Prevalence of β-Thalassemia Carriers among a high Health Institute Students in Sana’a, Yemen (2013-2016)

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B.Sc. (Honors) of Medical Laboratory Sciences University of Sana’a College (1995)

A Dissertation Submitted to the University of Gezira in Fulfillment of the Requirements for the Award of the Degree of Master of Science in Haematology and Immunohematology

Department of Haematology Faculty of Medical Laboratory Sciences University Of Gezira

March 2016
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_Date: 28/ 3/2016_
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Date: 28/3/2016
بسم الله الرحمن الرحيم

قال تعالى:

"وَلَيَخْشَ الَّذِينَ لَوْ تَرَكُوا مِنْ خَلْفِهِمْ ذُرِّيَّةً ضِعَافًا خَافُوا عَلَيْهِمْ فَلْيَتَّقُوا اللهََّ وَلْيَقُولُوا قَوْلًً سَدِيدًا"

صدق الله العظيم

سورة النساء الآية (9)
DEDICATION

To Mom and Dad

Tomy family an my Clan
Tomy teachers
Tomy colleagues
Toburncandlesforlightsto others
To who taught me a letter
ACKNOWLEDGEMENT

I extend my thanks to my teacher, and Supervisor Dr. A. Salma Othman Taha who provides me with necessary knowledge. Our thanks is also to the administration of University of Gezira.

I would like to express my gratitude to all those who have helped me to write this work paper especially to my Dr. A. Salma Othman Taha.

I am very grateful to my respectable Dr. A.AmaniMohammed Ali Shamshair. Thanks to Dr. AlbadawiAbdelbagi Talha, great who has shouldered the burden of teaching, training, and leading me in the right path of life. I am also very thankful to Ustaz Badreldein, and my friends who helped and advised me.

I take pleasure in recording my gratitude to all doctors, laboratory technologists and the staff of central laboratory in sana,a.

Great thanks to the volunteers who participated willingly in this study.
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Abstract

The thalassemias are characterised by reduced synthesis of one or more of the globin chains that form the oxygen-carrying hemoglobin molecules found in red blood cells. Hemoglobins are tetrameric molecules, with 2 α-like and 2 β-like globin polypeptide chains, each associated with a heme group. In normal adult, HbA (α2β2) accounts for around 97.5% of the hemoglobin in erythrocytes; there is another component called HbA2 (α2δ2), which normally constitutes <2.5% of total hemoglobin. Fetal hemoglobin or HbF (α2γ2) is the major hemoglobin synthesised before birth, but normal adult have <1% Hb F. β-thalassemia constitutes one of the most serious health problems worldwide, accounting for a major number of childhood deaths per year primarily in regions of the world endemic for malaria. It is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia. It results from a variety of molecular defects that reduce (β+-thalassemia) or abolish (β0-thalassemia) the synthesis of the β-globin chains of hemoglobin.

A representative sample of a thousand volunteer institute students was screened for evidence of thalassemia minor. Complete blood counts using automated blood cell analysers and blood smears were examined. Patients having anemia, abnormal red cell indices or morphological features of thalassemia minor like hypochromia, microcytosis, target cells, erythrocytosis, and family history of thalassemia were then investigated for determination of HbA2 & HbF levels. Estimation of hemoglobin A2 was performed by micro-column chromatography while HbF was done using alkali denaturation. (forty eight )out of the thousand samples tested positive for thalassemia minor. They all showed a hemoglobin A2 of more than 3.6 percent and higher, associated in most of the cases with mild anemia, erythrocytosis and hypochromic microcytic red cells. We reached to the conclusion that the prevalence of thalassemia minor in our community, represented at institute students at fertile age, to be 4.8%. We hope that similar figures could be made available in the future for the rest of Yemen and the so that a national figure could be presented to the world literature.
انتشار β-الثلاسيميا للحاملين في المعهد العالي للعلوم الصحية الطلاب في صنعاء اليمن (2016-2017)

