therefore accurate characterization of

haematological features would help to select appropriate molecular genetic tests to determine carrier states. Finding a relationship between the genotype and first line haematological investigations such as the FBC and blood picture will be especially important in low resource settings and to guide for further investigations cost effectively. Establishing a relationship between genotype and HPLC pattern will also help in accurate diagnosis. Therefore findings of this study will ultimately help to prevent new cases of thalassaemia by accurate carrier detection. Very little work has been done regarding this matter and this study will help to expand the current level of understanding.

3 OBJECTIVES

Objectives of this study were

- To describe phenotypic characteristics of individuals with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions
- To determine the relationship between red cell parameters, HPLC parameters and red cell morphology with the genotype

4 METHODOLOGY

4.1 Study Design

This is a descriptive study.

4.2 Study Setting

This study was carried out at Human Genetics Unit (HGU), Faculty of Medicine, Colombo. Patients were interviewed and examined in the HGU clinic room and blood was taken at the bleeding room.

4.3 Study Population

Patients referred for genetic testing for α thalassaemia to the HGU by Consultant Haematologists/ Paediatricians/ Physicians and General Practitoners, from 1st October, 2012.

4.4 Inclusion criteria

Individuals who meet following criteria were recruited to the study.

- Individuals referred for alpha thalassaemia genetic studies who have had a full haematological work up for thalassaemia
- Sri Lankans by descent
- Individuals who give written informed consent directly or by proxy (parents/ guardians)
- Following genetic testing in the HGU laboratory those who were found to have $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions

4.5 Exclusion criteria

Individuals who met following criteria was excluded from the study

- Non Sri Lankan patients
- Individuals who did not agree to give written informed consent.
- Patients with normal HPLC levels with co-existing iron deficiency
- Patients who had not had quantification of haemoglobin types by HPLC
- Following genetic testing in the HGU laboratory those who were found not to have $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions

4.6 Obtaining of written informed consent

Written informed consent was taken from all the participants before the enrolment to the study**.** Each participant was given the information sheet containing essential details about the study and a consent form. The information sheet and the consent form was available in Sinhala, Tamil and English languages. They were given sufficient time to read and understand the information sheet. They were also given sufficient time to ask questions from the researchers and to take the decision on participation. In the cases minors below 18 years, informed consent was obtained from the parents.

Any participant were explained that they are free to withdraw his/her consent to participate in the study at any time, with no penalty or effect on medical care or loss of benefits. All participants were given contact details of the investigators and the Ethics Review Committee (ERC) for clarification of any doubts about the study.

4.7 Ethical considerations

Ethical clearance was obtained from Ethical Review Committee (ERC), Faculty of Medicine, University of Colombo (ERC 14/063). Appropriate measures were taken to ensure that consent is obtained in an ethical manner from all study participants. The patients were interviewed privately in a room to ensure privacy. Informed written consent was obtained after providing the necessary information and giving them time to make a decision in private.

The data collection booklet was designed to ensure confidentiality of information gathered. Soon after collecting the personal information, the identification pages were removed and filed separately. Only the identification number was used in the rest of the booklet. The electronic database containing the data only has the subject study number thus ensuring confidentiality. The database was password protected. The risk of minor bruising, pain and discomfort as a result of venaepuncture was minimized by performing under aseptic conditions by a trained phlebotomist or a doctor.

4.8 Data collection

Data was collected using an interviewer administered data collection booklet by the principal investigator at the time of recruitment. Relevant data was obtained directly from participants, parents or guardians and indirectly by referral letters, clinic notes, diagnostic cards and investigation reports. Collected data was entered to an electronic database, which was kept password protected. Personal details like name, address, date of birth, etc. were collected in order to keep a track on the participant and they were kept separately by the principal investigator under lock and key. These personal details that lead to identification of individuals were not entered in to the Database.

4.9 Sample collection

One sample of five milliliters of peripheral venous blood was drawn under sterile conditions at HGU by a doctor or a nurse**.** Blood samplee was put into an ethylene diaminetetraacetic acid (EDTA) container. The EDTA sample was stored at -80 ^{o}C until DNA extraction was done.

4.10 Haematological parameters

Red blood cell parameters

Following red cell parameters were obtained from full blood count reports available with patients which were performed using automated analyzers.

- Haemoglobin (Hb)
- Red blood cell count (RBC)
- Mean cell volume (MCV)
- Mean cell Hb(MCH)
- Mean cell Hb concentration (MCH)
- Red cell distribution width (RDW)

Full blood reports at the time of initial diagnosis and on follow up clinic visits/ admissions were used to obtain haematological parameters. In patients treated for iron deficiency anaemia, FBC reports after the correction of iron deficiency were obtained.

4.11 Haemoglobin quantification

Haemoglobin quantification data was obtained from HPLC reports performed using BioRad automated HPLC system. These tests had been done during work up for thalasaaemia, mainly to exclude beta thalassaemia.

4.12 Red cell morphology

Red blood cell morphology data were obtained from blood picture reports available with patients which were reported by a Consultant Haematologist.

4.13 Genotyping

4.13.1 DNA extraction

DNA extraction was done by QIAamp DNA Mini Kit™ using standard protocol according to manufacturer guidelines.DNA extraction was done using 200 µl of peripheral venous blood.

Digestion

Protinase K was used for proteolysis. According to manufacture'rs protocol, the activity of the protinase K solution was 600mAU/ml solution. Twenty microliters of protinase K was added to bottom of the 1.5 ml microcentrifuge tubes(Eppedorf tubes).Two hundred micro liters of whole blood and 200 µl of AL buffer solution was added to the tube and mixed by pulse vortexing for 15 seconds. The mixture was incubated for 10 minutes at 56 ^{0}C.

Binding

The mixture was briefly centrifuged to remove drops from the inside of the lid and 200 µl of 100% alcohol was added. It was mixed again by pulse vortexing for 15S and briefly centrifuged. Then the mixture was applied to the QIAamp Spin Column tube without wetting the rim. After closing the cap it was centrifuged for at 8000 rpm for one minute. Then the spin column was placed in a 2ml collection tube and the tube containing filtrate was discarded.

Washing

The spin column was opened , 500 µl of buffer AW1 solution added and centrifuged at 8000 rpm for 1 minute. Again the filtrate was discarded and the spin column was placed in another 2ml collection tube. Five hundred microliters of buffer AW2 solution was added and centrifuged at 14000 rpm for 3 minutes.

Elution

Filtrate was discarded and the spin column was placed in a 1.5 ml microcentrifuge tube . Two hundred microliters of buffer AE (elution buffer) was added to the spin column and incubated for 5 minutes at room temperature. Then it was centrifuged at 8000 rpm for 1 minute and the spin column was discarded. Microcentrifuge tube containing genomic DNA was stored at -20^0C.

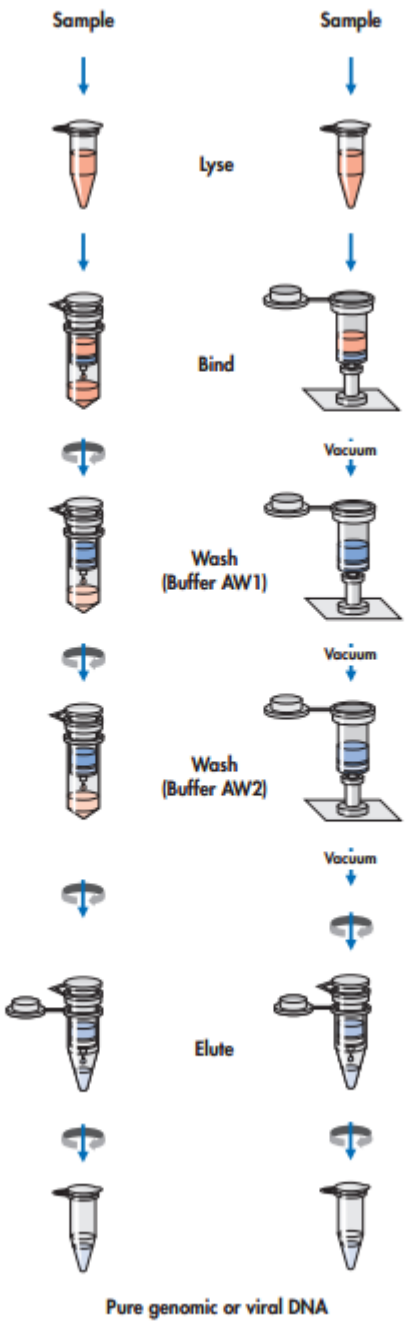

Figure 16: Steps in DNA Extraction

Abstracted from QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook© 1999-2003QIAGEN)

4.13.2 Genotyping

Genotyping for the –α3.7 and –α4.2 deletions were done using multiflex gap polymerase chain reaction (gap-PCR) methodology described by Chong *et al (*2000)[119].

Following set of primers were used in this PCR reaction.

α2/3.7-F	-	CCCCTCGCCAAGTCCACCC
3.7/20.5-R	-	AAAGCACTCTAGGGTCCAGCG
α2/R	-	AGACCAGGAAGGGCCGGTG
4.2-F	-	GGTTTACCCATGTGGTGCCTC
4.2-R	-	CCCGTTGGATCTTCTCATTTCCC

All the primers were initially reconstituted to the concentration of 100µM and diluted to the concentration of 10µM to be used in the PCR reaction.

Mixture for the PCR was made using following components

- dH_2O 5 µl
- MgCl2 (25mM) 1.5 µl
- 5 x PCR Buffer 5 µl
- dNTP's (1mM) 1.25 µl
- Betaine (5 M)[dilute 0.58g in 1ml of TE] 3.75 µl
- DMSO (10%) 1.25 µl
- α2/3.7-F 1.0 µl

- α2/20.5-R 1.0 µl
- α2-R 0.25 µl
- 4.2-F 1.0 µl
- 4.2-R 1.5 µl
- Taq polymerase 0.2 µl
- DNA 2.5 µl

The total volume of the PCR reaction was 25 µl

Polymerase chain reaction

PCR cycle was set to following conditions

Initial denaturation at 95˚C for 5 min.

Denaturation at 97 ˚C for 45 sec.

Annealing at 64˚C for 90 sec. 30 cycles

Extension at 72 ˚C for 2min. 15 sec.

Final extension at 72 ˚C for 3 min.

Cooling at 4 ˚C ∞

The PCR run consisted of 30 cycles.

Gel electrophoresis

Two percent agarose gel was made using 0.5 g of Agarose dissolved in 25 ml of 1x TBE buffer. Three microliters of ethidium bromide was added to the solution. Ten micro liters of PCR product was used in the gel run together with 3 µl of 1 kb ladder. Gel electrophoresis was set

at 70 mV for 45 minutes. At the end of the run gel image was captured under UV light using MegaCapt® software.

Interpretation of the gel image

The -$\alpha^{3.7}$ deletion in mutation produces a band with a size of 2020 bp while the –$\alpha^{4.2}$ deletion produces a band with a size of 1628 bp. A 1800 bp size band is produced in the absence of two deletions mentioned above[125].

Figure 17: Gel interpretation in multiplex gap-PCR for -$\alpha^{3.7}$ and –$\alpha^{4.2}$ deletions

4.14 Statistical analysis

Statistical Analysis was carried out using software SPSS statistics 17.0(SPSS inc). A p- value <0.05 was considered as statistically significant.

5 RESULTS

Thirty one individuals with four different genotypes($-\alpha^{3.7}/-\alpha^{3.7}$,$\alpha\alpha/-\alpha^{3.7}$, $\alpha\alpha/-\alpha^{4.2}$ and $-\alpha^{3.7}/-\alpha^{4.2}$) were analyzed in this study. Their age ranged between 12 months to 684 months. The mean age was 134.58(SD 178.36) months. Table 4 illustrates the sex composition of the subjects and Table 5 illustrates the ethnic composition of the subjects.

Table 4: Sex composition of the study subjects

Sex	Frequency	Percentage
Male	17	54.8%
Female	14	45.2%
Total	31	100%

Table 5: Ethnic composition of the study subjects

Ethnicity	Frequency	Percentage
Sinhalese	17	54.8%
Tamil	2	6.5%
Muslims	12	38.7%
Total	31	100%

Indication for testing for alpha thalassaemia were incidental finding of abnormal red cell indices suggestive of alpha thalassaemia(23, 74%), investigation of symptomatic patients(5,16%) and screening of family members (3, 10%). Figure 22 illustrates the indication for testing for alpha thalassaemia.

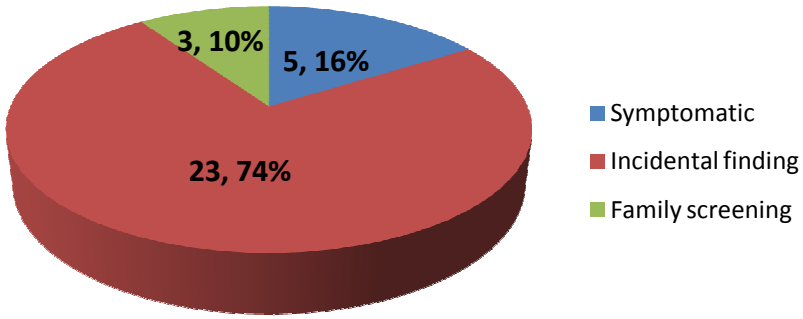

Figure 18: Indication for testing for alpha thalassaemia

Summary of each case of the cohort is given below . Reference range for RBC parameters were taken from Dacie and Lewis: Practical Haematology, 10th edition[131]

Case no: 1

Seven year old child is the third child born to a non consanguineous parents. He was found to have anaemia and abnormal red cell indices when investigated for fever and vomiting. Blood picture showed hypochromic microcytic red cells with many target cells. At diagnosis co-existing iron deficiency was suspected and treated with iron suppliments After six months of iron treatment his serum ferritin level found to be elevated[185ng/dl (6.24-137)]. HbH inclusions were detected and HPLC report was normal with no Hb variant detcted(92.4% of HbA, 1.6% of HbA_2 and 0.0 % of HbF). There was no evidence for heaptosplenomegaly. His RBC parameters are given below in Table 06.

Table 06: RBC parameters of case no:1

RBC parameter		Ref. range
Hb (g/dl)	9.3	13.5±2
RBC count(10^6/µl)	4.7	4.6±6
MCV(fl)	68.30	86±9
MCH (pg)	19.80	29±4
MCHC (g/dl)	32.20	34±3
RDW- CV(%)	15.8	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 2

Thirt four year old primi gravida was found to have persistently low Hb level. Haematinic deficiencies and other causes for anaemia were excluded. She is a product of non consanguineous marriage and there is no family history of thalassaemia or other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with few tear drop cells, target cells and occasional stomatocytes. Her HPLC report was normal with no Hb variant detcted (90% of HbA, 2.2% of HbA_2 and 0.6 % of HbF). Serum ferritin level was normal [17ng/ml(6.24-137ng/ml)].

Her RBC parameters are given below in the Table 7.

Table 7 : RBC parameters of case no:2

RBC parameter	Initial	Follow up	Ref. range
Hb (g/dl)	9.5	10.2	13.5±1.5 (in pregnancy 10.6-10.9)
RBC count(10^6/µl)	3.76	4.05	4.3±O.5
MCV(fl)	80.3	79.3	92±9
MCH (pg)	25.2	25.1	29.5±2.5
MCHC (g/dl)	31.5	31.7	33±1.5
RDW- CV(%)	15	16.1	12.8±1.2

She was presented for genetic studies at 33 weeks of gestation. Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 3

Three year old boy was found to have anaemia when investigated for fever. He is the first child born to a non consanguineous couple. There is no family history of thalassaemia or other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with few target cells. His serum ferritin level was normal [39.7 ng/ml(10-140)]. HPLC report was normal with no Hb variant detcted (86.9% of HbA, 2.6% of HbA_2 and 0.8 % of HbF).

His RBC parameters are given below in the Table 8.

Table 8: RBC parameters of case no: 3

RBC parameter		Ref. range
Hb (g/dl)	9.9	12.5±1.5
RBC count(10^6/µl)	4.71	4.6 ±0.6
MCV(fl)	68.7	81±6
MCH (pg)	21.0	27±3
MCHC (g/dl)	30.6	34±3
RDW- CV(%)	14.9	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 4

Two year old girl was found to have anaemia when investigated for fever. She is the second child born to a non consanguineous couple and there is no family history of thalassaemia or other haemoglobinopathy. Her blood picture showed hypochromic microcytic red cells with irregularly contracted, partially haemoglobinized cells, target cells and few pencil cells. Her serum ferritin level was normal [33.2 ng/ml(7-140)] and HbH inclusions were not detected. HPLC report was normal with no Hb variant detcted (85.2% of HbA, 2.4% of HbA_2 and 3 % of HbF).

Her RBC parameters are given below in the Table 9.

Table 09: RBC parameters of case no: 4

RBC parameter		Ref. range
Hb (g/dl)	10.1	12.5±1.5
RBC count(10^6/μl)	4.75	4.6 ±0.6
MCV(fl)	65.2	81±6
MCH (pg)	21.2	27±3
MCHC (g/dl)	32.6	34±3
RDW- CV(%)	16.6	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 5

Three year old boy was found to have anaemia when investigated for fever. He is the first child born to a non consanguineous parents and there is no family history of thalassaemia or other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with target cells. He was treated with haematinics for 8 months. His serum ferritin level after the iron treatment became normal [39.7 ng/ml(10-140)]. HPLC reportafter iron treatment was normal with no Hb variant detcted (89.2% of HbA, 2.8% of HbA_2 and 0.3 % of HbF).

His initial RBC parameters and post iron treatment parameters are given below in the Table 10.

Table 10: RBC parameters of case no: 5

RBC parameter	Initial	Post iron treatment	Normal values
Hb (g/dl)	7.9	10.0	12.5±1.5
RBC count(10^6/µl)	5.59	5.85	4.6 ±0.6
MCV(fl)	45.4	46.4	81±6
MCH (pg)	11.1	17.1	27±3
MCHC (g/dl)	31.1	35.0	34±3
RDW- CV(%)	27.90	22.8	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 6 , Case no: 7 and Case no: 8

One year and one month old child was found to have anaemia when investigated for fever. He is the third child born to a non consanguineous family and there is no family history of thalassaemia or any other haemoglobinopathy.

Blood picture showed hypochromic microcytic red cells with few target cells, tear drop cells and irregularly contracted cells. On HPLC his HbA_2 level was 2.9%.His serum ferritn level at the time of HPLC was normal [29.9 ng/ml (20-400)]. Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is a compound heterozygous for these two deletions(genotype $-\alpha^{3.7}/-\alpha^{4.2}$).

Father (Case no: 7) and mother (Case no: 8) of this child were also studied. Both parents had their Hb levels within in the normal range but had low MCH levels. Father (Case no: 7) had marginally low MCV level and high RBC count. Both father and mother had normal HPLC patterns and their serum ferritin levels at the time of HPLC were within the normal range(61

ng/ml and 33.1 ng/ml respectively). Blood picture of the case no: 7 showed normochromic normocytic red cells with a minor population of hypochromic microcytic cells. Case no : 8 also had a blood picture with predominantly normochromic normocytic red cells with occasional hypochromic microcytic cells. Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed the genotype $\alpha\alpha/-\alpha^{4.2}$ for case no: 7 and genotype $\alpha\alpha/-\alpha^{3.7}$ for case no:8.

Table 11 illustrates the RBC parameters of these individuals with reference ranges . Table 12 illstrates HPLC parameters of these three individuals.

Table 11: RBC parameters of case no: 6,7 and 8

RBC parameter	Case no: 6	Case no:7	Case no: 8
Hb (g/dl)	10.6(12.5±1.5)	14.8(15±2)	12.5(13.5±1.5)
RBC count(10^6/µl)	5.59(4.5±0.6)	5.88(5±0.5)	4.82(4.3±0.5)
MCV(fl)	56.2 (78±6)	79.6(92±9)	82.4(92±9)
MCH (pg)	19(27±2)	25.3(29.5±2.5)	25.9(29.5±2.5)
MCHC (g/dl)	33.8(34±2)	31.7(33±15)	31.4(33±15)
RDW- CV(%)	19.2	11.5(12.8±1.2)	13.1(12.8±1.2)

Table 12: HPLC parameters of case no: 6,7 and 8

HPLC parameter	Case no: 6	Case no:7	Case no: 8
HbA(%)	86	86	85.7
HbA_2 (%)	2.9	2.7	2.4
HbF (%)	0.7	0.2	0.2

Case no: 9

Two year old girl found to have anaemia when investigated for fever. She is the second child born to a non consanguineous family. There is no family history of thalassaemia or other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with few target cells. Serum ferritin level was normal [19 µg/l (12-190)]. HPLC report was normal with no Hb variant detcted[86.9% of HbA, 2.4% of HbA_2 and 1.4 % of HbF].

Her RBC parameters are given below in the Table 13.

Table 13 : RBC parameters of case no: 9

RBC parameter		Ref. range
Hb (g/dl)	8.8	12.5±1.5
RBC count(10^6/µl)	4.89	4.6 ±0.6
MCV(fl)	62.4	81±6
MCH (pg)	17.9	27±3
MCHC (g/dl)	28.80	34±3
RDW- CV(%)	15.2	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 10

Two year old boy was found to have abnormal red cell indices when investigated during an episode of febrile convulsion. He is the fourth child born to a non consanguineous family. There is no family history of thalassaemia or haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with target cells and partially haemoglobinized cells. HbH

inclusions were not detected. Serum ferritin level was normal [89.8 ng/ml(28-365)]. HPLC report was normal with no Hb variant detcted(86.3% of HbA, 2.4% of HbA_2 and 0.2 % of HbF).

His RBC parameters are given in the Table 14.

Table 14: RBC parameters of case no: 10

RBC parameter		Ref. range
Hb (g/dl)	11.5	12.5±1.5
RBC count(10^6/µl)	5.2	4.6 ±0.6
MCV(fl)	68.7	81±6
MCH (pg)	22.9	27±3
MCHC (g/dl)	33.4	34±3
RDW- CV(%)	15.7	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that he is heterozygous for $-\alpha^{4.2}$ ($\alpha\alpha/-\alpha^{4.2}$).

Case no:11

One year and five month old male child was found to have low haemoglobin levels while being investigated for hepatosplenomegaly. He is the second child born to a non consanguineous family. There is no family history of haemoglobinopathy. He was first came to medical attention due to abdominal distension. Ultrasound scan of the abdomen showed that liver is within the upper normal range(10cm) and minimal splenomegaly (9cm). His initial blood picture showed hypochromic microcytic red cells with acanthocytes. White blood cells showed lymphocyte predominance and many abnormal blasts. He underwent bone marrow aspiration

biopsy which showed active marrow with erythroid hyperplasia with no evidence suggestive of acute leukaemia. It also suggested to exclude a possibility of a haemoglobinopathy.

Follow up blood picture after one month was suggestive of iron deficiency anaemia with or without thalassaemia trait. Red cells were hypochromic and microcytic with some pencil cells, tear drop cells and polychromatic cells. Serum ferritin level was normal(26.1 ng/ml). HPLC was normal with no Hb variant detected(87.8% of HbA, 2.4% of HbA_2 and 0.9% of HbF).

Table 15 illustrates the RBC parameters of case no 11.

Table 15: RBC parameters of case no: 11

RBC parameter		Ref. range
Hb (g/dl)	10.8	12.5±1.5
RBC count(10^6/µl)	4.28	4.5±0.6
MCV(fl)	62.1	78±6
MCH (pg)	25.3	27±2
MCHC (g/dl)	40.8	34±2
RDW- CV(%)	19.8	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 12

Twenty four year old lady is the mother of case no : 11. She is the third child born to non consanguineous parents. There is no family history of anaemia, thalasseamia or haemoglobinopathy. She was found to have low Hb and low RBC indices while investigated for her son's anaemia. Blood picture showed hypochromic microcytic red cells with a sub population of normochromic normocytic cells ,few pencil cells and irregularly contracted cells.

Her serum ferritin level was normal [73.6 ng/ml(15-200] and HPLC was normal with no Hb varint detected(88.5% of HbA, 2.6% of HbA_2 and 0.1 % of HbF).

Table 16 illustrates her RBC parameters.

Table 16: RBC parameters of case no : 12

RBC parameter		Ref. range
Hb (g/dl)	10.8	13.5±1.5
RBC count(10^6/μl)	5.12	4.3±0.5
MCV(fl)	68.7	92±9
MCH (pg)	21.2	29.5±2.5
MCHC (g/dl)	30.8	33±1.5
RDW- CV(%)	13.4	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 13

Seven year old girl found to have anaemia when investigated for fever. She is the first child born to a non consanguineous parents. She had a Hb of 7.5 g/dl on admission and lowered to 6.5 g/dl 5 days after the admission. On admission her reticulocyte count found to be elevated(9.8%).There was no hepatosplenomegaly or jaundice. She did not give a preveous history of episodes of haemolysis.

Blood picture during acute episode showed predominantly hypochromic microcytic red cells irregularly contracted cells , target cells and nucleated red blood cells. Many polychromatic cells were also seen. This blood picture was compatible with a ongoing haemolysis. Repeat FBC and blood picture during same admission found to have a relatively high red cell count with hypochromic microcytic cells, target cells, tear drop poikilocytes, pencil cells, irregularly contracted cells and occasional partially haemoglobinized cells. Testing for HbH inclusions were not done. On discharge she was started on iron treatment. After three months of iron treatment her serum ferritin level was 187 ng/ml(10-60 ng/ml) and iron treatment was discontinued. HPLC was normal with no Hb varints(84.2% of HbA, 1.9% of HbA_2 and 0.2 % of HbF). Co-existing acquired and hereditary causes for haemolysis was not found.

Table 17 shows her RBC parameters and reticulocyte counts.

Table 17: RBC parameters and reticulocyte count values of case no:13

RBC parameter	9/7/13	7/8/13	Ref.range
Hb (g/dl)	7.5	10.9	13.5±2
RBC count(10^6/µl)	3.52	4.44	4.6±6
MCV(fl)	75	81	86±9
MCH (pg)	22.6	24.5	29±4
MCHC (g/dl)	28.4	30.3	34±3
RDW- CV(%)	20.8	20.4	12.8±1.2
	-	-	
Reticulocyte Count	9.8	13.2	

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that she is heterozygous for $-\alpha^{3.7}$ ($\alpha\alpha/-\alpha^{3.7}$).

Case no: 14

Three year old boy found to have anaemia and abnormal red cell indices incidentally while being investigated for failure to thrive. He was the second child born to a non consanguineous parents. There is no family history of thalassaemia or other haemoglobinopathy. He was delivered at 31 weeks of gestation with a birth weight of 1.95 kg due to maternal pre-eclampsia complicated pregnancy.

His Hb level at presentation was 10.9 g/dl. Blood picture was compatible with thalassaemia trait and showed hypochromic microcytic red cells with pencil cells, target cells and partially haemoglobinized cells. His serum ferritin level was found to be low normal[25.7 µg/l(25-240)]. HPLC was normal with no Hb Variants[85.7% of HbA, 2.5% of HbA_2 and 0.4 % of HbF].

Table 18 illustrates the RBC parameters of case no: 14.

Table 18: RBC parameters of case no: 14

RBC parameter		Ref. range
Hb (g/dl)	10.9	12.5±1.5
RBC count(10^6/µl)	5.6	4.6 ±0.6
MCV(fl)	62.60	81±6
MCH (pg)	19.40	27±3
MCHC (g/dl)	31.10	34±3
RDW- CV(%)	15.80	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 15

This one year seven month old boy was found to have a hypochromic microcytic blood picture while being investigated for a bleeding diathesis. He had prolonged bleeding following circumcision and ecchymosis. He was subsequently diagnosed as having haemophilia A(factor VIII level- 0.9%).

Blood picture showed predominantly hypochromic microcytic red cells with occasional pencil cells and tear drop cells. Testing for HbH inclusion bodies found to be negative. His serum ferritin level was normal [17.9 ng/ml(7-140)]. HPLC was normal with no Hb variants[86.4% of HbA, 2.7% of HbA_2 and 1 % of HbF].

Table 19 illustrates his RBC parameters.

Table 19: RBC parameters of case no:15

RBC parameter		Ref. range
Hb (g/dl)	11.1	12.5±1.5
RBC count(10^6/μl)	4.4	4.5±0.6
MCV(fl)	66.7	78±6
MCH (pg)	20.8	27±2
MCHC (g/dl)	32	34±2
RDW- CV(%)	16.6	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that he is heterozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no:16

Fourteen year old boy was found to have anaemia incidentally while being investigated for poor weight gain and poor tolerance of physical activities. He is the first child born to a non

consanguineous parents and he has younger brother who is healthy. There is no family history of thalassaemia or other haemoglobinopathy.

His initial blood picture showed mildly hypochromic red cells with target cells and acanthocytes. His serum ferritin levels found to be low and he was started on iron treatment. Serum ferritin had increased from 11.3 ng/ml to 28.2 ng/ml after 9 months of iron treatment. Repeat blood picture after the iron treatment showed predominantly normochromic normocytic red cells with a small population of hypochromic microcytic cells. HPLC after iron treatment was normal [86.8% of HbA, 2.5% of HbA_2 and 0.0 % of HbF]. HbH inclusions were not detected.

Table 20 illustrates his RBC parameters.

Table 20: RBC parameters of case no: 16

RBC parameter	Initial	Post iron treatment	Ref. range
Hb (g/dl)	10.5	12.3	15±2
RBC count(10^6/µl)	5.29	5.56	5±0.5
MCV(fl)	67	67.2	92±9
MCH (pg)	19.8	21.8	29.5±2.5
MCHC (g/dl)	29.6	32.5	33±1.5
RDW- CV(%)	15.3	14.8	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$)

Case no: 17

One year five month old boy was found to have anaemia (7.7 g/dl) while being investigated prior to an orchidopexy surgery at the age of one year and two months. His RBC indices were also found to be abnormal. He is the second child born to a non consanguineous family. Apart from his paternal grandmother is a diagnosed β thalassaemia carrier. She had been diagnosed at the age of 55 year. He has developmental delay, growth faltering, chronic constipation, atrial septal defect and left sided undescended testis. He also has a disproportionally large head but ultrasound scan of the brain did not show evidence of hydrocephalus.

Blood picture showed predominantly hypochromic microcytic red cells with some target cells, macrocytes, many polychromatic cells and few nucleated red cells. Serum ferritin was normal [291 ng/ml(20-400)]. HPLC report showed elevated HbF level[5.3% of HbA, 1.8% of HbA_2 and 96.2 % of HbF] compatible with either hereditary persistence of fetal haemoglobin(HPFH) or a homozygous δβ thalassaemia.

His initial RBC parameters are given in the Table 21

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Table 21 : RBC parameters of case no: 17

RBC parameter		Ref. range
Hb (g/dl)	7.7	12.5±1.5
RBC count(10^6/μl)	3.36	4.5±0.6
MCV(fl)	71.7	78±6
MCH (pg)	22.9	27±2
MCHC (g/dl)	32.0	34±2
RDW- CV(%)	17.1	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 18

One year four month old child was found to have anaemia and abnormal red cell indices while being investigated for fever at six months of age. She is the first child born to a non consanguineous parents. Her initial Hb level was 9.5 g/dl and blood picture showed hypochromic microcytic red cells. She was treated with haematinics for 6 months but there was only a little improvement in her Hb level with minor changes in red cell indices.HPLC report was mormal[86.0% of HbA, 2.7% of HbA_2 and 1.9 % of HbF].

Her initial and post iron treatment RBC parameters are given in the Table 22 .

Table 22: RBC parameters of case no: 18

RBC parameter	Initial	Post iron treatment	Ref. range
Hb (g/dl)	9.5	10.2	12.5±1.5
RBC count(10^6/µl)	4.14	4.21	4.5±0.6
MCV(fl)	74.3	73.8	78±6
MCH (pg)	22.9	24.1	27±2
MCHC (g/dl)	30.8	32.7	34±2
RDW- CV(%)	18.4	16.3	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 19

Three year three month old girl was incidentally found to have anaemia and abnormal red cell indices when investigated for fever. She is the third child born to a non consanguineous family. Her elder brother was also being investigated for similar red cell abnormalities.

Her blood picture showed hypochromic microcytic red cells with occasional contracted cells and target cells. She was treated with iron suppliments for six months. HPLC report was normal[85.7% of HbA, 2.9% of HbA_2 and 1.7 % of HbF]. Serum ferritin level was within the normal range[56 ng/ml(20-400)]

Her RBC parameters are given below in the Table 23.

Table 23: RBC parameters of case no: 19.

RBC parameter	Initial	Post iron treatment	Ref. range
Hb (g/dl)	10.2	11.5	12.5±1.5
RBC count(10^6/μl)	5.03	5.59	4.6±0.5
MCV(fl)	65.6	65.5	81±6
MCH (pg)	20.3	20.6	27±3
MCHC (g/dl)	30.9	31.5	34±3
RDW- CV(%)	17.2	18	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that he is heterozygous for $-\alpha^{3.7}$ deletion ($\alpha\alpha/-\alpha^{3.7}$).

Case no: 20

This six year old boy found to have anaemia and low red cell indices when investigated for fever. He is the third child born to a non consanguineous parents .Blood picture showed markedly hypochromic microcytic red cells with occasional pencil cells, fragmented red cells, polychromatic cells and oval macrocytes. His serum ferritin level was low [2.98 ng/ml(10-60)] and he was given haematinic supplements for 8 months. Post iron treatment serum ferritin level was 19.2 ng/ml and HPLC report was normal[87% of HbA, 2.3% of HbA_2 and 0.0% of HbF]. His initial and post iron treatment RBC parameters are given in the Table 24.

Table 24: RBC parameters of case no: 20

RBC parameter	Initial	Post iron treatment	Normal values
Hb (g/dl)	9.3	10.5	12.5±1.5
RBC count(10^6/µl)	5.85	4.89	4.6±0.6
MCV(fl)	51.7	63	81±6
MCH (pg)	15.9	21	27±3
MCHC (g/dl)	30.8	34.1	34±3
RDW- CV(%)	20.2	16	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that he is heterozygous for $-\alpha^{3.7}$ deletion ($\alpha\alpha/-\alpha^{3.7}$).

Case no:21

Eight year old boy was incidentally found to have low Hb and abnormal red cell indices when investigated for fever. He is the first child born to non consanguineous parents. There was no

family history of thalassaemia or any other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with few contracted red cells and tear drop cells. His HPLC report was normal[85% of HbA, 2.9% of HbA_2 and 0.8% of HbF]. Serum ferritin level was normal (30.2 ng/ml).

His RBC parameters are given below in the Table 25.

Table 25: RBC parameters of case 21

RBC parameter		Ref. range
Hb (g/dl)	11	13.5±2
RBC count(10^6/µl)	4.57	4.6±0.6
MCV(fl)	71.8	86±9
MCH (pg)	24.1	29±4
MCHC (g/dl)	33.6	34±3
RDW- CV(%)	18.3	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that he is homozygous for $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 22

Two year and two month old girl found to have low haemoglobin levels when investing for fever. She is the first child born to a consanguineous family. There is no family history of thalassaemia orhaemoglobinopathy.. Blood picture showed hypochromic microcytic red blood

cells. HPLC performed after 6 months of iron treatment was normal [84.7% of HbA , 2.6% of HbA_2 and 0.5% of HbF].

Table 26 illustrates initial and post iron treatment RBC parameters.

Table 26: RBC parameters of case no: 22

RBC parameter	At presentation	Post iron treatment	Ref. range
Hb (g/dl)	9	11.8	12.5±1.5
RBC count(10^6/µl)	4.35	4.62	4.6±0.5
MCV(fl)	67.9	76.7	81±6
MCH (pg)	20.6	25.6	27±3
MCHC (g/dl)	30.4	33.4	34±3
RDW- CV(%)		16.9	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is heterozygous for $-\alpha^{4.2}$ ($\alpha\alpha/-\alpha^{4.2}$).

Case no: 23

Two year eight month old girl is the first child born to a non consanguineous parents. She was found to have anaemia at age one year of age. She was started on haematinics and it was discontinued after two months due to poor tolerance. After two months of iron treatment her Hb level was increased from 7 to 8.8 g/dl .

At the age of two years and eight months she had been admitted to the local hospital with fever, pallor and abdominal distension. On admission her Hb level was 5.1 g/dl with a retic count of 3.5% and two units of packed red cells were transfused. Blood picture showed severe

hypochromic microcytic red cells. She had hepatosplenomegaly. Blood sample for HPLC was taken prior to transfusin and it was compatible with HbH disease. It showed 11.7% and 14.5% of Hb at the retention times of 44s and 68s respectively and 68% of HbA and 0.8% of HbA_2. Her serum ferritin level was 152 ng/ml(8-140 ng/ml). Her Initial RBC parameters are given below in the Table 27.

Table 27: RBC parameters of case no:23.