إسماعيل عبد الله أحمد سعد الله

ملخص الدراسة

تمييز الثلاسيميا بالانخفاض في تكوين وانتاج واحداً وآخراً من سلسل غلوبين يشكل جزئياً خضاب الدم القادر على عمل الأكسجين والتي توجد في خلايا الدم الحمراء، هي عبارة عن جزيئات رابعة القسيمات، تتكون من سلسلتين α وسلسلتين β وجسجم السلاسل ترتبط بمجموعات هيم أ، HBA تمثل حوالي %97.5 من خضاب الدم في كريات الدم الحمراء. وهناك عاصر آخر يسمى هيموغلوبين 2 (α2α2)β2 HBA2، والتي تشكل عادةً 2.5% مناجم ال β خضاب الدم. خضاب الدم الجنيني HBF (α2γ2) β وهو خضاب دم نموذجي للولادة في الولادة ويكون >1% في البالغين، وهو غلوبين وتشكل β-الثلاسيميا واحدة من أخطر المشاكل الصحية ب全世界، وهو يؤدي إلى وفيات عدد كبير من الأطفال سنوياً في المقام الأول في مناطق متعددة من العالم والموبوءة بالملاريا وهو مرض وراثي مثلمي يتميز بحجم كريات الدم الحمراء وفقر الدم الانحلالي، وهو يتضمن مجموعة متنوعة من العيوب الجزيئية التي يكون فيها إنتاج سلسلة غلوبين أحادي السلاسل والثلاسيميا في خضاب الدم والذين يتولى الي عجز كل (β) أو عجز كلي (β) في تركيب سلسلة غلوبين التي تدخل في تكوين خضاب الدم. وفي هذه الدراسة تم اخذ عيناته من 1000 طالب وتالبة من طلاب المعهد العالي للعلوم الصحية بصنعاء، وتم إجراء السحح لإعداد نسبة انتشارها وتفشي حامل الثلاسيميا الصغرى. وقد تم إجراء فحص التحليل الكامل لمكونات الدم وإجراء فحص فلم الدم وقد تم فرز الحالات للطلاب الذين يعتلون من فقر الدم وذلك عندما يكون حجم الكريات الحمراء أو نسبة شبعها بالخضاب أقل من الحد الطبيعي، كذلك عندما يكون عدد الكريات الحمراء أكثر من الحد الطبيعي، وخصوصاً عندما يصاحب ذلك تاريخ عائلى لدى الطالب بفقر الدم (β0، لكل هذه الحالات اجرت فحص تحديد نسبة خضاب الدم بو (A2) وخضاب الدم نوع (F) أعدد أن 48 طالباً من العينة المدروسة يحملون صفة الوراثية لفقر الدم الحموي وجميعهم ظهروا نتائج خضاب الدم أكثر من %3، 4% يصاحب في معظم الحالات فقد درج خفي أو انتقال مستوى لزمنيد دم الحموي، وهو من جمعيات ومثل هبط قبل المعهد الصحي في عموم المعلومية وهو %4 ونأمل أن تتوفر دراسات وأرقام مماثلة لهذه الدراسة في المستقبل القريب لكافة أنحاء اليمن ومن ثم الحصول على أرقام وطنية تقدم للبحث العالمية.
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<td>CBC</td>
<td>Complete blood count</td>
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<td>Hb</td>
<td>Haemoglobin</td>
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<td>HBB</td>
<td>Beta globin</td>
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<tr>
<td>Hcp</td>
<td>Health care provide</td>
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<td>Hplc</td>
<td>High performance liquid chromatog</td>
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<td>MCV</td>
<td>Mean corpuscular volume</td>
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<tr>
<td>MCH</td>
<td>Mean corpuscular volume</td>
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<td>PC</td>
<td>Premarital counselor villus sampling</td>
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<td>CVS</td>
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<td>Pcv</td>
<td>Packed cell volume</td>
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Chapter One

1. Introduction

1.1 Background:

Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals (flint et, 1998). The total annual incidence of symptomatic individuals was estimated to be in 100,000 throughout the world and 1 in 10,000 people in the European Union (Vichinsky, 2005). Three main forms have been described: thalassemia major, thalassemia intermedia and thalassemia minor (Galanello, et al, 1979). Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular red blood cell (RBC) transfusions. Findings in untreated or poorly transfused individuals with thalassemia major, as seen in some developing countries, are growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow. Regular transfusion therapy leads to iron overload-related complications including endocrine complication growth retardation, failure of sexual maturation, diabetes mellitus, and insufficiency of the parathyroid, thyroid, pituitary, and less commonly, adrenal glands, dilated cardiomyopathy, liver fibrosis and cirrhosis. Patients with thalassemia intermedia present later in life with moderate anemia and do not require regular transfusions (Giardinia, et al, 2007). Main clinical features in these patients are hypertrophy of erythroid marrow with medullary and extramedullary hematopoiesis and its complications (osteoporosis, masses of erythropoietic tissue that primarily affect the spleen, liver, lymph nodes, chest and spine, bone deformities with typical facial changes), gallstones, painful leg ulcers and increased predisposition to thrombosis. Thalassemia minor is clinically asymptomatic but some subjects may have moderate anemia. Beta-thalassemias are caused by point mutations or, more rarely, deletions in the beta globin gene on chromosome 11, leading to reduced (β+) or absent (β−) synthesis of the beta chains of hemoglobin (Hb). Transmission is autosomal recessive; however, dominant mutations have also been reported. Diagnosis of thalassemia is based on hematologic and molecular genetic testing.
Differential diagnosis include genetic sideroblastic anemias, congenital dyserythropoietic anemias, and other conditions with high levels of HbF (such as juvenile myelomonocytic leukemia and aplastic anemia). Genetic counseling is important and prenatal diagnosis can be offered. Treatment of thalassemia major includes regular RBC transfusions, iron chelation and management of secondary complications of iron overload. In some circumstances, spleen removal may be required. Bone marrow transplantation remains the only definitive cure currently available. Individuals with thalassemia intermedia may require splenectomy, folic acid supplementation, treatment of extramedullary erythropoietic masses and leg ulcers, prevention and therapy of thromboembolic events. Prognosis for individuals with beta-thalassemia has improved substantially in the last 20 years following recent medical advances in transfusion, iron chelation and bone marrow transplantation therapy. However, cardiac disease remains the main cause of death in patients with iron overload.

1. **Justification:**

It is a medical fact that Thalassemia cases in Yemen is in a dramatic increase (Association Thalassemia of Yemen). According to the (WHO) report about 1 in 16 Yemeni suffers from Thalassemia and requires multiple blood transfusion resulting in highly social and economic impact on Yemens. On the other hand, the researcher tries to find out educational activities to reflect the danger to the non-patients to try out premarital check for Thalassemia as a preventive step.

1. **Objectives:**

* General Objectives:*
To determine the prevalence of B Thalassemia Trait (BTT) among students of High Health Institute of Sanaa.

* Specific Objectives:*
1- To assess Hb A2 level of β Thalassemia that trait (BTT) among students in Health Institute of Sanaa.
2- To determine Thalassemia by early medical investigation.
3- To assess the association of hematological indices with β Thalassemia trait (carriers).
4- To establish a database registry of all affected students.
5- To identify the geographic and variation of the disease.
Chapter Two

2. Literature Review

The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood). Beta-thalassemia includes three main forms:

1. Thalassemia Major, variably referred to as "Cooley's Anemia" and "Mediterranean Anemia".
2. Thalassemia Intermedia.
3. Thalassemia Minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia".

Apart from the rare dominant forms, subjects with thalassemia major are homozygotes or compound heterozygotes for $\beta^-$ or $\beta^+$ genes, subjects with thalassemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes.

Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis, resulting in reduced Hb in red blood cells (RBC), decreased RBC production and anemia.  

- Beta-thalassemia with associated Hb anomalies
  - HbC/Beta-thalassemia
  - HbE/Beta-thalassemia
  - HbS/Beta-thalassemia (clinical condition more similar to sickle cell disease than to thalassemia major or intermedia)

- Hereditary persistence of fetal Hb and beta-thalassemia

- Autosomal dominant forms

- Beta-thalassemia associated with other manifestations
  - Beta-thalassemia-tricotriothydystrophy
  - X-linked thrombocytopenia with thalassemia (Flint J et al 1998)

2.1 Epidemiology:
Globally, Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia. The high gene frequency of
beta-thalassemia in these regions is most likely related to the selective pressure from plasmodium falciparum malaria population migration and intermarriage between different ethnic groups has introduced thalassemia in almost every country of the world, including Northern Europe where thalassemia was previously absent. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union. However, accurate data on carrier rates in many populations are lacking, particularly in areas of the world known or expected to be heavily affected. (Vichinsky EP-2005) According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world.(http://www.thalassemia.org.cy-2008). The most common combination of beta-thalassemia with abnormal Hb or structural Hb variant with thalassemic properties is HbE/beta-thalassemia which is most prevalent in Southeast Asia where the carrier frequency is around 50%. (Borgna-etal 2004).