RBC parameter		Ref. range
Hb (g/dl)	7.0	12.5±1.5
RBC count(10^6/µl)	4.38	4.6±0.5
MCV(fl)	52.8	81±6
MCH (pg)	15.9	27±3
MCHC (g/dl)	30.3	34±3
RDW- CV(%)	26.6	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is heterozygous for $-\alpha^{3/7}(\alpha\alpha/-\alpha^{3.7})$.

Case no: 24

Seventeen year old girl found to low Hb while being investigated for faintishness. She was also having menorrhagia which lasts for 8 days or more. She is the third child born to a non consanguineous parents and there is no family history of thalasseamia or other haemoglobinopathy. Her Hb level at presentation was 8g/dl. Blood picture showed markedly hypochromic microcytic red cells with pencil cells. Her serum ferritin level was low [7 ng/ml(8-

140)] and she was given iron treatment for 6 months.. HPLC following iron treatment was normal[87.1%. of HbA, 2.5% of HbA_2 and 0.5% of HbF]. Table 28 illustrates the RBC parameters post iron treatment.

Table 28: RBC parameters of case no: 24 after iron treatment

RBC parameter		Ref. range
Hb (g/dl)	11.4	13.5±1.5
RBC count(10^6/µl)	4.81	4.3±0.5
MCV(fl)	70.1	92±9
MCH (pg)	23.60	29.5±2.5
MCHC (g/dl)	33.70	33±15
RDW- CV(%)	27.2	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is heterozygous for $-\alpha^{4.2}$ ($\alpha\alpha/-\alpha^{4.2}$).

Case no: 25

One year and five month old girl found to have anaemia while being investigated for failure to thrive. She is the third child born to a non consanguineous parents. Her initial Hb level was10.3 g/dl and red cell indices were low. Blood picture showed hypochromic microcytic red cells with few target cells and irregularly contracted cells. She was treated for iron deficiency anaemia for 6 months and post iron treatment HPLC was normal [87.6 % of HbA, 2.3% of HbA_2 and 0.6% of HbF] and serum ferritin level was 69 ng/ml, which was within the normal range.

Her initial and post iron treatment RBC parameters are given in the Table 29.

Table 29: RBC parameters of case no:25

RBC parameter	Initial	Post iron treatment	Ref. range
Hb (g/dl)	10.3	12.2	12.6±1.5
RBC count(10^6/µl)	5.3	5.9	4.5±0.6
MCV(fl)	62.3	62.6	78±6
MCH (pg)	19.4	20.6	27±2
MCHC (g/dl)	31.2	33	34±2
RDW- CV(%)	18	16.9	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is heterozygous for $-\alpha^{4.2}$ ($\alpha\alpha/-\alpha^{4.2}$).

Case no: 26

Twenty eight year old female with mild anaemia since childhood and had been on haematinics on and off. However she had not undergone investigations for anaemia before. She is the eldest child born to a non consanguineous parents with four children. Her mother and a maternal; aunt also gives a similar history of mild anaemia. Blood picture showed hypochromic microcytic cells with few target cells , irregularly contracted cells and acanthocytes. Her serum ferritin level was low [3.45 ng/ml_(5-148)]. HPLC report following iron treatment was normal [89.1% of HbA, 2.4% of HbA_2 and 0.0% of HbF]

Table 30 illustrates RBC parameters of case no 26.

Table 30: RBC parameters of case no: 26

RBC parameter		Ref. range
Hb (g/dl)	10.9	13.5±1.5
RBC count(10^6/µl)	4.22	4.3±0.5
MCV(fl)	77.5	92±9
MCH (pg)	25.8	29.5±2.5
MCHC (g/dl)	33.3	33±15
RDW- CV(%)	19.8	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed she is heterozygous for $-\alpha^{3.7}$ ($\alpha\alpha/-\alpha^{3.7}$).

Case no: 27

Six year old child , who was initially investigated for fever found to have abnormal red cell indices. He is the first child born to a non consanguineous family. There is a no family history of thalassaemia or other haemoglobinopathy. Blood picture showed hypochromic microcytic red cells with few target, tear drop and partially haemoglobinized cells. Serum ferritin level normal [54 ng/ml(25-240)]. HPLC report was normal (87.2% of HbA , 2.3% of HbA_2 and 0.9% of HbF).

Table 31 illustrates his RBC parameters.

Table 31: RBC parameters of case no:27

RBC parameter		Ref. range
Hb (g/dl)	11.6	13.5±2
RBC count(10^6/µl)	5.4	4.6±0.6
MCV(fl)	69.6	86±9
MCH (pg)	21.4	29±4
MCHC (g/dl)	30.8	34±3
RDW- CV(%)	13.7	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed he is a compound heterozygote. ($-\alpha^{3.7}/-\alpha^{4.2}$).

Case no: 28

One year four month old child was found to have low Hb and low red cell indices when investigated for fever. He is the first child born to a non consanguineous parents. There is no family history of thalassaemia or other haemoglobinopathies. Blood picture showed predominantly hypochromic microcytic red cells. with some elongated poikilocytes and oval macrocytes. Serum ferritin level was normal [42.4 ng/ml(8-140 ng/ml)]. HPLC report was normal(85.2% of HbA , 2.5% of HbA_2 and 1.5% of HbF).

Table 32 illustrates the RBC parameters of case no: 28.

Table 323: RBC parameters of case no: 28

RBC parameter		Ref. range
Hb (g/dl)	10.2	12.6±1.5
RBC count(10^6/µl)	5.11	4.5±0.6
MCV(fl)	63.4	78±6
MCH (pg)	20.0	27±2
MCHC (g/dl)	31.5	34±2
RDW- CV(%)	15.9	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed that she is heterozygous for $-\alpha^{3.7}$ ($\alpha\alpha/-\alpha^{3.7}$).

Case no : 29

Forty one year old person was the fifth child born to a non consanguineous parents. There is no family history of anaemia or haemoglobinpathy. He was free of any anaemic symptoms throughout his childhood an early adulthood until anaemia(Hb 6.6 gldl) was detected incidentally at the age of 23 years when he presented to the hospital with a history of fever of four days. On examination he had pallor, cervical and axillary lymphadenopathy and hepatosplenomegaly. Blood picture and bone marrow biopsy showed features suggestive of severe iron deficiency anaemia with megaloblastoid changes. He had been given haematinics. He remained asymptomatic for another eleven years and at the age of 34 he was admitted to the hospital with a severe headache and fever of 2 weeks duration. On examination he was pale, icteric and had hepatosplenomegaly. His haemoglobin level had been 7.5g/dl and blood picture showed features suggestive of thalassaemia intermedia. HbH inclusions were detected following

staining with brilliantcresyl blue. HPLC report was normal(86.2% of HbA , 1.9% of HbA_2 and 0.3 % of HbF).

At the age of 38 years he was admitted to the hospital with pallor, icterus and excertional dyspnoea.His Hb level was 6.7 g/dl and he was transfused with three units of packed red cells. Since then he had been transfused during six episodes and each times three units packed red cells was transfused. His serum ferritin level found to be elevated [2112 ng/ml (20-400)] and started on oral deferisirox. After 9 months of deferasirox treatment his ferritin levels was lowered to 1024 ng /ml. At present he is on deferasirox 1600 mg daily. Following cardiological referral 2D echocardiogram was performed and it was found to be normal. Ultrasound scan of the abdomen showed mild hepatomegaly with moderately enlarged spleen . He was last transfused in May 2014 following he presented to the hospital with pallor, icterus and excertional dyspnoea. His Hb level was 5.3 g/dl. Blood picture showed severe hypochromic microcytic red cells with some target cells, tear drop cells and partially haemoglobinzed cells He is also a diagnosed patient with diabetes mellitus for two years and currently on insulin mixtrad.

Table 33 shows his RBC parameters prior to a transfusion.

Table 33: RBC parameters of case no: 29

RBC parameter		Ref. range
Hb (g/dl)	6.10	15±2
RBC count(10^6/μl)	4.36	5±0.5
MCV(fl)	55.7	92±9
MCH (pg)	14.0	29.5±2.5
MCHC (g/dl)	25.1	33±1.5
RDW- CV(%)	25.5	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$)

Case no:30

One year three month old boy found to have low red cell indices when investigated for fever. He is the first child born to a non consanguineous family. There is no family history of thalassaemia or other haemoglobinopathy. Blood picture of this child showed predominantly normochromic normocyti red cells with some hypochromic microcytic cells, acanthocytes and occasional partially haemoglobinized cells. Serum ferritin level was normal (34 μg/l). HPLC report was normal(87.7% of HbA , 2.7% of HbA_2 and 0.4 % of HbF).

His RBC parameters are given below in the Table 34.

Table 34: RBC parameters of case no: 30

RBC parameter		Ref. range
Hb (g/dl)	11.6	12.6±1.5
RBC count(10^6/μl)	5.55	4.5±0.6
MCV(fl)	64.6	78±6
MCH (pg)	20.8	27±2
MCHC (g/dl)	32.3	34±2
RDW- CV(%)	17.2	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions showed he is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$)

Case no: 31

Fifty seven year old female found to have red cell indices suggestive of thalassaemia while being investigated for multiple joint pains. Her daughter and two of her grand children are

currently being investigated for anaemia. Hb levels improved after iron treatment. However rest of her family is not investigated further. Blood pictures showed hypochromic microcytic red cells with pencil cells. HPLC showed HbA 85.2% HbA_2- 2.3% and HbF- 0.5%.

Table 35 illustrates RBC parameters of case no: 31.

Table 35 : RBC parameters of case no: 31

RBC parameter		Ref. range
Hb (g/dl)	11	13.5±1.5
RBC count(10^6/µl)	5.0	4.3±0.5
MCV(fl)	69.2	92±9
MCH (pg)	22	29.5±2.5
MCHC (g/dl)	31.8	33±1.5
RDW- CV(%)	19	12.8±1.2

Genotyping for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ mutations showed that she is homozygous for $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$).

Table 36 illustrates the summary of all 31 individuals.

Table 36 : Summary of cases

No:	Age-months	Sex	Ethnicity	Indication	FBC parameters	HPLC	Blood picture	Genotype
1	84	M	Muslim	Incidental finding while investigated for fever	Hb -9.3 g/dl RBC- 4.7x 10^6/µl MCV- 68.3 fl MCH- 19.8 pg MCHC- 32.2 g/dl RDW- 15.8%	HbA-92.4% HbA2-1.6% HbF-0	hypochromic microcytic red cells with many target cells	$-\alpha^{3.7}/-\alpha^{3.7}$
2	364	F	Sinhalese	Incidental finding while investigated in Pregnancy	Hb -10.2 g/dl RBC- 4.05x10^6/µl MCV- 79.3 fl MCH- 25.1 pg MCHC- 31.7 g/dl RDW- 17.1%	HbA-90 % HbA_2-2.2% HbF-0.6 %	hypochromic microcytic red cells with few tear drop cells target cells and occasional stomatocytes	$-\alpha^{3.7}/-\alpha^{3.7}$
3	36	M	Sinhalese	Incidental finding while investigated for fever	Hb -9.9 g/dl RBC- 4.71x10^6/µl MCV- 68.7 fl MCH- 21pg MCHC- 30.6g/dl RDW- 14.9%	HbA-86.9 % HbA_2-2.6% HbF-0.8 %	hypochromic microcytic red cells with few target cells	$-\alpha^{3.7}/-\alpha^{3.7}$
4	24	F	Sinhalese	Incidental finding while investigated for fever	Hb - 10.1g/dl RBC- 4.57x10^6/µl MCV- 65.2 fl MCH- 21.2 pg MCHC- 32.6g/dl RDW-16.6 %	HbA- 85.2% HbA_2- 2.4% HbF- 3%	hypochromic microcytic red cells with irregularly contracted cells, partially haemoglobinized cells, target cells and few pencil cells	$-\alpha^{3.7}/-\alpha^{3.7}$

5	24	M	Sinhalese	Incidental finding while investigated for fever	Hb -10 g/dl RBC- $5.85x10^6/\mu l$ MCV- 46.4 fl MCH- 17.1 pg MCHC- 35 g/dl RDW- 22.8%	HbA-89.2 % HbA_2-2.8% HbF- 0.3%	hypochromic microcytic red cells with target cells	$-\alpha^{3.7}/-\alpha^{3.7}$
6	12	M	Muslim	Incidental finding while investigated for fever	Hb - 10.6 g/dl RBC- $5.59x10^6/\mu l$ MCV- 56.2 fl MCH- 19 pg MCHC- 33.8 g/dl RDW- 19.2%	HbA- 86% HbA_2-2.9% HbF- 0.7%	hypochromic microcytic red cells with few target cells, tear drop cells and irregularly contracted cells.	$-\alpha^{3.7}/-\alpha^{4.2}$
7	480	M	Muslim	Family screening	Hb -14.8 g/dl RBC- $5.88x10^6/\mu l$ MCV- 79.6 fl MCH- 25.3 pg MCHC- 31.7 g/dl RDW-11.5 %	HbA-86 % HbA_2- 2.7% HbF- 0.2%	normochromic normocytic red cells with a minor population of hypochromic microcytic cells	$\alpha\alpha/-\alpha^{4.2}$
8	372	F	Muslim	Family screening	Hb -12.5 g/dl RBC- 4.82 $x10^6/\mu l$ MCV- 82.4fl MCH- 25.9pg MCHC- 31.4 g/dl RDW- 13.1%	HbA-85.7 % HbA_2-2.4% HbF- 0.2%	predominantly normochromic normocytic red cells with occasional hypochromic microcytic	$\alpha\alpha/-\alpha^{3.7}$

9	24	F	Sinhalese	Incidental finding while investigated for fever	Hb -8.8 g/dl RBC- $4.89x10^6/\mu l$ MCV- 62.4 fl MCH- 17.9pg MCHC-28.2 g/dl RDW- 15.2%	HbA-86.9 % HbA_2-2.4% HbF- 1.4%	hypochromic microcytic red cells with few target cells	$-\alpha^{3.7}/-\alpha^{3.7}$
10	24	M	Muslim	Incidental finding while investigated for fever	Hb - 11.5g/dl RBC- $5.2x10^6/\mu l$ MCV- 68.7 fl MCH- 22.9 pg MCHC- 33.4 g/dl RDW-15.7 %	HbA- 86.3% HbA_2-2.4% HbF-0.2 %	hypochromic microcytic red cells with target cells and partially haemoglobinized cells	$\alpha\alpha/-\alpha^{4.2}$
11	12	M	Muslim	Investigated for hepatosplenomegaly	Hb -10.8 g/dl RBC- $4.28x10^6/\mu l$ MCV- 62.1 fl MCH- 25.3pg MCHC- 40.8 g/dl RDW- 19.8%	HbA-87.8 % HbA_2-2.4% HbF-0.9 %	hypochromic microcytic red cells with some pencil cells, tear drop cells and polychromatic cells	$-\alpha^{3.7}/-\alpha^{3.7}$
12	288	F	Muslim	Family Screening	Hb - g/dl RBC- $10^6/\mu l$ MCV- fl MCH- pg MCHC- g/dl RDW- %	HbA- 88.5% HbA_2-2.6% HbF-0.1 %	hypochromic microcytic red cells with a sub population of normochromic normocytic cells ,few pencil cells and irregularly contracted cells	$-\alpha^{3.7}/-\alpha^{3.7}$
13	84	F	Sinhalese	Incidental finding while investigated for fever	Hb -10.9 g/dl RBC- $4.44x10^6/\mu l$ MCV- 81fl MCH- 24.5 pg MCHC- 30.3g/dl RDW- 20.4%	HbA-84.2 % HbA_2-1.9% HbF-0.2 %	hypochromic microcytic cells, target cells, tear drop cells, pencil cells irregularly contracted cells and occasional partially haemoglobinized cells	$\alpha\alpha/-\alpha^{3.7}$

14	36	M	Sinhalese	Incidental finding while investigated for failure to thrive	Hb - 10.9g/dl RBC- $5.6x10^6/\mu l$ MCV- 62.6 fl MCH- 19.4pg MCHC- 31.1 g/dl RDW-15.8 %	HbA- 85.7% HbA_2-2.5% HbF- 0.4%	hypochromic microcytic red cells with pencil cells, target cells, partially haemoglobinized cells and irregularly contracted cells	$-\alpha^{3.7}/-\alpha^{3.7}$
15	19	M	Muslim	Incidental finding while investigated for bleeding disorder	Hb -11.1 g/dl RBC- $4.4x10^6/\mu l$ MCV- 66.7 fl MCH- 20.8 pg MCHC- 32 g/dl RDW- 16.6%	HbA- 86.4% HbA_2-2.7% HbF- 1%	predominantly hypochromic microcytic red cells with occasional pencil cells and tear drop cells	$-\alpha^{3.7}/-\alpha^{3.7}$
16	168	M	Sinhala	Incidental finding while investigated for failure to thrive	Hb -12.3 g/dl RBC- $5.56x10^6/\mu l$ MCV- 67.2 fl MCH- 21.8pg MCHC- 32.5 g/dl RDW-14.8 %	HbA- 86.8% HbA_2-2.5% HbF-0 %	predominantly normochromic normocytic red cells with a sub population of hypochromic microcytic cells	$-\alpha^{3.7}/-\alpha^{3.7}$
17	17	M	Muslim	Incidental finding while investigated before a surgery	Hb -7.7 g/dl RBC- $3.36x10^6/\mu l$ MCV- 71.7fl MCH- 22.9 pg MCHC- 32g/dl RDW- 17.1%	HbA- 5.3% HbA_2-1.8% HbF- 96.2%	predominantly hypochromic microcytic red cells with some target cells, macrocytes, many polychromatic cells and few nucleated red cells	$-\alpha^{3.7}/-\alpha^{3.7}$
18	16	F	Muslim	Incidental finding while investigated for fever	Hb -10.2 g/dl RBC- $4.21x10^6/\mu l$ MCV- 73.8fl MCH- 24.1 pg MCHC- 32.7 g/dl RDW- 16.3%	HbA-86 % HbA_2-2.7% HbF- 1.9%	hypochromic microcytic red cells	$-\alpha^{3.7}/-\alpha^{3.7}$