2.2 Etiology:
More than 200 mutations have been so far reported; the large majority are point mutations in functionally important regions of the beta globin gene. (Giardine B-et al - 2007). Deletions of the beta globin gene are uncommon. The beta globin gene mutations cause a reduced or absent production of beta globin chains.(Giardine B etal 2007).

2.3 Genetic Modifiers:
Modifier genes are defined as genetic variants that lead to differences in disease phenotype. In homozygous beta-thalassemia, primary genetic modifiers, affecting the clinical severity of the disease, include genetic variants able to reduce the globin chain imbalance, therefore resulting in a milder form of thalassemia. These factors are the presence of silent or mild beta-thalassemia alleles associated with a high residual output of beta globin, the co-inheritance of alpha thalassemia and/or of genetic determinants able to sustain a continuous production of gamma globin chains (HbF) in adult life, (Galanello R, Cao A,et al-1998).
Some beta-thalassemia mutations (i.e. deletion and non deletion delta beta-thalassemia, deletions of the 5' region of the beta globin gene) increase "per se" the gamma globin gene output. Other mutations increasing HbF production are those associated with deletional and non-deletional HPFH linked to the beta globin gene cluster. Recently, the genome-wide association approach, particularly studying quantitative trait loci (QTL) which cause elevated HbF, have revealed genetic elements (i.e. polymorphism in BCL11A gene and in the HBS1LCMYB intergenic region) unlinked to beta globin gene cluster, able to modify the severity of the homozygous beta zero thalassemia. The clinical phenotype of homozygous beta-thalassemia may also be modified by the co-inheritance of other genetic variants mapping outside the globin clusters. These secondary genetic modifiers influence mainly the complications of the thalassemia phenotype. Several secondary genetic modifiers have been identified in the recent years. The presence of (TA)7 polymorphism in the promoter region of the uridine diphosphate glucuronosyl transferase gene, which in the homozygous state is associated with the Gilbert syndrome, is a risk factor for the development of cholelithiasis in thalassemia major and intermedia patients (Galanello R, et al -1997).

Other candidate genes for modification of the thalassemia phenotype are the apolipoprotein E ε4 allele and some HLA haplotypes, which seem to be genetic risk factors for left ventricular failure in homozygous beta-thalassemia (Kremastinos DT, et al -1999).

Less consistent data have been reported for genes involved in iron metabolism (i.e. C282Y and H63D HFE gene mutations), probably because their effect on iron overload is hidden as a result of treatment (i.e. secondary iron overload from red cell transfusion and iron chelation), and for genes associated with bone metabolism . (Longo F, et al -1999).

Recently, a polymorphism in glutathione-Transferase M1 gene has been associated with an increased risk of heart iron overload in thalassemia major . (Origa R, et al - 2008).

In some instances, heterozygous beta-thalassemia may lead to the thalassemia intermedia phenotype instead of the asymptomatic carrier state. Most of these patients have excess functional alpha globin genes (alpha gene triplication or quadruplication) which increases the imbalance in the ratio of alpha/non-alpha globin chain synthesis. (Sollaino MC, et al 2009).
2.\textsuperscript{4} Pathophysiology:

The reduced amount (\(\beta^+\)) or absence (\(\beta^-\)) of beta globin chains result in a relative excess of unbound alpha globin chains that precipitate in erythroid precursors in the bone marrow, leading to their premature death and hence to ineffective erythropoiesis. The degree of globin chain reduction is determined by the nature of the mutation at the beta globin gene located on chromosome. Peripheral hemolysis contributing to anemia is less prominent in thalassemia major than in thalassemia intermedia, and occurs when insoluble alpha globin chains induce membrane damage to the peripheral erythrocytes. Anemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the bone marrow (up 25 to 30 times normal), which in turn causes the typical previously described bone deformities. Prolonged and severe anemia and increased erythropoietic drive also result in hepatosplenomegaly and extramedullary erythropoiesis. (Galanello R, 2010).