19	39	F	Sinhalese	Incidental finding while investigated for fever	Hb - 11.5g/dl RBC- $5.59x10^6/\mu l$ MCV- 65.5fl MCH- 20.6pg MCHC- 31.5 g/dl RDW- 18%	HbA-85.7 % HbA_2-2.9% HbF- 1.7%	hypochromic microcytic red cells with occasional contracted cells and target cells	$\alpha\alpha/-\alpha^{3.7}$
20	72	M	Muslim	Incidental finding while investigated for fever	Hb -10.5 g/dl RBC- $4.89x10^6/\mu l$ MCV- 63 fl MCH- 21 pg MCHC- 34.1 g/dl RDW- 16%	HbA-87 % HbA_2-2.3% HbF-0 %	markedly hypochromic microcytic red cells Few pencil cells, fragmented red cells, polychromatic cells and some macrocytes	$\alpha\alpha/-\alpha^{3.7}$
21	96	M	Sinhalese	Incidental finding while investigated for fever	Hb - g/dl RBC- $10^6/\mu l$ MCV- fl MCH- pg MCHC- g/dl RDW- %	HbA-85 % HbA_2-2.9% HbF- 0.8%	hypochromic microcytic red cells with few contracted cells and tear drop cells.	$-\alpha^{3.7}/-\alpha^{3.7}$
22	26	F	Sinhalese	Incidental finding while investigated for fever	Hb -11.8 g/dl RBC-$4.62x10^6/\mu l$ MCV- 76.7 fl MCH- 25.6 pg MCHC-33.4 g/dl RDW- 16.9%	HbA- 84.7% HbA_2-2.6% HbF-0.5 %	hypochromic microcytic red blood cells	$\alpha\alpha/-\alpha^{4.2}$
23	36	F	Muslim	Investigated for fever and hepatosplenomegaly	Hb - 7g/dl RBC-4.38x $10^6/\mu l$ MCV-52.8 fl MCH-15.9 pg MCHC- 30.3 g/dl RDW- 26.6%	HbA-68 % HbA_2-0.8% HbF- %	Severe hypochromic microcytosis	$\alpha\alpha/-\alpha^{3.7}$

24	216	F	Sinhalese	Investigated for tiredness	Hb - 11.4g/dl RBC- $4.81x10^6/\mu l$ MCV- 70.1 fl MCH- 23.60 pg MCHC-33.7 g/dl RDW- 27.2%	HbA-87.1 % HbA_2-2.5% HbF-0.5 %	markedly hypochromic microcytic red cells with pencil cells	$\alpha\alpha/-\alpha^{4.2}$
25	18	F	Sinhalese	Incidental finding while investigated for failure to thrive	Hb -12.2 g/dl RBC- $5.9x10^6/\mu l$ MCV- 62.6 fl MCH- 20.6 pg MCHC- 33g/dl RDW- 16.9%	HbA-87.6 % HbA_2-2.3% HbF-0.6 %	hypochromic microcytic red cells with few target cells and irregularly contracted cells	$\alpha\alpha/-\alpha^{4.2}$
26	341	F	Sinhalese	Investigated for pallor	Hb -10.9 g/dl RBC- $4.22x10^6/\mu l$ MCV- 77.5 fl MCH- 25.8 pg MCHC- 33.3 g/dl RDW-19.8 %	HbA-89.1 % HbA_2-2.4% HbF-0 %	hypochromic microcytic cells with few target cells , irregularly contracted cells and acanthocytes	$\alpha\alpha/-\alpha^{3.7}$
27	48	M	Tamil	Incidental finding while investigated for fever	Hb -11.6 g/dl RBC- $5.4x10^6/\mu l$ MCV-69.6 fl MCH- 21.4 pg MCHC- 30.8g/dl RDW-13.7 %	HbA- 87.2% HbA_2-2.3% HbF- 0.9%	hypochromic microcytic red cells with few target, tear drop and partially haemoglobinized cells	$-\alpha^{3.7/}-\alpha^{4.2}$
28	16	M	Tamil	Incidental finding while investigated for fever	Hb - 10.2g/dl RBC- $5.11x10^6/\mu l$ MCV- 63.4 fl MCH- 20 pg MCHC- 31.5 g/dl RDW-15.9 %	HbA-85.2 % HbA_2-2.5% HbF-1.5 %	predominantly hypochromic microcytic red cells with some elongated cells and oval macrocytes	$\alpha\alpha/-\alpha^{3.7}$

29	481	M	Sinhalese	Investigated for pallor and hepatosplenomegaly	Hb - 6.1g/dl RBC- $4.36x10^6/\mu l$ MCV- 55.7 fl MCH- 14pg MCHC-25.1 g/dl RDW- 25.5%	HbA- 86.2% HbA_2-1.9% HbF-0.3 %	hypochromic microcytic red cells with some target, tear drop cells and partially haemoglobinzed cells	$-\alpha^{3.7/}-\alpha^{3.7}$
30	15	M	Sinhalese	Incidental finding while investigated for fever	Hb -11.6 g/dl RBC- $5.55x10^6/\mu l$ MCV- 64.6 fl MCH- 20.8 pg MCHC- 32.3 g/dl RDW-17.2 %	HbA-87.7 % HbA_2-2.7% HbF-0.4 %	predominantly normochromic normocyti red cells with some hypochromic microcytic cells, acanthocytes and occasional partially haemoglobinized cells	$-\alpha^{3.7/}-\alpha^{3.7}$
31	684	F	Sinhalese	Incidental finding while investigated for fever	Hb - 11g/dl RBC- $5x10^6/\mu l$ MCV-69.2 fl MCH- 22 pg MCHC-31.8 g/dl RDW-19 %	HbA- % HbA_2-% HbF- %	hypochromic microcytic anaemia with pencil cells	$-\alpha^{3.7}/-\alpha^{3.7}$

Table 37 illustrates the summary of RBC parameters of the subjects with their reference ranges. In individuals with iron deficiency anaemia, RBC after the correction of the iron deficiency was used and in other individuals FBC reports with highest Hb value was used in the analysis. Table 38 gives the summary of HPLC data.

Table 37: RBC parameters of the study subjects

RBC parameter	Reference range - Male	Reference range- Female	Minimum	Maximum	Mean	SD
Hb (g/dl)	13.5-18	11.5-16	6.10	14.80	10.62	1.65
RBC ($1x10^6$/µl)	4.5-6.5	3.8-5.8	3.36	5.90	4.90	0.61
MCV (fl)	78-100	78-100	46.40	82.40	67.53	8.28
MCH (pg)	27-32	27-32	14.00	25.90	21.65	2.98
MCHC (g/dl)	31-37	31-37	25.10	40.80	32.19	2.43
RDW –CV (%)	11.5-15	11.5-15	11.50	27.20	17.62	3.74

The mean Hb level of the study cohort was 10.62 g/dl and the reference range reported by the laboratories was 11.5-18 g/dl. The mean RBC count of the study cohort was 4.90 $x10^6$/µl and it was within the normal reference range. The mean MCV value was 67.53 fl and the reference range was 78-100 fl. The mean MCH was 21.65 pg and all the MCH values were below the lower limit of the reference range(27-32 pg). The mean MCHC level was 32.19 g/dl and it was within the normal range(31-37 g/dl). The mean RDW was 17.62% and the reference range was 11.5-15%.

In this study group The Hb, RBC count,MCV and MCH had shown mild anaemia, normal RBC count, low MCV with significantly low MCH levels compared to normal reference range. The mean RDW level was 17.62% and it was above the upper normal limit.

Table 38: Summary of HPLC data of study subjects

Hb variant	Minimum	Maximum	Mean	SD
HbA(%)	5.30	92.40	83.61	14.98
HbA_2 (%)	0.80	2.90	2.38	0.43
HbF (%)	0.00	96.20	3.74	17.17

The mean HbA_2 value of the study cohort was 2.38 %. The laboratory normal reference range for HbA_2 was 2.2-3.5%.

Four genotypes ($\alpha\alpha/-\alpha^{3.7}$, $-\alpha^{3.7}/-\alpha^{3.7}$, $\alpha\alpha/-\alpha^{4.2}$, $-\alpha^{3.7}/-\alpha^{4.2}$) were reported in the study. Table 39 gives the frequency for each genotype.

Table 39: Genotype frequencies

Genotype	Frequency	Percentage
$\alpha\alpha/-\alpha^{3.7}$	9	29.0 %
$-\alpha^{3.7}/-\alpha^{3.7}$	15	48.4 %
$\alpha\alpha/-\alpha^{4.2}$	5	16.1%
$-\alpha^{3.7}/-\alpha^{4.2}$	2	6.5 %
Total	31	100%l

The $-\alpha^{3.7}/-\alpha^{3.7}$ genotype was the commonest(48.4%) genotype in the cohort and the $\alpha^{3.7}/-\alpha^{4.2}$ genotype was rare (6.5%). The other possible genotype, homozygosity for $-\alpha^{4.2}$ ($-\alpha^{4.2}/-\alpha^{4.2}$) was not detected in this cohort.

Table 40 shows the mean values of RBC parameters for each genotype with standard deviations.

Table 40: Mean values and standard deviations for RBC parameters of each genotype

Genotype	Hb	RBC	MCV	MCH	MCHC	RDW
$\alpha\alpha/-\alpha^{3.7}$	10.53 (SD 1.50)	4.70 (SD 0.45)	69.57 (SD 9.77)	22.12 (SD 3.26)	31.90 (SD1.28)	18.08 (SD 3.88)
$-\alpha^{3.7}/-\alpha^{3.7}$	10.03 (SD 1.57)	4.82 (SD 0.67)	65.59 (SD 7.58)	20.90 (SD 3.023)	32.06 (SD3.29)	17.49 (SD3.22)
$\alpha\alpha/-\alpha^{4.2}$	12.34 (SD 1.41)	5.28 (SD 0.59)	71.54 (SD 6.74)	23.60 (SD 2.02)	33.04 (SD 0.79)	17.64 (SD5.79)
$-\alpha^{3.7}/-\alpha^{4.2}$	11.10 (SD 0.71)	5.49 (SD 0.13)	62.90 (SD 9.48)	20.20 (SD 1.70)	32.30 (SD 2.12)	16.45 (SD 3.89)

The $\alpha\alpha/-\alpha^{4.2}$ genotype had the highest mean Hb , MCV, MCH and MCHC. The $-\alpha^{3.7}/-\alpha^{3.7}$ had lowest mean Hb value. Lowest mean MCV and MCH values were observed in the $-\alpha^{3.7}/-\alpha^{4.2}$ genotype. The highest RDW was observed in the $\alpha\alpha/-\alpha^{3.7}$ genotype and lowest in the $-\alpha^{3.7}/-\alpha^{4.2}$ genotype**.** A one –way between groups analysis of variance (ANOVA) was conducted to compare the mean values of different RBC parameters and results are shown in Table 41.

Table 41: Comparison of mean values of different RBC parameters between genotypes using ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Hb	Between Groups	20.483	3	6.828	3.024	0.047
	Within Groups	60.965	27	2.258		
	Total	81.448	30			
RBC	Between Groups	1.900	3	0.633	1.853	0.161
	Within Groups	9.228	27	0.342		
	Total	11.128	30			
MCV	Between Groups	216.866	3	72.289	1.061	0.382
	Within Groups	1839.401	27	68.126		
	Total	2056.268	30			
MCH	Between Groups	33.513	3	11.171	1.299	0.295
	Within Groups	232.265	27	8.602		
	Total	265.777	30			
MCHC	Between Groups	4.647	3	1.549	0.243	0.865
	Within Groups	172.008	27	6.371		
	Total	176.655	30			
RDW	Between Groups	4.892	3	1.631	0.106	0.956
	Within Groups	414.470	27	15.351		
	Total	419.362	30			

There was statistically significant differences in the Hb levels between genotypes (p- 0.047) . In all the other RBC parameters, there was no statistically significant difference(p- > 0.05).

Further analysis was done by dividing the study subjects in to two groups depending on the number of defective genes [one defective gene(αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$), Two defective gene (-$\alpha^{3.7}$/-

$\alpha^{4.2}$ and $\alpha^{3.7}/-\alpha^{3.7}$)]. Mean values for each group for RBC parameters with standard deviations are given in the Table 42.

Table 42 : Summary of RBC parameters according to number of defective genes.

	Frequency	%	Hb	RBC	MCV	MCH	MCHC	RDW
One defective gene	14	45.16 %	11.18 (SD 1.68)	4.90 (SD 0.56)	70.27 (SD 8.58)	22.65 (SD2.89)	32.31 (SD1.23)	17.92 (SD4.43)
Two defective genes	17	54.84 %	10.16 (SD1.52)	4.90 (SD0.66)	65.28 (SD7.53)	20.82 (SD2.87)	32.09 (SD3.13)	17.36 (SD3.18)

The group with one defective gene ($\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$) had higher mean Hb, MCV, MCH ,MCHC values and RDW values. Mean RBC count values were equal in both groups. An independent-samples t-test was conducted to compare means between two groups and the results are given in the Table 43.

Table 43: Comparison of mean values of different RBC parameters between two groups using independent t-test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Hb	EVA	0.002	0.963	1.775	29	0.086	1.01975	0.57442	-.15508	2.19458
RBC	EVA	0.334	0.568	0.009	29	0.993	0.00206	0.22356	-.45517	0.45929
MCV	EVA	0.796	0.380	1.726	29	0.095	4.99496	2.89401	-.92396	10.91387
MCH	EVA	0.180	0.674	1.759	29	0.089	1.82647	1.03860	-.29771	3.95065
MCHC	EVA	1.539	0.225	0.246	29	0.807	0.21891	0.88982	-1.60098	2.03880
RDW	EVA	0.806	0.377	0.407	29	0.687	0.55672	1.36852	-2.24222	3.35567

EVA- Equal variance assumed

There was no statistically significant difference between two groups for any of the FBC parameters(p- >0.05).

Table 44 illustrates the mean values and standard deviations for HPLC parameters according to each genotype.

Table 44: Summary of HPLC parameters for each genotype

Genotype	HbA	HbA2	HbF
αα/-$\alpha^{3.7}$	84.26 (SD5.88)	2.29 (SD0.63)	0.72 (SD0.80)
-$\alpha^{3.7}$/-$\alpha^{3.7}$	81.92 (SD21.29)	2.37 (SD0.37)	7.05 (SD24.67)
αα/-$\alpha^{4.2}$	86.34 (SD1.11)	2.50 (SD0.16)	0.40 (SD0.19)
-$\alpha^{3.7}$/-$\alpha^{4.2}$	86.60 (SD0.85)	2.60 (SD0.42)	0.80 (SD0.14)

The -$\alpha^{3.7}$/-$\alpha^{4.2}$ genotype had highest mean values for HbA and HbA_2 and the -$\alpha^{3.7}$/-$\alpha^{3.7}$ genotype had the highest mean value for HbF.

A one –way between groups analysis of variance (ANOVA) was conducted to compare the means values HPLC parameters and results are given in Table 45.

Table 45: Comparison of mean values of HPLC parameters between genotypes using ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
HbA	Between Groups	101.737	3	33.912	0.138	0.936
	Within Groups	6629.178	27	245.525		
	Total	6730.915	30			
HbA2	Between Groups	0.244	3	0.081	0.412	0.745
	Within Groups	5.318	27	0.197		
	Total	5.562	30			
HbF	Between Groups	319.038	3	106.346	0.337	0.799
	Within Groups	8529.073	27	315.892		
	Total	8848.111	30			

There is no statistically significant difference between genotypes for any of the HPLC parameters.

Further analysis was done by dividing the study subjects in to two groups depending on the number of defective genes as above [one defective gene(αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$), Two defective gene(-$\alpha^{3.7}$/-α:$^{4.2}$ and $\alpha^{3.7}$/-$\alpha^{3.7}$)]. Mean values are given in Table 46.

Table 46: Summary of HPLC parameters according to number of defective genes

Genotype	Frequency	%	HbA	HbA2	HbF
One defective gene (αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$)	14	46.7%	85.00 (SD 4.77)	2.36 (SD 0.51)	0.61 (SD 0.66)
Two defective genes(-$\alpha^{3.7}$/-$\alpha^{4.2}$ and $\alpha^{3.7}$/-$\alpha^{3.7}$)	17	53,3%	87.47 (SD1.98)	2.40 (SD 0.37)	6.31 (SD 23.17)

The group with two defective genes had higher values for all 3 HPLC parameters. An independent-samples t-test was conducted to compare means of HPLC parameters between two groups. Results are given in the Table 47.

Table 47: Comparison of mean values of HPLC parameters between two groups using independent t-test

		t-test for equality of means								
									95% CI of the difference	
		F	Sig.	T	df	Sig(2-tailed)	Mean difference	Std.Error of Difference	Lower	Upper
HbA	EVA	1.887	0.180	0.462	29	0.65	2.53	5.48	-8.67	13.73
HbA2	EVA	0.106	0.748	-0.226	29	0.82	-0.04	0.16	-0.36	0.29
HbF	EVA	3.360	0.077	-0.918	29	0.37	-5.70	6.21	-18.41	7.01

EVA- Equal variance assumed

There was no statistically significant difference in means for any of the HPLC parameters between two groups

Since case no: 17 has an abnormally elevated HbF level it can be considered as an outlier for HPLC parameters. We re-analyzed the HPLC parameter data after removing the case no:17.

Mean values for HPLC parameter with standard deviations for each genotype is given in the Table 48.

Table 48 : Summary of HPLC parameters after excluding the outlier

Genotype	Frequency	%	HbA	HbA_2	HbF
αα/-$\alpha^{3.7}$	9	30.0	84.26 (SD 5.88)	2.29 (SD 0.63)	0.72 (SD 0.80)
-$\alpha^{3.7}$/-$\alpha^{3.7}$	14	46.7	87.39 (SD 2.10)	2.41 (SD 0.34)	0.68 (SD 0.77)
αα/-$\alpha^{4.2}$	5	16.7	86.34 (SD 1.11)	2.50 (SD 0.16)	0.40 (SD 0.19)
-$\alpha^{3.7}$/-$\alpha^{4.2}$	2	6.7	86.60 (SD 0.85)	2.60 (SD0.42)	0.80 (SD 0.14)

A one –way between groups analysis of variance (ANOVA) was conducted to compare the mean values of HPLC parameters of different genotypes and results are given in Table 49.

Table 49 : Comparison of mean values of HPLC parameters between genotypes using ANOVA after excluding the outlier

		Sum of Squares	Df	Mean Square	F	Sig.
HbA	Between Groups	54.350	3	18.117	1.389	0.268
	Within Groups	339.224	26	13.047		
	Total	393.574	29			
HbA2	Between Groups	0.244	3	0.081	0.425	0.737
	Within Groups	4.966	26	0.191		
	Total	5.210	29			
HbF	Between Groups	0.416	3	0.139	0.277	0.842
	Within Groups	13.019	26	0.501		
	Total	13.435	29			

There is no statistically significant difference between genotypes for any of the HPLC parameters.

This group was further analyzed by dividing the study subjects in to two groups depending on the number of defective genes[one defective gene(αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$), Two defective gene(-$\alpha^{3.7}$/-$\alpha^{4.2}$ and $\alpha^{3.7}$/-$\alpha^{3.7}$)]. Frequency and mean values with standard deviations given in Table 50.

Table 50: Summary of HPLC parameters according to number of defective genes after excluding the outlier

Genotype	Frequency	%	HbA	HbA2	HbF
One defective gene ($\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$)	14	46.7%	85.00 (SD 4.77)	2.36 (SD 0.51)	0.61 (SD 0.66)
Two defective genes($-\alpha^{3.7}/-\alpha^{4.2}$ and $\alpha^{3.7}/-\alpha^{3.7}$)	16	53,3%	87.29 (SD1.98)	2.44 (SD 0.34)	0.69 (SD0.72)

An independent-samples t-test was conducted to compare means of HPLC parameters between two groups. Results are given in the Table 51.

Table 51: Comparison of mean values of HPLC parameters between two groups using independent t-test after excluding the outlier

		t-test for equality of means								
									95% CI of the difference	
		F	Sig.	t	Df	Sig(2-tailed)	Mean difference	Std.Error of Difference	Lower	Upper
HbA	EVA	0.823	0.372	-1.762	28	0.09	-2.29	1.30	-4.96	0.37
HbA2	EVA	0.249	0.622	-0.466	28	0.64	-0.07	0.16	-0.40	0.25
HbF	EVA	0.160	0.692	-0.342	28	0.74	-0.09	0.25	-0.60	0.43

EVA- Equal variance assumed

There is no statistically significant difference of HPLC parameter mean values between two groups.

Blood picture data of individuals were analysed. The appearance of the red blood cells is shown in the Figure 23

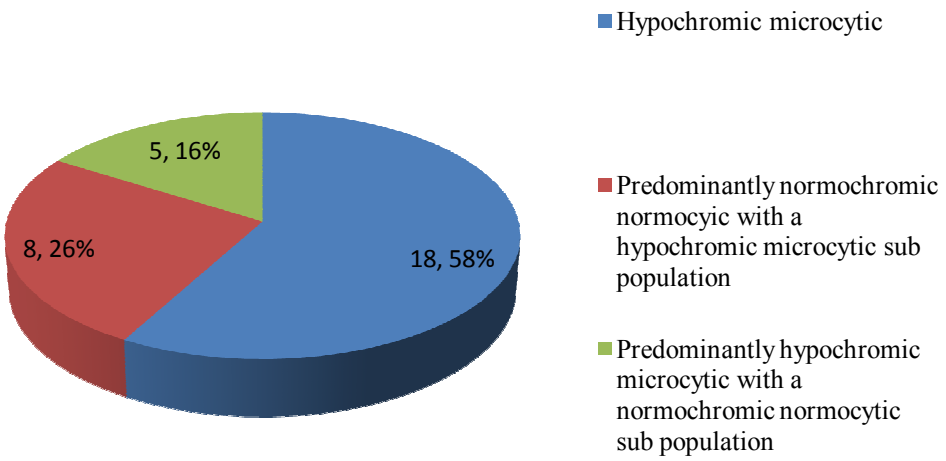

Figure 23 : Appearance of red blood cells in the blood picture

Figire 24 illustrates the frequency of different morphological cell types seen in the blood pictures.

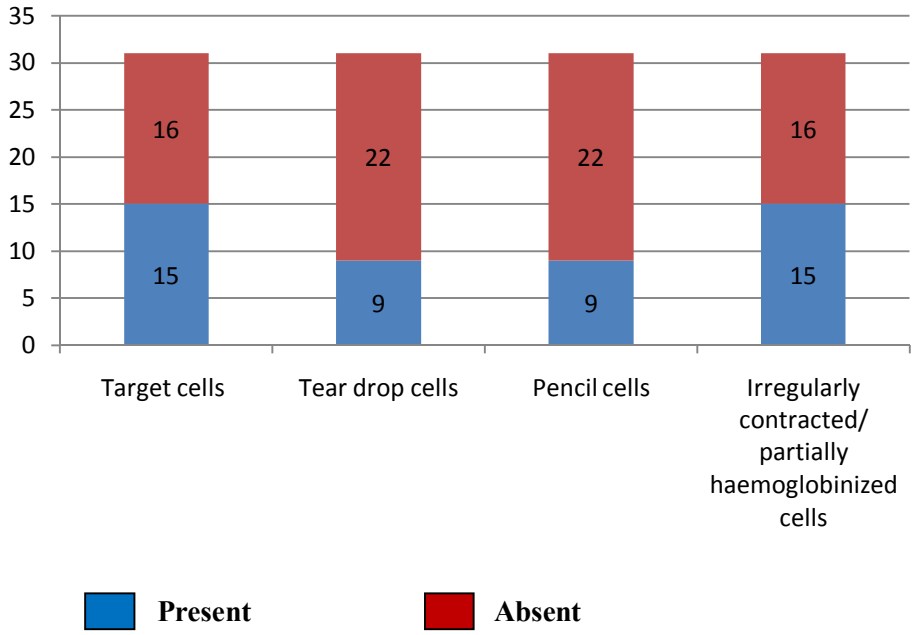

Figure 24: Frequency of different morpfological red cell types

Table 52 illustrates the frequency of blood pictures with different morphological cell types.

Table 52: Frequency of blood pictures with different morphological cell types.

Genotype	Frequency	Target cells	Tear drop cells	Pencil cells	Irregularly contracted/ partially haemoglobinized cells
αα/-$\alpha^{3.7}$	9	2 (22.2%)	2 (22.2%)	3 (33.3%)	4 (44.4%)
-$\alpha^{3.7}$/-$\alpha^{3.7}$	15	9 (60%)	5 (33.3%)	5 (33.3%)	7 (46.6%)
αα/-$\alpha^{4.2}$	5	2 (40%)	0 (0%)	1 (20%)	2 (40%)
-$\alpha^{3.7}$/-$\alpha^{4.2}$	2	2 (100%)	2 (100%)	0 (0%)	2 (100%)
Total	31	15	9	9	15

Chi – square test was applied to find out wether there is a statistically significant difference in the presence of different morphological cell types between different genotypes. Table 53 illustrates the result of the chi-square analysis .

Table 53: Chi-square test results between genotypes

Cell type	Value	Df	Asymp. Sig. (2-sided)
Target cells	5.551	3	0.136
Tear drop cells	7.272	3	0.064
Pencil cells	1.232	3	0.745
Irregularly contracted/ partially haemoglobinized cells	2.348	3	0.503

There was no statistically significant difference($p- < 0.05$) between genotypes for any of the cell types studied.

Further analysis was done by dividing the study subjects in to two groups depending on the number of defective genes as above [one defective gene($\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$), Two defective gene($-\alpha^{3.7}/-\alpha^{4.2}$ and $\alpha^{3.7}/-\alpha^{3.7}$)] and chi –square test was applied to see wether there is a difference in the presence of different morphological cell types between two groups. Table 54 illustrates the frequency of blood pictures with different morphological cell types.

Table 54 : Frequency of blood pictures with different morphological cell types according to number of defective genes.

Genotype	Frequency	Target cells	Tear drop cells	Pencil cells	Irregularly contracted/ partially haemoglobinized cells
One defective gene	14	4 (28.6%)	2 (14.3%)	4 (28.6%)	6 (42.9%)
Two defective genes	17	11 (64.7%)	7 (41.2%)	5 (29.4%)	9 (52.9%)
Total	31	15	9	9	15

Target cells and tear drop cells were present in higher percentage (64.7% and 41.2% respectively)of blood pictures in the group with two defective genes than the group with one defective genes(28.6% and 14.3% respectively). Pencil cells were present in similar percentage of blood pictures in both groups and the group with two defective genes had higher percentage(52.9%) of blood pictures with Irregularly contracted/ partially haemoglobinized cells than the group with one defective genes. Chi-square test was applied to find a statistical significance in the presence of different morphological cell types between two groups. Table 55 illustrates results obtained from chi-square test.

Table 55 : Chi square test results according to number of defective genes.

Cell type	Value	Df	Asymp. Sig. (2-sided)
Target cells	4.014	1	.045
Tear drop cells	2.695	1	.101
Pencil cells	0.003	1	.959
Irregularly contracted/ partially haemoglobinized cells	0.313	1	.576

The presence of target cells were significantly high in the group with two defective genes (p- 0.045). For other cell types there was no statistically significant difference($p > 0.05$) between two groups.

6. DISCUSSION

Case no:1 had low Hb, MCV, MCH normal RBC count and MCHC with elevated RDW. He was also positive for HbH inclusions. Some times HbH inclusions may be present in alpha thalassaemia traits also[122]. Therefore homozygosity for $-\alpha^{3.7}$ deletion may explains the phenotype. However further molecular genetic studies are needed to exclude HbH disease.

In case no:2 Hb level was below the level expected in pregnancy. MCV and MCH levels were slightly low and MCHC and RBC count was within the normal range. RDW was high. Homozygosity for $-\alpha^{3.7}$ and haemodilution during pregnancy can explain the phenotype.

Case no:3 had low Hb, low MCV, low MCH and low MCHC with normal RBC count and high RDW. Homozygosity for $-\alpha^{3.7}$ can explain the phenotype.

Case no :4 had low Hb, low MCV, low MCH , normal RBC count and MCHC with high RDW. Homozygosity for $-\alpha^{3.7}$ can explain the phenotype.

Case no: 5 had low Hb, elevated RBC count, very low MCV, low MCH normal MCHC with high RDW. Homozygosity for $-\alpha^{3.7}$ can explain the phenotype.

Case no:6 had low Hb, elevated RBC count low MCV, low MCH, normal MCHC with high RDW. His phenotype can be explained by compound heterozygosity for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ which gives rise to an alpha thalassaemia trait. Case no: 7, father of case no: 6 who is heterozygous for $-\alpha^{4.2}$ deletion had normal Hb, elevated RBC count, slightly low MCV and MCH with normal MCHC and RDW. Case no: 8 mother of case no: 6 who is a heterozygous for $-\alpha^{3.7}$ deletion had normal Hb level. Except for marginally low MCH(25.9 pg) other red cell indices can be considered normal. Both case no : 7 and 8 had predominantly normochromic normocytic red

cells with a sub population of hypochromic microcytic cells. Both parents phenotypes are compatible with alpha thalassaemia silent carrier state. In $-\alpha^{4.2}$ deletion *HBA2* gene is deleted while in $-\alpha^{3.7}$ deletion parts of both *HBA1* and *HBA2* genes are deleted and a fusion gene is created. *HBA2* gene has higher transcription and translation rate than *HBA1* and the fusion gene has an intermediate rate[15]. Although a higher Hb level can be expected in case no:8 than in case no:7 , the male sex and other compounding factors may have contributed to the higher Hb levels in case no: 7. However case no: 8 has slightly better MCV and MCH than case no: 7.

Case no:9 had low Hb ,low MCV, low MCH, low MCHC with normal RBC count. Her low Hb level (8.8 g/dl) is not compatible with her genotype ($-\alpha^{3.7}/-\alpha^{3.7}$)as this genotype usually results in a mildly low haemoglobin level. This patient will need further testing to exclude co-existing alpha and beta gene defects

Case no:10 had normal Hb, RBC count within the upper normal range, low MCV , low MCH, normal MCHC with high RDW. His phenotype is compatible with the genotype ($\alpha\alpha/-\alpha^{4.2}$) which gives rise to silent carrier state.

Case no: 11 had low Hb, normal RBC count, low MCV, normal MCH, high MCHC with a high RDW. His genotype($-\alpha^{3.7}/-\alpha^{3.7}$) can explain the phenotype.

Case no: 12 had low Hb, slightly elevated RBC count, low MCV,MCH, MCHC and normal RDW. Her genotype ($-\alpha^{3.7}/-\alpha^{3.7}$) can explain the phenotype.

Case no: 13 initially presented with an acute haemolytic episode. Her Hb was low and RBC count was normal. MCV was normal while MCH and MCHC was slightly lower. RDW was high. HbH disease is associated with haemolytic episodes and in this patient HbH disease is not

excluded. Her present genotype ($\alpha\alpha/-\alpha^{3.7}$) cannot explain her phenotype and further genetic testing is essential. Since co-existing common acquired and hereditary haemolytic conditions has been excluded HbH disease still remains as a differential diagnosis. A large deletion on the other chromosome can be ruled out due to presence of 1800 bp size amplicon for the wild type. A non deletional mutation of the regulatory elements of the alpha gene cluster inactivating both alpha genes or two mutations affecting *HBA1* and *HBA2* genes are other possible explanations. Therefore sequencing the entire alpha gene cluster should be the next most appropriate method of genotyping. Furthermore this child should be followed up and monitored regularly for a possible iron overload. She is also at risk of developing acute haemolytic crisis during infections.

Case no: 14 had marginally low Hb, elevated RBC count, elevated MCV, MCH and normal MCHC. Homozygosity for $-\alpha^{3.7}$ deletion can explain his RBC parameters and blood picture.

Case no: 15 had normal Hb, normal RBC count, low MCV, low MCH , normal MCHC and high RDW. His phenotype can be explained by his genotype($\alpha\alpha/-\alpha^{3.7}$).

In case no : 16 who had a co existing iron deficiency Hb level was improved significantly after iron treatment but red cell indices were not changed significantly. Only MCH and MCHC were increased slightly and change in MCV and RDW was minimal. RBC count following iron treatment was slightly above the upper normal level. His phenotype can be explained by the genotype ($-\alpha^{3.7}/-\alpha^{3.7}$).

Case no: 17 had an atypical HPLC report which was compatible with either hereditary persistence of fetal haemoglobin(HPFH) or a homozygous $\delta\beta$ thalassaemia. She had low Hb (7.7 g/dl) and low RBC count(3.36 $x10^6/\mu l$) ,marginally low MCV, low MCH and normal MCHC.

Blood picture was compatible with thalassaemia major phenotype. However HPLC shows abnormally elevated levels of HbF (96.2%) and low HbA (5.3%). The heterozygous state for HPFH is characterized by presence of 10-40% HbF. This does not cause any consequence in healthy individual but it can ameliorate the effects of conditions affecting β globin production such as β thalassaemia major [52] as in this patient. Abnormally high levels of HbF is associated with deletion of the *HBB* gene. It is also caused by upstream point mutations of the *HBG* gene such as Xmn-1 polymorphism (Gγ 158 C>T, rs7482144) [30,52]. A polymorphism in BCL11A gene on chromosome 2p16(rs 11886868) and HBS1L-MYB intragenic region are the other well known genetic variants associated with HPFH[52]. This patient is homozygous for $-\alpha^{3.7}$ deletion and thus the reduction of α chains may have also contributed to less severe phenotype. Further studies in β gene cluster by sequencing may reveal causative *HBB* gene mutations for β thalassaemia major phenotype. Genotyping for variants causing increased HbF levels may help to explain the phenotype completely.

Case no: 18 had low Hb, normal RBC count, lower normal MCV, low MCH and high RDW. Following iron treatment there was only a little improvement in Hb levels minor changes in red cell indices. Her genotype ($-\alpha^{3.7}/-\alpha^{3.7}$) can explain the phenotype.

Case no:19 had low Hb and low MCH, MCV,normal RBC count , marginally low MCHC and high RDW . Following iron treatment his Hb levels improved by 1.3g/dl and RBC count was also increased. But there were minimal changes in other red cell indices. His genotype ($\alpha\alpha/-\alpha^{3.7}$) can explain the phenotype.

Case no 20: had low Hb, high RBC count, low MCV, MCH and MCHC with high RDW. With iron treatment his Hb level was raised by1.2 g/dl with some improvement in MCV,MCH and

MCHC values. Although the reduction in the RDW can be explained by the correction of iron deficiency, the RBC count is usually expected to increase, unless it is a laboratory error this cannot be explained. Apart from the RBC count irregularity the phenotype following iron treatment is compatible with a silent carrier sate and the genotype was heterozygosity for –α3.7 deletion (αα/–α3.7).

Case no 21: had low Hb, normal RBC count, low MCV, low MCH, normal MCHC and high RDW. His genotype, homozygosity for $-\alpha^{3.7}$ deletion which give rise to alpha thalassaemia trait state can explain the phenotype in this patient.

Case no:22 initially had low Hb , normal RBC count and low red cell indices. After treating co-existing iron deficiency his Hb level was increased by 2.8 g/dl to the lower normal range. MCV, MCH and MCHC values were also improved. His phenotype can be explained by his genotype($\alpha\alpha/-\alpha^{4.2}$) which gives rise to a silent carrier state.

Case no: 23 had HPLC evidence suggestive of HbH disease. Her Hb level is very low(7 g/dl) and is compatible with HbH phenotype. For confirmation of HbH disease testing for HbH inclusions should be done. However her genotype ($\alpha\alpha/-\alpha^{3.7}$) only results in a silent carrier state. Therefore further genetic studies are needed to identify the causative mutations in this patient.

Case no: 24 had low Hb, normal RBC count , low MCV, low MCH , normal MCHC and high RDW. His phenotype can be explained by the genotype ($\alpha\alpha/-\alpha^{4.2}$) which results in silent carrier state.

In case no: 25 Hb level was improved by 1.9 g/dl following iron treatment. RBC count and MCHC was also increased. Changes in MCV and MCH was minimal. After iron deficiency is corrected her phenotype can be explained by the genotype($\alpha\alpha/-\alpha^{4.2}$).

Case no: 26 had low Hb, normal RBC count, low MCV, low MCH . normal MCHC and high RDW. Her genotype($\alpha\alpha/-\alpha^{3.7}$) gives rise to a silent carrier state. Her phenotype can be explained by the genotype alone.

Case no: 27 had lower normal Hb low MCV, low MCH, marginally low MCHC and upper normal RDW . His blood picture also showed hypochromic microcytic red cells. His phenotype can be explained by the compound heterozygosity for $-\alpha^{3.7}$ and $-\alpha^{4.2}$ which gives rise to an alpha thalassaemia trait state.

Case no: 28 had low Hb , marginally elevated RBC count, low MCV, low MCH , marginally low MCHC and marginally elevated RDW can be explained as there is evidence of mixed deficiency anaemia in the blood film. However his phenotype can be explained by the genotype($\alpha\alpha/-\alpha^{3.7}$).

Case no 29: had very low Hb, marginally low RBC count, low MCV, low MCH, low MCHC and high RDW. HbH inclusions were also detected. His genotype ($-\alpha^{3.7}/-\alpha^{3.7}$) does not cause HbH disease. He has thalassaemia intermedia phenotype. Further genetic testing of both HAA and HBB genes are needed to look for additional genetic defects**.**

Case no: 30 had normal Hb with high RBC count, low MCV, low MCH, normal MCHC and high RDW. Incidentally found low RBC indices led further investigations in this child and his genotype ($-\alpha^{3.7}/-\alpha^{3.7}$) is compatible with the phenotype.