2.\textsuperscript{5} Hereditary Transmission:

The beta-thalassemias are inherited in an autosomal recessive manner. The parents of an affected child are obligate heterozygotes and carry a single copy of a disease-causing beta globin gene mutation. At conception, each child of heterozygotes parents has 25\% chance of being affected, 50\% chance of being an asymptomatic carrier, and 25\% chance of being unaffected and not carrier. The parents of the proband have a 1 in 4 (25\%) risk of having further affected children in each pregnancy. Dominant forms of beta-thalassemia, associated with mutations that result in the production of highly unstable beta globulin variants and leading to a clinically manifesting phenotype of beta-thalassemia in heterozygotes, have been discussed above in the clinical description section (Thein SL, 1992).

2.\textsuperscript{6} Diagnosis:

Clinical Diagnosis

Thalassemia major is usually suspected in an infant younger than two years of age with severe microcytic anemia, mild jaundice and hepatosplenomegaly. Thalassemia intermedia presents at a later age with similar but milder clinical findings. Carriers are usually asymptomatic, but sometimes may have mild anemia. Hematologic diagnosis RBC indices show microcytic anemia. Thalassemia major is characterized by reduced Hb level (<7 g/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean corpuscular Hb (MCH) > 12< 20 pg. Thalassemia intermedia is characterized by Hb
level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased Hb A2 level (Galanello R, et al, 1979).

2-V Peripheral Blood Smear:

- Affected individuals show RBC morphologic changes [microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells)], and nucleated RBC (i.e., erythroblasts). The number of erythroblasts is related to the degree of anemia and is markedly increased after splenectomy.
- Carriers have less severe RBC morphologic changes than affected individuals. Erythroblasts are normally not seen.
- Qualitative and quantitative Hb analysis (by cellulose acetate electrophoresis and DE-52 microchromatography or HPLC) identifies the amount and type of Hb present. The Hb pattern in beta-thalassemia varies according to beta-thalassemia type. In beta0 thalassemia, homozygotes HbA is absent and HbF constitutes the 92-95% of the total Hb. In beta+ thalassemia homozygotes and beta+/ beta0 genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA2 is variable in beta thalassemia homozygotes and it is enhanced in beta thalassemia minor. Hb electrophoresis and HPLC also detect other hemoglobinopathies (S, C, E, OArab, Lepore) that may interact with beta-thalassemia. Molecular Genetic Analysis.
- The prevalence of a limited number of mutations in each population has greatly facilitated molecular genetic testing. Commonly occurring mutations of the beta globin gene are detected by PCR-based procedures (Vrettou C, 2003).
- The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.
- If targeted mutation analysis fails to detect the mutation, beta globin gene sequence analysis can be used to detect mutations in the beta globin gene (Jeanl. Mark, 1989).
Differential Diagnosis:

Few conditions share similarities with homozygous betathalassemia:

- The genetically-determined sideroblastic anemias are easily differentiated because of ring sideroblasts in the bone marrow and variably elevated serum concentration of erythrocyte protoporphyrin. Most sideroblastic anemias are associated with defects in the heme biosynthetic pathway, especially delta-aminolevulinic acid synthase.

- Congenital dyserythropoietic anemias do not have high HbF and do have other distinctive features, such as multinuclearity of the red blood cell precursors.

- A few acquired conditions associated with high HbF (juvenile chronic myelomonocytic leukemia with normal karyotype, aplastic anemia both congenital and acquired during the recovery phase) may be mistaken for betathalassemia, even though they have very characteristic clinical and hematological features.

Typical beta-thalassemia carriers are identified by analysis of RBC indices, which shows microcytosis (low MCV) and reduced content of Hb per red cell (low MCH), and by qualitative and quantitative Hb analysis, which displays the increase of HbA2.

Pitfalls in carrier identification by hematologic testing are:

- Coinheritance of alpha-thalassemia, which may normalize the RBC indices. However, in alpha/beta double heterozygotes, the HbA2 concentration remains in the beta-thalassemia carrier range and thus is diagnostic. Therefore, HbA2 determination should always be performed for betathalassemia carrier identification.

- Coinheritance of delta-thalassemia, which may reduce to normal the increased Hb A2 levels typical of the beta-thalassemia carrier state. Double heterozygosity for delta- and betathalassemia can be distinguished from the most common alpha-thalassemia carrier state by globin chain synthesis or globin gene analysis.