Case no: 31 had low Hb, marginally elevated RBC count low MCV, low MCH, normal MCHC and high RDW. Her phenotype is compatible with the genotype of $-\alpha^{3.7}/-\alpha^{3.7}$.

In summary majority (N = 26 ,84 %) had a phenotype compatible with the genotype. Those with lower haemoglobin levels and atypical phenotypes need to be investigated further.

When variability of the haemoglobin level and the red cell parameters of the four different genotypes were compared, the haemoglobin level showed a statistical significance. The $\alpha\alpha/-\alpha^{4.2}$ genotype had the highest mean Hb levels [12.34 g/dl (±1.41)]. When mean values of each genotypes were compared, there is a statistically significant difference [p value- 0.047(<0.05)] [Table 40] The highest value for Hb (14.8 g/dl) belonged to an individual with the $\alpha\alpha/-\alpha^{4.2}$ genotype and only 5 individuals (16.1%) belonged to this particular genotype. When the effect on the chain production of the two genotypes $\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$ are considered the $\alpha\alpha/-\alpha^{3.7}$genotype results in relatively more alpha chains since the $-\alpha^{3.7}$ deletion produces a fusion gene which has lower expression rate than *HBA2* but higher expression rate than *HBA1.* In $-\alpha^{4.2}$ deletion *HBA2* gene is deleted on the affected chromosome [15]. This statistically significant finding has to be verified by carrying out further studies using larger patient co-horts.

Individuals carrying the $-\alpha^{4.2}$ mutation, either as ~~a~~ heterozygote or compound heterozygote had higher mean RBC count ($5.28x10^6$/µl and $5.49x10^6$/µlfor $\alpha\alpha/-\alpha^{4.2}$and $-\alpha^{3.7}/-\alpha^{4.2}$ genotypes respectively) than $\alpha\alpha/-\alpha^{3.7}$ and $-\alpha^{3.7}/-\alpha^{3.7}$ genotypes($4.70x10^6$/µl and $4.82x10^6$/µl respectively)[Table 40]. However there is a no statistically significant difference in RBC count between genotypes.

Some of the previous studies have shown statistically significant difference inthe MCV and MCH levels between different genotypes causing alpha thalassaemia [33,58, 115]. In this cohort αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$ genotypes had higher mean values for both MCV and MCH than-$\alpha^{3.7}$/-$\alpha^{3.7}$and -$\alpha^{3.7}$/-$\alpha^{4.2}$ genotypes. However there is no statistically significant difference in mean values between the genotypes [Table 40,41]. Small sample size in this cohort would have resulted in this difference. The MCHC and RDW mean values also did not have statistically significant differences between different genotypes[Table 41]

In this cohort of patients MCH of all the patients remained below 27 pg. This finding is compatible with previously published data [114]. The highest MCH value, 25.9 pg was found in a female patient heterozygous for –$\alpha^{3.7}$ deletion who had normal MCV(82.4 fl) and normal Hb(12.5 g/dl). The lowest MCH , 14 pg was found in a patient with thalassaemia intermedia phenotype who had a MCV of 55 fl and Hb of 6.1 g/dl).This patient was positive for HbH inclusions and he is homozygous for –$\alpha^{3.7}$ deletion.

When the presence of the number of defective genes were considered [one defective gene(αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$), two defective genes in homozygous or compound heterozygous state (-$\alpha^{3.7}$/-$\alpha^{4.2}$ and $\alpha^{3.7}$/-$\alpha^{3.7}$)] the mean Hb , MCV and MCH values were higher in the group with one defective gene(αα/-$\alpha^{3.7}$ and αα/-$\alpha^{4.2}$) than the group with two defective genes (-$\alpha^{3.7}$/-$\alpha^{4.2}$ and $\alpha^{3.7}$/-$\alpha^{3.7}$). Mean values for RBC count, MCHC and RDW showed minor or no difference. However there was no statistically significant difference for any of the RBC parameters between these two groups.[Table 42,43]

There was no statistically significant difference in HbA, HbA_2 or HbF levels between the four genotypes.[Table 48,49] Similarly there was no statistically significant difference in any of the HPLC parameters when number of defective genes were considered [one defective gene($\alpha\alpha/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{4.2}$), two defective gene($-\alpha^{3.7}/-\alpha^{4.2}$ and $\alpha^{3.7}/-\alpha^{3.7}$)].[Table 50,51]

Analysis of the red cell morphology findings based on the different genotypes and number of affected genes, all groups showed presence of population of hypochromic microcytic cells and minor populations of target cells, tear drop poikilocytes, and partially haemoglobinzed / irregularly contracted cells. These are in keeping with published data on the red cell morphology in alpha thalassaemia[15,28,35]. However although the presence of target cells in the blood picture was significantly higher(p- 0.045) in those who had two defective genes than one defective genes. However there was no statistically significant difference in the presence of target cells, tear drop poikilocytes, and partially haemoglobinzed / irregularly contracted cells between the genotypes.[Table 53,55]

In this cohort three patients had a phenotype suggestive of HbH disease (Case 1,23 and 29). Case no:1 and 29 were positive for HbH inclusions but in case no:23 HbH disease was suspected based on HPLC pattern alone. Both case no:1 and 29 were homozygous for the $-\alpha^{3.7}$ deletion while case no: 23 was heterozygous for the $-\alpha^{3.7}$ deletion. As usually HbH disease is a result of the compound heterozygous state for α^{o}-thalassemia and α^{+}-thalassemia these patients should have additional genetic defects which have to be tested for . In HbH disease three alpha genes should be defective and only one should be active. However$-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions are α^{+}thalassaemia causing deletions and therefore do not result in HbH disease theoretically.

Therefore further genotyping is needed to find out the complete genetic basis of these patients' phenotypes In case no:1 and 29 sequencing of alpha gene cluster may help to identify the third mutation, such as a non-deletional mutation. In case no: 23 presence of an α^0 mutation in the chromosome without $-\alpha^{3.7}$ deletion is a possibility. However it is highly unlikely to be a large deletion like $--^{SEA}$ and $--^{MED}$ **or** previously reported $--^{SL}$ since such a large deletion would have abolished the possibility of amplification of 1800 bp size amplicon for the wild type. Therefore sequencing of entire alpha cluster including regulatory regions should be the method for further genotyping.

Iron deficiency seems to be an important factor determining the haematological phenotype among individuals who has $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions. Initial low haemoglobin levels have improved after an adequate course of haematinics in many patients. However their response to iron treatment shows great variations. In case no: 16 who is homozygous for $-\alpha^{3.7}$ deletion, after nine months of iron treatment his Hb level was improved by 1.8 g/dl and MCH by 2 pg but there was no significant change in MCV values. In case no: 19 who is heterozygous for $-\alpha^{3.7}$ deletion, there was only 1.3 g/dl increase in Hb level and no significant changes in MCV and MCH values. However in case no: 20 who is also heterozygous for $-\alpha^{3.7}$ deletion, both MCV and MCH values improved significantly despite only 1.2 g/dl increase in Hb. In case no: 25 who is heterozygous for $-\alpha^{4.2}$ deletion there was 1.9 g/dl increase in Hb levels but minimal changes in MCV and MCH values. The underline genetic defect may have a minimal effects in the response to iron treatment but other compounding genetic and environmental factors may have contributed to this variable response.

Ethnic Muslims who represent only 7% of the Sri Lankan population accounts for 38.7% of the patients in this cohort. Since this is not a population based study and owing small sample size, it is not possible to conclude that these two deletions are common among Muslims. However inbreeding is a common practice among Sri Lankan Muslims. Inbreeding and founder effect may have contributed to the high frequency of these two mutations among Muslims. Further studies recruiting representative samples of adequate size from each ethnic group are needed to find the prevalence of these mutations among different Sri Lankan ethnic groups.

6 CONCLUSIONS

Based on the clinical and red blood cell parameters, types of haemoglobin estimation, and genotyping data of this cohort of patients several conclusions can be arrived.

- The alpha thalassaemia silent carriers and traits showed the typical haematological phenotypes as described to other published alpha thalassaemia patient cohorts in the literature.
- Consistent change in the red cell parameters was the low MCH level and presence of a hypochromic microcytic population of red cells detected in the blood film. These parameters can be used to screen for alpha thalassaemia silent carrier states and alpha thalassaemia carriers.
- Red cell parameters and morphology have little or no role in differentiating genotypes
- Apart from detecting those with HbH syndrome, HPLC plays no role in the diagnosis of alpha thalassaemia silent carrier states and alpha thalassaemia carriers.
- Molecular genetic testing strategies to understand the genetic basis of alpha thalassaemia especially mutations causing HbH disease need to be developed further for the Sri Lankan population

7 LIMITATIONS

Following limitation was identified in this study.

- Not obtaining statistically significant results (especially in relation to red cell morphology findings) may have been due to the small sample size

8 FURTHER STUDIES

- Continuing the study further to include a larger patient co-hort.
- Further molecular genetic studies in patients with unexplained phenotypes may help to identify other mutations including novel mutations causing alpha thalassaemia in Sri Lankans.
- Studies on effects of possible modifier genes will help to understand the phenotypic variability among the individuals with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions .

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APPENDIX 1: List of Abbreviations

ARMS	-	Amplification Refractory Mutation System
BCB	-	Brilliant Cresyl Blue
BP	-	Blood Picture
CE-HPLC	-	Cation Exchange High Performance Liquid Chromatography
CFU	-	Colony Forming Unit
CV	-	Coefficient of Variation
FBC	-	Full Blood Count
Gap-PCR	-	Gap Polymerase Chain Reaction
Hb	-	Haemoglobin
HPLC	-	High Performance Liquid Chromatography
HSC	-	Hematopoietic Stem Cells
IDA	-	Iron Deficiency Anaemia
IVS	-	Intervening Sequences
Kb	-	Kilo base
LCR	-	Locus Control Region
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCS	-	Multi species Conserved Sequence
MCV	-	Mean Corpuscular Volume
MLPA	-	Multiplex Ligation-dependant Probe Amplification
PCR	-	Polymerase Chain Reaction
PCV	-	Packed Cell Volume
RBC	-	Red Blood Cell

RDW	-	Red cell Distribution Width
TI	-	Thalassaemia Intermedia
TM	-	Thalassaemia Major
TT	-	Thalassaemia Trait
G6PD	-	Glucose-6-Phosphate Dehydrogenase
EVA	-	Equal variance assumed
SD	-	Standard deviation

APPENDIX 2: Documents used for subject recruitment

This appendix contains English, Sinhala, Tamil versions of documents used for subject recruitment

- Information sheets- (English, Sinhala, Tamil versions)
- Consent forms- (English, Sinhala, Tamil versions)
- Data collection booklet

Information Sheet

Molecular and Phenotypic Characterization of Alpha Thalassaemia patients in Sri Lanka.

I'm, Dr. S.M.A.Jayawardana, a post graduate student attached to the Human Genetics Unit, Faculty of Medicine, University of Colombo. I'm inviting you/ your child to take a part in a research study titled "**Molecular and Phenotypic Characterization of Alpha Thalassaemia patients in Sri Lanka"** conducted by myself under the supervision of Prof**.** Vajira H.W. Dissanayake and Dr. Hemali W.W. Goonasekera of the Human Genetics Unit, Faculty of Medicine , University of Colombo.

1. Purpose of the study

Genes are responsible for the transmission of features from parents to children. Genes determine how we look and also determine all our bodily functions. Some diseases can be inherited through genes. Thalassaemia is such an inherited disease.

In our red blood cells haemoglobin carries oxygen to all the cells in the body. Some defects in genes coding haemoglobin production cause thalassaemia which results in reduced haemoglobin production. There are two major types thalassaemia; alpha and beta thalassaemia. Diagnosis of alpha thalasaaemia carriers is not as straightforward as beta thalassaemia. It requires genetic testing in addition to other routine blood tests. Therefore there is always a delay in diagnosis of alpha thalassaemia carriers. Sometimes they are detected only after two carriers marry and have an affected child.

The purpose of this study is to identify the of gene defects in the alpha thalassaemia patients in Sri Lanka and to find out how these gene defects affect your disease (Eg: level of haemoglobin). These findings can be used to improve the way doctors diagnose alpha thalassaemia patients and carriers in a more efficient way and may help early diagnosis of the condition.

2. Voluntary participation

To participate or not to participate (you/your child) in this study is your own voluntary decision. You can withdraw your consent from the study at any given time. Withdrawal from the study or your wish to not to participate will not affect the quality of medical care you are/ your child is routinely entitled to receive.

3. Duration ,procedures of the study and the participants responsibility

The study duration is one year**. Five milliliters (5ml) of blood will be withdrawn once from an arm vein by a nurse/ doctor at the Human Genetics Unit. The sample** will be labeled by a unique number and details like name, address and age will not be written to maintain your anonymity. All the current tests we are planning to do will be done in the genetic laboratory of the Human Genetics Unit. If additional genetic tests are required, we might send samples to a foreign laboratory . **This sample will be used in this research and other future studies related to thalassaemia.**

We need to collect data on clinical details of thalassaemia ,results of investigations done pertaining to thalassaemia, and family history of thalassaemia or any other related illnesses. Data will be collected and recorded in a data collection booklet by the investigator following an interview. We request you to give consent for us to draw 5 ml of venous blood for genetic testing and data collection as stated above from you / your child. (Please see section 7 for more details)

4. Potential benefits

All participants will receive a genetic test report of thalassaemia at the end of the study. Additionally all the participants and family members will be provided with genetic counseling regarding test results.
By participating in this study you will help to add new knowledge to the genetic basis of alpha thalassaemia and thereby help doctors to diagnose alpha thalassaemia more easily.

5. Risks, Hazards and Discomforts

During or following drawing blood from the arm vein, there may be pain and discomfort and also bruising may occur around the site, especially if it was difficult to draw blood. Very rarely infection at the site of blood draw can occur. All these would be prevented or would be minimized by performing the blood sampling by a trained nurse or a doctor using sterile disposable needles and syringes and following clean (sterile) technique to draw blood.

6. **Reimbursements**

There will be no reimbursement for participating in this study.

7. Confidentiality

All participants of this study will be identified only by a number, and all details of identification contained in the data collection booklet will be separated from the rest of the data collection booklet and will be stored separately. The data will be electronically saved in a computer as password protected documents with restricted access to only the three investigators.

We hope to publish the results of this study in scientific journals or present as communications in scientific forums as individual case reports or study results as a whole. At any of these instances you/ your child will not be named individually or the identification will not be divulged ,and strict confidentiality will be maintained.

8. Termination of participation

You can withdraw your consent to participate in this study at any time without giving reasons, with no penalty or effect on medical care or loss of benefits. However it will not be possible for you to withdraw once the results are sent for publication or once the results are published.

9. Clarification

We hope this information sheet has given you sufficient information regarding the study for you to give consent. However if you have any further questions at anytime following consent please feel free to contact me or Dr. Goonasekera. Our contact details are given below.

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තොරතුරු පත්‍රිකාව

ශ්‍රී ලංකාවේ ඇල්ෆා තැලසීමියා රෝගීන්ගේ ජානමය වෙනස්කම් හා රූපානුදර්ශය අධ්‍යයනය කිරීම

කොළඹ වෛද්‍ය පීඨයේ මානව ප්‍රවේණි විද්‍යා ඒකකයට අනුබද්ධව පශ්චාත් උපාධිය හදාරණ වෛද්‍ය S.M.A.ජයවර්ධන (ප්‍රධාන පර්යේෂක) වන මම **ශ්‍රී ලංකාවේ ඇල්ෆා තැලසීමියා රෝගීන්ගේ ජානමය වෙනස්කම් හා රූපානුදර්ශය අධ්‍යනය කිරීම** (Molecular and Phenotypic Characterization of Alpha Thalassaemia Patients in Sri Lanka) යන අධ්‍යයනය සඳහා සහභාගී වන ලෙස ඔබට ඇරයුම් කර සිටිමි. මෙම අධ්‍යයනය කොළඹ වෛද්‍ය පීඨයේ මානව ප්‍රවේණි විද්‍යා ඒකකයේ මහාචාර්ය චජිර H .W දිසානායක හා ජ්ෙෂ්ඨ කථිකාචාර්ය වෛද්‍ය හේමාලි W.W. ගුණසේකර විසින් අධීක්ෂණය කරනු ලැබේ.

01' මෙම අධ්‍යයනයේ අරමුණු

ජාන මගින් එක් පරම්පරාවක ලක්ෂණ තවත් පරම්පරාවකට ගෙනයාම ප්‍රවේණිය නම් වේ.අපගේ හැඩරුව හා ශරීරයේ සියළුම ක්‍රියාවන් පාලනය කරනුයේ මෙම ජාන මගිනි. ඇතැම් රෝගද ප්‍රවේණිගත (ආරයට යන රෝග/ පරම්පරාවට යන රෝග) විය හැක. තැලසීමියාව යනු එලෙස ප්‍රවේණියෙන් වැළදෙන රෝගයකි.

අපගේ රුධිරයේ රතු රුධිර සෛල තුළ හිමොග්ලොබින් සිරුරේ සියළුම සෛල වලට ඔක්ෂිජන් ලබා දීමට වැදගත් වේ. තැලසීමියාව සෑදෙන්නේ හිමොග්ලොබින් ප්‍රෝටීනය සාදන ජාන වල ඇතිවන වෙනස්කම් (ජාන විකෘති) නිසාය. ඇල්ෆා තැලසීමියා හා බීටා තැලසීමියා ලෙස ප්‍රධාන තැලසීමියා වර්ග දෙකක් පවතී. ඇල්ෆා තැලසීමියා වාහකයින් නිර්ණය කිරීම බීටා තැලසීමියා වාහකයින් නිර්ණයට වඩා අපහසු කටයුත්තකි. ඒ සඳහා සාමාන්‍ය රුධිර පරීක්ෂණ වලට අමතරව ජාන පරීක්ෂණද අවශ්‍ය වේ. එමනිසාම ඇල්ෆා තැලසීමියා වාහකයින් හඳුනාගැනීමේදී බොහෝ ප්‍රමාදයන් ඇතිවේ. ඇතැම්විට ඔවුන් හඳුනාගනු ලැබෙන්නේ වාහකයින් දෙදෙනෙකු විවාහ වී රෝගී දරුවෙකුද ලැබුණු පසුය.

මෙම අධ්‍යයනයේ අරමුණු වන්නේ ජාන තාක්ෂණ ක්‍රමවේදයන් යොදා ගෙන අප රටේ ඇල්ෆා තැලසීමියා රෝගය සැදීමට හේතු වන ජාන වෙනස්කම් හඳුනා ගැනීමත් එම විවිධ ජානමය වෙනස්කම් හා රෝග ලක්ෂණ වල ස්වභාවයන් අතර (උදා: ඔබගේ හිමොග්ලොබින් මට්ටම) සම්බන්ධයක් තිබේද යන්නත් සොයා බැලීමත්ය. මෙම තොරතුරු වෛද්‍යවරුන් විසින් ඇල්ෆා තැලසීමියා රෝගීන් හා වාහකයින් නිර්ණය කිරීම කාර්යක්ෂමව සිදුකිරීමට ඉවහල් වන අතර ඉක්මනින් වාහක තත්වයන් නිර්ණය කිරීමටද .හේතුවේ.

02' ස්වේච්ඡා සහභාගිත්වය

මෙම අධ්‍යයනයට ඔබගේ /ඔබගේ දරුවාගේ සහභාගී වීම සිය කැමැත්තෙන්ම සිදු වන්නකි. මෙයට සහභාගී නොවී සිටීමට ඔබට පූර්ණ අයිතියක් ඇත. එසේම ඕනෑම අවස්ථාවක හේතු දැක්වීමකින් තොරව අධ්‍යයනයෙන්

ඉවත් වීමටද ඔබට අයිතියක් ඇත.තවද ඔබගේ / ඔබගේ දරුවාගේ ඉවත්වීම හෝ සහභාගී නොවීම ඔබට/ ඔබේ දරුවාට ලැබිය යුතු වෛද්‍ය ප්‍රතිකාර සඳහා කිසිම අයුරකින් බලපෑම් ඇති නොකරයි.

03' කාල සීමාව , අධ්‍යයනයේ ක්‍රියා පිළිවෙල හා සහභාගී වන්නන්ගේ වගකීම්.

මෙම අධ්‍යයනය වසරක් පුරා ක්‍රියාත්මක වේ.මේ සඳහා එක්වරක් පමණක් ඔබගේ වම් හෝ දකුණු අතේ නහරයකින් මිලි ලීටර පහක (5) ලේ සාම්පලයක් ලබාගත යුතුය. මෙම සාම්පලය අංකයකින් පමණක් හඳුන්වන අතර ඔබගේ නම , ලිපිනය හෝ වයස වැනි ඔබව හඳුනාගැනීමට හේතුවන කිසිදු තොරතුරක් එහි සඳහන් නොකෙරේ. එය කොළඹ විශ්ව විද්‍යාලයේ වෛද්‍ය පීඨයේ මානව ප්‍රවේණි ඒකකයේ ප්‍රවේණි විද්‍යාගාරයේදී සියළුම පරීක්ෂණ වලට ලක්කෙරෙනු ඇත. අවශ්‍යතාවක් ඇතිවුවහොත් වැඩිදුරටත් තැලසීමියා ජාන පරීක්ෂාව සඳහා විදේශ රසයනගාරයන්ට සාම්පල යැවීමටද සිදුවිය හැක. තවද මෙම සාම්පලය මෙම අධ්‍යනයට හා තලසිමියාව පිලිබඳ තවත් අධ්‍යනයන් සඳහා යොදා ගැනෙනු ඇත.

ඔබව / ඔබේ දරුවාව පරීක්ෂා කිරීමට , ඔබගෙන්/ඔහුගෙන්/ඇයගෙන් ප්‍රශ්න ඇසීමට හා අදාල සායනික තොරතුරු ලබා ගැනීමට අපට ඔබගේ අවසරය අවශ්‍ය වේ. මෙම අධ්‍යයනය සඳහාඅපි ඔබගේ/ ඔබෙගේ දරුවාගේ තැලසීමියා රෝගී තත්වය පිලිබඳ සායනික තොරතුරු , ඒ සඳහා කරන ලද පරීක්ෂණ ප්‍රතිඵල හා පවුලේ සාමාජිකයින් අතර තැලසිමියාව හා ඒ හා සම්බන්ධ අනෙකුත් රෝග පිළිබඳ තොරතුරුද ලබා ගන්නෙමු. පර්යේෂක විසින් රෝගියා / මව හෝ පියා/ භාරකරු හමුවී සාකච්ඡාකර ඔවුන්ගෙන්ලබාගන්නා අදාළ තොරතුරු,සායනික හා රෝහල් වාර්තා වල ඇති තොරතුරු හා පරීක්ෂණ වාර්තා දත්ත එකතු කිරීමේ පොත් පිංච තුළ සටහන් කරගනු ලැබේ.
තවද ඔබෙන්/ ඔබේ දරුවාගෙන් ලේ සාම්පලයක් ලබා ගැනීමටත්, ජාන පරීක්ෂණ සිදුකිරීමටත් ඔබගේ කැමැත්ත අවශ්‍ය වේ.

04' මින් ලද හැකි ප්‍රතිලාභ

මෙම අධ්‍යයනය අවසානයේදී ඔබේ/ ඔබේ දරුවාගේ තැලසීමියා ජාන වාර්තාවක් ලබා දෙනු ඇත. එයට කිසිදු අයකිරීමක් නොකෙරේ. එසේම ඔබටත් ඔබ පවුලේ සාමාජිකයින්ටත් එම ප්‍රතිඵල පිළිබඳව පැහැදිලි කිරීමක්ද කෙරෙනු ඇත.

මෙම අධ්‍යයනයට සහභාගී වීම තුළින් ඔබට ශ්‍රී ලංකාවේ තැලසීමියා රෝගයට හේතුවන ජානමය වෙනස්කම් පිළිබඳව පවතින දැනුම දියුණු කිරීමට දායක විය හැකි අතර එය රෝගය නිර්ණය කරන ක්‍රම හා රෝග ප්‍රතිකාර දියුණු කිරීමට ඉවහල් වේ.

05' අවදානම් හා අපහසුතා

මෙම අධ්‍යයනය සඳහා මි.ලී. 5 ක රුධිර සාම්පලයක් ලබා ගන්නා විටදී ඔබට යම් සුළු වේදනාවක් හෝ අපහසුතාවක් ඇති විය හැක. මෙහිදී රුධිරය ලබාගත් ස්ථානයේ ලේ පැල්ලමක් ඇති වීමටත් ඉතාමත්ම කලාතුරකින් ආසාදනයක් ඇතිවීමටත් අවදානමක් ඇත. ලේ ලබා ගැනීම හොඳින් පුහුණුවූ පුද්ගලයන් හෝ වෛද්‍යවරුන් විසින් සිදු කරනු ලබයි. මෙහිදී මනාව විෂබීජ ඉවත් කරන ලද තත්ත්ව යටතේ වරක් පමණක් භාවිතාකරන සිරින්ජ හා එන්නත් කටු යොදාගැනේ. එමගින් ඇතිවිය හැකි අවදානම හා අපහසුතා අවම කෙරේ.

06' දීමනා

මෙම අධ්‍යයනයට සහභාගී වීම නිසා ඔබට කිසිම දීමනාවක් නොලැබේ.

07' රහස්‍ය භාවය

ඔබ / ඔබේ දරුවා හඳුනා ගැනීම සඳහා අනන්‍ය වූ අංකයක් පමණක් යොදා ගැනේ. අදාළපුද්ගලයාහඳුනා ගැනීමට ඉවහල් වන තොරතුරු දත්ත එක් කිරීමේ පොත් පිංවෙහි පළමු පිටුවට ඇතුලත් කරන අතර එය සෙසු පිටු වලින් වෙන්කර සුරක්ෂිතව තැන්පත් කර තැබේ.සියළු තොරතුරු පරිඝනක ගතකර මුරපදයක්(Password) යොදා අනවසරයෙන් ලබාගත නොහැකි පරිදි සුරක්ෂිත කරනු ලැබේ. එම තොරතුරු පරිහරණය කිරීමට හැකිවන්නේ ප්‍රධාන පර්යේෂක වන මා හටත් අධීක්ෂක වරුන්ටත් පමණි.

මෙම පර්යේෂණයේ ප්‍රතිඵල සායනික වාර්තා (case report)ලෙස හෝ සමස්ත ඛාණ්ඩයම ඇතුලත් පර්යේෂණ පත්‍රිකා (research paper) ලෙස විද්‍යාත්මක සභරා වල පල කිරීමටත් විද්‍යාත්මක සැසි වල ඉදිරිපත් කිරීමටත් බලාපොරොත්තු වේ. කිසිම අවස්ථාවක ඔබගේ/ ඔබෙගේ දරුවාගේ අනන්‍යතාව හෙළි නොකරන අතර සියළුම තොරතුරුවල රහස්‍ය භාවය උපරිමයෙන් ආරක්ෂා කෙරේ.අප විසින් ලබා ගන්නා දත්ත විද්‍යාත්මක සභරාවක පළකිරීමට ද අපට ඔබගේ අවසරය අවශ්‍ය වේ.මෙසේ පළකරන විට ඔබගේ නම හෝ ඔබව හඳුනා ගත හැකි කිසිම තොරතුරක් යොදාගැනීමෙන් වළකින බව සලකන්න.

08' අධ්‍යයනයෙන් ඉවත්වීම

අධ්‍යයනයට සහභාගී වීමට ලබාදුන් කැමැත්ත කවර අදියරකදී හෝ හේතු දැක්වීමකින් තොරව වෙනස් කර ඉවත් වීමට ඔබට හැකිය. ඔබගේ / ඔබගේ දරුවාගේ නිසා කිසිම අලාභයක් ගෙවීමට සිදුවීමක් , වෛද්‍ය ප්‍රතිකාර හෝ අනෙකුත් වරප්‍රසාද අහිමි වීමක් හෝ සිදු නොවේ. ඔබ ඉවත්වීමට තීරණය කළ විගස අපට ඔබගේ තීරණය දැනුම් දෙන මෙන් ඉල්ලා සිටිමු . නමුත් ප්‍රතිඵල ප්‍රකාශයට පත්කළ පසු හෝ ප්‍රකාශයට පත් කිරීමට පෙර සමාලෝචනය සඳහා භාර දීමෙන් පසුව හෝ ඉන් ඉවත් වීමට නොහැකි බව කරුණාවෙන් සලකන්න.

09' වැඩිදුර තොරතුරු

මෙම පත්‍රිකාව මඟින් අධ්‍යයනය පිළිබඳව ප්‍රමාණවත් තොරතුරු ඔබට ලැබෙන්නට ඇතැයි අපි විශ්වාස කරමු. නමුත් මෙම අධ්‍යනයට කැමැත්ත දීමෙන් පසු ඔබට තව දුරටත් දැනගැනීමට යමක් ඇතිනම් පහත දී ඇති

දුරකථන අංක ඔස්සේ ඕනෑම අවස්ථාවක මා හෝ වෛද්‍ය හේමාලි ගුණසේකර අමතා එය නිරවුල් කර ගත හැක. අපගේ ලිපිනයන් හා දුරකථන අංක පහත දක්වා ඇත.

වෛද්‍ය S .M .A .ජයවර්ධන

මානව ප්‍රවේණි විද්‍යා ඒකකය
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தகவல்பத்திரம்

இலங்கையில் உள்ள தலசீமியா நோயாளிகள் மற்றும் இந்நோயைக் காவும் நபர்களில் மூலகூற்று மற்றும் தோற்றவமைப்பு இயல்பாய்வு

Dr.S.M.A.ஜெயவர்த்தன ஆகிய நான் மனிதமரபியல் பிரிவு, கொழும்பு மருத்துவ பீடத்தில் மருத்துவ மரபியலில் பட்டப் பின்படிப்பு மாணவனாக இருக்கின்றேன். "இலங்கையில் உள்ள தலசீமியா நோயாளிகள் மற்றும் இந்நோயைக் காவும் நபர்களில் மூலகூற்று மற்றும் தோற்றவமைப்பு இயல்பாய்வு" எனும் தலைப்பில் நடைபெறும் இந்த ஆய்வில் பங்குபெற உங்களை/உங்கள் குழந்தையை அழைக்கின்றேன். என்னால் நடத்தப்படும் இந்த ஆய்வானது மனித மரபியல் பிரிவு, கொழும்பு மருத்துவ பீடத்தைச் சேர்ந்த பேராசிரியர்.வஜிர H.W. திசாநாயக மற்றும் Dr. ஹேமாலி குணசேகர ஆகியோரின் மேற்பார்வையின் கீழ் நடத்தப்படுகின்றது.

1. இந்த ஆய்வின் குறிக்கோள்

பெற்றோரிடமிருந்து அவர்களது பிள்ளைகளுக்கு பரிமாற்றப்படும் அமசங்களுக்கு காரணமானதாக உள்ளவை மரபணுக்கள் ஆகும். எமது தோற்றவமைப்புகளும் உடற்தொழில்பாடுகளும் எமது மரபணுக்களால் தீர்மானிக்கப்படுகின்றன. சில நோய்கள் பரம்பரை வழியாக மரபணுக்கள் மூலம் கடத்தப்படக் கூடியவை. இதன் அடிப்படையில் தலசீமியாவும் ஒரு பரம்பரையாக கடத்தப்படும் நோயாகும்

எமது இரத்தக் கலங்களில் காணப்படும் ஈமோகுளோபின் ஒட்சிசன் வாயுவை ஏனைய உடற்கலங்களுக்கு எடுத்துச் செல்லும். ஈமோகுளோபின் உருவாகுவதற்கு தேவையான மரபணுக்களில் ஏற்படும் மரபியல் குறைபாடுகளினால் தலசீமியா ஏற்படுகின்றது. இதனால் ஈமோகுளோபின் புரதத்தின் உற்பத்தியளவு குறைகின்றது. தலசீமியாக்களில் இரண்டு பிரதான வகைகள் உண்டு; அவை அல்பா மற்றும் பீட்டா தலசீமியாக்களாகும்.

அல்பா தலசீமியா நோயை காவும் நோயாளிகளை கண்டறிவது பீட்டா தலசீமியா நோய் காவுவோரை கண்டறிவது போல் ஒரு நேரடியான விடயம் அல்ல. இரத்தப் பரிசோதனைகளுக்கும் மேலதிகமாக மரபியல் சோதனைகளும் இதற்க்கு அவசியம். ஆகவே, அல்பா தலசீமியா நோயை காவுவோரை இனங்காண்பதில் தாமதம் ஏற்படுகிறது., சிலநேரங்களில், இரண்டு நோய் காவுவோர் திருமணம் முடித்து, அவர்களுக்கு இந்நோயினால் பாதிக்கப்பட்ட குழந்தை பிறக்கும் பொழுதே அவர்கள் நோயைக் காவுகின்றனர் என அடையாளம் காணப்படுகின்றனர்..

ஆகவே, இந்த ஆய்வின் நோக்கமானது, இலங்கையில் காணப்படும் அல்பா தலசீமியா நோயாளிகளில் உள்ள மரபணு குறைபாடுகளை கண்டறிந்து, அந்தக் குறைபாடுகள் எவ்வாறு இந்நோயைப் பாதிக்கின்றது (உதாரணம்: ஈமொகுளோபினுடைய அளவு) என்பதை

அறிவதேயாகும். இந்த கண்டுபிடிப்புக்கள், வைத்தியர்கள் அல்பா தலசீமியா நோயாளிகள் மற்றும் நோயை காவுவோரை காலதாமதம் இன்றியும் திறம்படனும் கண்டறிய உதவும்.

2. தன்னார்வ பங்கேற்ப்பு

இந்த ஆய்வில் பங்குபற்றுவதோ அல்லது பங்குபற்றாமல் இருப்பதோ (நீங்கள்/ உங்களது குழந்தை/) உங்களுடைய தனிப்பட்ட முடிவாகும். முன்னர் நீங்கள் இவ் ஆய்வில் பங்குபெற சம்மதம் தெரிவித்திருந்தாலும், எவ்வேளையிலும் உங்களால் இந்த ஆய்விலிருந்து அறிவித்தலுடன் விலகிக் கொள்ள முடியும். மேலும் நீங்கள் விலகிக் கொண்டாலோ அல்லது பங்குபெற்றாவிட்டாலோ, அது உங்களுக்கு/உங்களது குழந்தைக்கு வழங்கப்படும் மருத்துவ சிகிச்சையையோ அதன் தரத்தையோ பாதிக்காது.

3. ஆய்வின் காலம், செயல்முறை மற்றும் பங்குபெறுவோரின் கடமைகள்.

இந்த ஆய்வு ஒருவருட காலத்திற்கு நடைபெறும். உங்களது கையிலுள்ள இரத்த நரம்பிலிருந்து 5 ml இரத்தம் மனித மரபியல் பிரிவில் உள்ள ஒரு மருத்துவ தாதி/வைத்தியரினால் பெறப்படும். உங்கள் இரத்த மாதிரியானது பிரத்தியேகமான இலக்கம் மூலம் பெயரிடப்பட்டு, உங்களது பெயர், விலாசம் மற்றும் வயது போன்ற உங்களை அடையாளம் காட்டும் எந்தவொரு தகவலும் குறிப்பிடப்படாமல் பரிசோதனை ஆய்வுகூடத்திற்கு அனுப்பப்படும். தேவை ஏற்படின் மேலதிக மரபியல் பரிசோதனைகளுக்காக உங்களது இரத்தம் வெளிநாடுகளுக்கு அனுப்பப்படலாம். உங்களது இரத்த மாதிரி இந்த ஆய்விலும் வேறு தலசீமியா தொடர்பான ஆய்வுகளிலும் பயன்படுத்தப்படும்.

தலசீமியா பற்றிய மருத்துவ விவரங்கள், இந்நோயைச் சார்ந்த மருத்துவ விசாரணை அல்லது சோதனை முடிவுகள் மற்றும் தலசீமியாவுடனோ அல்லது அதனை சார்ந்த வேறு நோய்களுடன் சம்பந்தப்பட்ட குடும்ப வரலாறு போன்ற தகவல்கள் உங்களிடம் இருந்து பெறப்படும்.

உங்களை நேர்கண்டு கதைத்த பின்னர், உங்களிடமிருந்து பெறப்பட்ட தகவல்கள் ஆய்வாளரினால் தகவல் சேகரிப்புப் புத்தகத்தில் பதியப்படும். தங்களின்/ தங்கள் குழந்தையின் கை இரத்த நரம்பிலிருந்து 5 ml இரத்தம் எடுப்பதற்கு, அந்த இரத்த மாதிரியை மரபியல் பரிசோதனைக்கு உட்படுத்துவதற்கு மற்றும் மேலே குறிப்பிட்ட வகையில் தகவல் சேகரிப்பதற்கு உங்களது சம்மதம் கோருகின்றோம்.

நன்மைகள்

பங்குபெற்றுவோர் தலசீமியாவிற்கான மரபியல் சோதனை முடிவுகளை இந்த ஆய்வின் முடிவில் இலவசமாக பெற்றுக்கொள்ளலாம். மேலும், இந்த ஆய்வில் பங்குபெற்றும்

அனைவருக்கும் மற்றும் அவர்களது குடும்பத்தினர்களுக்கும் சோதனை முடிவுகள் பற்றிய கருத்துரை/ஆலோசனைஇலவசமாக வழங்கப்படும்.

நீங்கள் இந்த ஆய்வில் பங்குபெற்றுவதன் மூலம், தலசீமியாவின் மரபியல் அடிப்படை பற்றிய புது தகவல்களையும் அறிவையும் பெற உதவுவதுடன் தலசீமியாவை கண்டறிந்து குணப்படுத்தும் முறைகளை மேம்படுத்தவும் பங்களிப்புச் செய்வீர்கள்.

4. அபாயங்கள், தீங்குகள்மற்றும்உபாதைகள்.

மரபியல் பரிசோதனைகளுக்காக கையிலுள்ள இரத்த நரம்பிலிருந்து 5 ml இரத்தம் எடுக்கப்படும் போதோ பின்னரோ சிலவேளைகளில் வலி, சிறு உபாதைகள் ஏற்படலாம். இரத்தம் எடுப்பது கடினமாக இருக்கும் பட்சத்தில் ஊசி செலுத்தப்பட்ட இடத்தில் சிலசமயம் சிராய்ப்பு ஏற்படலாம். மிகவும் அரிதாக ஊசி குத்தப்பட்ட இடத்தில் தொற்றுக்கள் உண்டாகலாம்.

பயிற்றுவிக்கபபட்ட மருத்துவ தாதி அல்லது வைத்தியர் ஒருவரினால் கிருமிகள் அற்ற நிலையில் தொற்று நீக்கப்பட்ட ஒரு தரம் மாத்திரமே பாவிக்கப்படும் ஊசியினை பயன்படுத்தி இரத்தம் எடுக்கப்படும் போது இந்த அபாயங்கள் மற்றும் உபாதைகள் தடுக்கப்படும் அல்லது குறைக்கப்படும்.

5. செலவுஈடுகள்

இந்த ஆய்வில் பங்குபெறுவதற்கு எந்தவித செலவும் ஈடுசெய்யப்பட மாட்டாது.

6. இரகசியத்தன்மை

நீங்கள்/உங்களது குழந்தை ஒரு இலக்கத்தைக் கொண்டே அடையாளப்படுத்தப்படுவீர்கள். உங்களை அடையாளம் காட்டும் வகையில் தகவல் சேகரிப்புப் புத்தகத்தில் உள்ள எல்லாத் தகவல்களும் மற்றைய தகவல்களில் இருந்து பிரிக்கப்பட்டு வேறாக வைக்கப்படும். சேகரிக்கப்பட்ட தகவல்கள் கணனியில் கடவுச்சொல் பாதுகாப்புடன் எனக்கும் மற்றைய ஆய்வாளர்களுக்குமாத்திரமே பார்க்கக்கூடியதாக இருக்கும்.

இந்த ஆய்வினுடைய முடிவுகளை தனிப்பட்ட அல்லது முடிவுகள் அனைத்தையும் ஒன்றுசேர்த்த அறிக்கையாக அறிவியல் இதழ்களில் மற்றும் கருத்தரங்குகளில் முன்னிலைப்படுத்த எதிர்பார்கின்றோம். இந்த எந்தவோர் சந்தர்பத்திலும் நீங்களோ/ உங்களது குழந்தையோ பெயரிடப்படவோ அல்லது அடையாளப்படுத்தப்படவோ மாட்டீர்கள். அதுமட்டும் இன்றி மிகவும் கண்டிப்பான இரகசியத்தன்மை நிச்சயமாகப் பேணப்படும்.

7. பங்குநிறுத்தம்

நீங்கள் இந்த ஆய்விலிருந்து காரணங்கள் எதுவும் கூறாமல், தண்டங்களோ அல்லது மருத்துவ பராமரிப்பில் பாதிப்புகளோ இல்லாமல் எவ்வேளையிலும் விலகிக்கொள்ள முடியும். இருப்பினும், ஆய்வு முடிவுகள் பிரசுரிக்கப்பட்ட பின்னரோ அல்லது பிரசுரிக்க அனுப்பிய பின்னரோ உங்களால் இந்த ஆய்விலிருந்து விலகிக்கொள்ள முடியாது.

8. விளக்கம்

இந்த தகவல் பத்திரம் உங்களுக்குத் தேவையான தகவல்களை அளித்திருக்கும் என எதிர்பார்க்கின்றோம். இருப்பினும், உங்களுக்கு மேலும் ஏதாவது கேள்விகள் கேட்க வேண்டியிருந்தால் எந்நேரமும், என்னையோ அல்லது Dr . குணசேகரவையோ தொடர்பு கொள்ளலாம். எமது தொடர்பு விவரங்கள் கீழே:

Dr. S.M.A .ஜெயவர்த்தன

மருத்துவ மரபியல் முதுநிலை கல்வி மாணவன்

மனிதமரபியல் பிரிவு,

கொழும்பு மருத்துவபீடம்.

0718076137

Dr. ஹேமாலி W. W. குணசேகர

மனிதமரபியல்பிரிவு,

கொழும்புமருத்துவபீடம்.

011-2689545

Study Identification No:

Consent Form

Molecular and Phenotypic Characterization of Alpha Thalassaemia patients in Sri Lanka.

Part A: (to be completed by the participants/parents/guardians)

Instructions: Please select "yes/no" for each question except in question no 5

1	Have you read and clearly understood the information given to you about the study?	Yes / No
2	Have you had an opportunity to discuss and ask questions about the study?	Yes / No
3	Were you given satisfactory answers to all your questions?	Yes / No
4	Have you received enough information about the study?	Yes / No
5	Who explained the study to you *(please write the name clearly)* Dr.……	
6	Do you understand that you are free to withdraw you/ your child/ your ward , from the study at any time without giving reasons and without affecting your/ your child's/your ward's future medical care?	Yes / No
7	Sections of your/ your child's /your ward's medical records,investigation reports and personal details may be examined by other researchers participating this study. All personal details will be treated as STRICTLY CONFIDENTIAL. Do you give your permission for these individuals to have access to your/your child's/your ward's records ?	Yes / No
8	Do you give consent to use left over blood samples to be used by prospective researchers after this study?	Yes / No
9	Do you give consent to send the blood/ DNA samples abroad?	Yes / No
10	Have you had sufficient time to come to your decision?	Yes / No
11	Do you agree to take part in this study?	Yes / No

(please turn over)

Name (participant)……..

Signature …… Date……..

Mother's/Father's/Guardian's name……

Signature …….. Date……..

Part B **(*to be completed by investigator*)**

I, Dr.,…….. have explained about the study to above mentioned volunteer/ parent or guardian of a child with thalassaemia who volunteered to enroll his/ her child in the study and he/she indicated his/her willingness to take part in the study.

Signature…… Date……..

Study Identification No:

අධ්‍යනයට සහභාගී වීමට කැමැත්ත ප්‍රකාශ කිරීම

“ ශ්‍රී ලංකාවේ ඇල්ෆා තැලසීමියා රෝගීන්ගේ ජානමය වෙනස්කම් හා රූපානුදර්ශය අධ්‍යයනය කිරීම”

“A" කොටස (සහභාගී වන්නන් විසින් පිරවීම සඳහා)

උපදෙස් : පස්වන ප්‍රශ්නය හැර සෑම ප්‍රශ්නයක් ඉදිරියේම ඔව් හෝ නැහැ යන්න ලකුණු කරන්න.

1	ඔබ මෙම **අධ්‍යයනය** පිලිබඳ තොරතුරු පත්‍රිකාව කියවා හොඳින් අවබෝධ කරගත්තාද?	ඔව් / නැහැ
2	මෙම **අධ්‍යයනය** ගැන ඔබට ඇතිවූ ප්‍රශ්න නිරාකරණය කරගැනීමට අවස්ථාවක් ලැබුණාද ?	ඔව් / නැහැ
3	ඔබගේ ප්‍රශ්න වලට සතුටුදායක පිළිතුරු ලැබුණේද?	ඔව් / නැහැ
4	මෙම **අධ්‍යයනය** පිලිබඳ ප්‍රමාණවත් තොරතුරු ලැබුණේද ?	ඔව් / නැහැ
5	ඔබට මෙම **අධ්‍යයනය** පිළිබඳව පැහැදිලි කර දුන්නේ කා විසින්ද ? (පැහැදිලිව නම ලියන්න)	
6	ඕනෑම අවස්ථාවකදී ඔබට මෙම **අධ්‍යයනයෙන්** හේතු දැක්වීමකින් තොරව ඉවත් වීමේ අයිතිය ඇති බවත් එසේ ඉවත් වීම ඔබට/ඔබේ දරුවාට/ ඔබ භාරකාරත්වය දරන දරුවාට ලැබෙන වෛද්‍ය ප්‍රතිකාර කෙරෙහි කිසි ලෙසකින් හෝ බල නොපාන බවත් ඔබ වටහා ගත්තද?	ඔව් / නැහැ
7	මෙම **අධ්‍යයනය** තුළදී ඔබේ / ඔබේ දරුවාගේ/ ඔබ භාරකාරත්වය දරණ දරුවාගේ සායනික වාර්තා , පරීක්ෂණ වාර්තා හා පෞද්ගලික තොරතුරු ලබා ගන්නා අතර සියළු පෞද්ගලික තොරතුරු වල රහස්‍ය භාවය තහවුරු කෙරේ. මෙම තොරතුරු ලබා ගැනීමට ඔබගේ අවසරය ලබාදෙන්නේද?	ඔව් / නැහැ
8	ඉතිරි රුධිර සාම්පල අනාගත **අධ්‍යයනයන්** සඳහා යොදා ගැනීමට ඔබේ අවසර ලබා දෙනවාද?	ඔව් / නැහැ
9	සාම්පල වැඩි දුර **අධ්‍යයනයන්** සඳහා විදේශ රටකට යැවීමට ඔබේ අවසර ලබාදෙනවාද?	ඔව් / නැහැ
10	තීරණයකට එළඹීමට ඔබට ප්‍රමාණවත් කාලයක් ලැබුනාද?	ඔව් / නැහැ
11	ඔබ මෙම අධ්‍යයනයට සහභාගී වීමට එකඟ වන්නේද?	ඔව් / නැහැ

(කරුණාකර අනෙක් පිට බලන්න)

නම:.....

අත්සන:..... දිනය:.....

මව/පියා /භාරකරු :.....

අත්සන:..... දිනය:.....

B" කොටස - පර්යේෂක විසින් පිරවීම සඳහා

වෛද්‍ය .....වන මවිසින් ඉහත සඳහන් ස්වේච්චාවෙන් සහභාගී වන පුද්ගලයාට/ මෙම අධ්‍යනයට තම දරුවා /තමා භාරකාරත්වය දරන දරුවා සහභාගී කිරීමට ස්වේච්චාවෙන් ඉදිරිපත් වූ මව/පියා/භාරකරුට අධ්‍යනය පිලිබඳ පැහැදිලි කර දෙන ලද අතර මෙම අධ්‍යයනයට සහසහභාගී වීමට ඔහු/ඇය කැමත්ත ලබා දෙන ලදී.

අත්සන:..... දිනය:.....

ஆய்வுஅடையாளஇலக்கம்

ஒப்புதல்படிவம்

இலங்கையரில் அல்பா தலசீமியா பற்றியமூலகூற்றுமற்றும்தோற்றவமைப்புஇயல்பாய்வு.

பகுதிA (பங்குபற்றுபவர், பெற்றோர்அல்லதுபாதுகாவலரினால்நிரப்பப்படவேண்டியது)

அறிவுறுத்தல்கள்: தயவு செய்து கேள்வி இலக்கம் ஐந்தைத் தவிர மற்றைய கேள்விகளுக்கு ஆம் அல்லது இல்லை என்ற பதிலுக்குபுள்ளடியிடவும்.

1	இந்தஆய்வைபற்றியதகவல்களைமுழுமையாகவாசித்துதெளிவாகவிளங்கிகொண்டீரா?	ஆம் /	இல்லை
2	இந்தஆய்வைபற்றிகலந்துரையாடமற்றும்வினாவஉங்களுக்குசந்தர்ப்பம்கிடைத்ததா?	ஆம் /	இல்லை
3	உங்கள்எல்லாக்கேள்விகளுக்கும்திருப்திகரமானபதில்கள்கிடைத்தனவா?	ஆம் /	இல்லை
4	இந்தஆய்வைப்பற்றிபோதுமானதகவல்கள்கிடைத்ததா?	ஆம் /	இல்லை
5	இந்தஆய்வைப் பற்றிஉங்களுக்குயார்விளக்கம்அளித்தது? (பெயரை தெளிவாக எழுதவும்)Dr.....		
6	உங்களுக்கு/உங்கள்குழந்தைக்கு/உங்கள்பாதுகாப்பில்உள்ள குழந்தைக்குஅளிக்கப்படும்மருத்துவசேவையில்எவ்விதபாதிப்பும்ஏற்படாமலும் எந்தவிதகாரணங்களும்கூறாமலும்உங்களால்இந்தஆய்விலிருந்துஎந்நேரமும் விலகிக்கொள்ளமுடியும்என்பதைபுரிந்துகொண்டுள்ளீரா?	ஆம் /	இல்லை
7	உங்களது/உங்கள்குழந்தையினது/ உங்கள்பாதுகாப்பில்உள்ள குழந்தையினதுமருத்துவப்பதிவுகள், பரிசோதனைஅறிக்கைகள்மற்றும்தனிப்பட்டவிவரங்கள்இந்தஆய்வில்பங்குபெற்றும்மற்றையஆராய்ச்சியாளர்களினால்சிலநேரங்களில்ஆய்வுசெய்யப்படலாம். அனைத்துதனிப்பட்டவிவரங்களும்நிச்சயமாகரகசியமாகபேணப்படும். இந்தஆய்வாளர்கள்இத்தகவல்களைஉபயோகிக்கநீங்கள்அனுமதிக்கின்றீர்களா?	ஆம் /	இல்லை
8	மீதமுள்ள இரத்த மாதிரிகளை வருங்காலஆய்வாளர்கள்இந்தஆய்வுக்குப் பின் பயன்படுத்த அனுமதிகின்றீர்களா?	ஆம் /	இல்லை
9	உங்கள்இரத்தமாதிரியைமேல்பரிசோதனைகளுக்காகவெளிநாடுகளுக்குஅனுப்பசம்மதிக்கின்றீர்களா?	ஆம் /	இல்லை
10	நீங்கள்உங்கள்முடிவுக்குவரபோதுமானநேரம்இருந்ததா?	ஆம் /	இல்லை
11	இந்தஆய்வில்பங்குபெற்றசம்மதிக்கின்றீர்களா?	ஆம் /	இல்லை

பங்குபெற்றுபவரின்பெயர்:.....

கையொப்பம்:.....திகதி:.....

தாய்/தந்தை/பாதுகாவலரின்பெயர்:.....

கையொப்பம்:.....திகதி:.....

பகுதிB (ஆய்வாளரினால்நிரப்பப்படவேண்டியது):

..... ஆகிய நான், இந்த ஆய்வை பற்றி மேலே குறிப்பிடப்பட்டுள்ளதன்னார்வபங்கேற்பாளருக்கு/ தலசீமியாவினால் பாதிக்கப்பட்டுள்ள தங்கள் குழந்தையை இந்த ஆய்வில் பங்குபெறச்செய்ய சுயமாக முன்வந்த பெற்றோர் அல்லது பாதுகாவலருக்கு விளக்கியுள்ளேன்.
இவர்இந்தஆய்வில்தங்கள்குழந்தையைபங்குபெறச்செய்யவிருப்பத்தைதெரிவித்துள்ளார்

கையொப்பம்.....திகதி.....

Molecular and Phenotypic Characterization of Alpha Thalassaemia Patients in Sri Lanka

Study Subject No : …… **Date(*dd/mm/yy*)……..**

Name: ……..

Date of Birth/ Age: (*dd/mm/yy*)…….

Gender: Male / Female

Ethnicity: Sinhala/ Tamil/ Muslim/ Other (please specify)……

Address: ……..

……..

Contact No: ……..

E-mail: …….

Referring consultant/ Doctor: Dr/Prof……..

……..

Hospital/Thal unit: …….

BHT/Clinic No: ……

Data Protection and Confidentiality

After completion of this page, ensure that the subject study number is entered on all pages of this booklet. Then detach this page and store separately from the remainder of the booklet.

Study subject No:……

1.Indication for referral :

1.1 Investigated for Alplha thalassaemia ☐

1.2 Investgated for other disease ☐(please specify……)

2.Clinical presentation

2.1Normal Hb, Family screening ☐

2.2Low Hb ☐

2.3 Anaemia , On occasional transfusion ☐

2.3.1 No of transfusions done ……

2.3.2.Events leading to transfusion Infection ☐

Surgery/ trauma ☐

Other (*Please specify*)….☐……..

2.4 Anaemia, on regular Transfusion ☐

2.4.1 Age at which transfusion started ……..

2.4.2 Frequency……

2.4.3 Iron chealation: Desferrioxamine/ deferipone/ other

3.0 Family History

Study subject No:……

3.1 Consanguinity

Yes	
No	

3.2 Family history of Haemoglobinopathy

Yes	
No	

3.3 pedigree information

I
II
III
IV
V

Additional pedigree information

Location in pedigree	Clinical or other information

Study subject No:……

4. Haematological investigations

4.1 Full blood count

	Initial	Follow up		
Date				
Hb				
PCV/HCT				
RBC count				
MCV				
MCH				
MCHC				
RDW-CV				
RDW-				
WBC				
N				
L				
Platelet				

4.2 Blood picture

	Initial	Follow up	
Date		Blood picture 1	Blood picture 2
RBC			
WBC			
Platelet			
Comment			

Study subject No:……

4.3 HbH inclusions - Present/Absent

4.4 Retic count ……..%

4.5 HPLC

Machine BioRad / other Date ___/ ____/20____

HbA HbA2 HbF Other Hb variants

Conclusion ……..

4.6 Bone marrow finding(*if available*) :

5.0 Iron studies

Test	Date	Value	Reference range
Serum ferritn			
Serum iron			
TIBC			
Transferin saturation			

7.0 Genetic testing Done/Not done

Genetic test/date	Index case	Family members				

Study subject No:……

8.0 Other important findings……
…… ……