- Silent mutations, i.e., very mild mutations associated with consistent residual output of Hb beta chains and with normal RBC indices and normal or borderline HbA2. The above reported groups of carriers are referred to as atypical carriers (see Table 1 β++ mutations). When the hematologic analysis
is abnormal, molecular genetic testing of beta globin gene is performed to identify the disease-causing mutation (Vrettou C-2003).


Carrier detection has been previously described. Genetic counseling provides information for individuals and at risk couples (i.e. both carriers) regarding the mode of inheritance, the genetic risk of having affected children and the natural history of the disease including the available treatment and therapies under investigation. Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis, usually performed at approximately 15-18 weeks' gestation or chorionic villi sampling at 11 weeks' gestation. Both disease-causing alleles must be identified before prenatal testing can be performed. Analysis of fetal cells in maternal blood and analysis of fetal DNA in maternal plasma for the presence of the father's mutation are currently under investigation (Mavrou A, et al, 2005). Preimplantation genetic diagnosis may be available for families in which the disease-causing mutations have been identified.

2.9. Transfusions:
The goals of transfusion therapy are correction of anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption, which occurs in non transfused patients as a consequence of increased, although ineffective, erythropoiesis. The decision to start transfusion in patients with confirmed diagnosis of thalassemia should be based on the presence of severe anemia (Hb < 7 g/dl for more than two weeks, excluding other contributory causes such as infections). However, also in patients with Hb > 7 g/dl, other factors should be considered, including facial changes, poor growth, evidence of bony expansion and increasing splenomegaly. When possible, the decision to start regular transfusions should not be delayed until after the second- third year, due to the risk of developing multiple red cell antibodies and subsequent difficulty in finding suitable blood donors. Several different transfusional regimens have been proposed over the years, but the most widely accepted aims at a pre-transfusional Hb level of 9 to 10 g/dl and a post-transfusion level of 13 to 14 g/dl. This prevents growth impairment, organ damage and bone deformities, allowing normal activity and quality of life (Philadelphia, et al, 2004).
The frequency of transfusion is usually every two to four weeks. Shorter intervals might further reduce the overall blood requirement, but are incompatible with an acceptable quality of life. The amount of blood to be transfused depends on several factors including weight of the patient, target increase in Hb level and hematocrit of blood unit. Appropriate graphs and formulae to calculate the amount of blood to be transfused are available (http://www.thalassemia.org.cy-200).

In general, the amount of transfused RBC should not exceed 15 to 20 ml/kg/day, infused at a maximum rate of 5 ml/kg/hour, to avoid a fast increase in blood volume. To monitor the effectiveness of transfusion therapy, some indices should be recorded at each transfusion, such as pre- and post-transfusion Hb, amount and hematocrit of the blood unit, daily Hb fall and transfusional interval. These measurements enable two important parameters to be calculated: red cell requirement and iron intake. Dedicated computerized programs (Webthal) are available to monitor transfused thalassemia patients accurately (Webthal, et al).

Although red cell transfusions are lifesavers for patients with thalassemia, they are responsible for a series of complications and expose the patients to a variety of risks. Iron overload is the most relevant complication associated with transfusion therapy (Porter, J, 2013).

2.1 Assessment and Treatment of Iron Overload:

Patients maintained on a regular transfusion regimen progressively develop clinical manifestations of iron overload: hypogonadism (35-55% of the patients), hypothyroidism (9-11%), hypoparathyroidism (4%), diabetes (6-10%), liver fibrosis, and heart dysfunction (33%) (Cunningham MJ, et al, 2004).

Iron status should be accurately assessed in order to evaluate its clinical relevance, the need for treatment, and the timing and monitoring of chelation therapy. The iron status of multitransfused patients can be assessed by several methods. Serum ferritin has in general been found to correlate with body iron stores (Brittenham GM, et al, 1993). However, as a single value it is not always reliable because, being an acute-phase reactant, it is influenced by other factors such as inflammatory disorders, liver disease, malignancy. Despite this, serial measurements of serum ferritin remain a reliable and the easiest method to evaluate iron overload and efficacy of chelation therapy. Determination of liver iron concentration in a liver biopsy specimen shows a high correlation with total body iron accumulation and is considered the gold standard.
for the evaluation of iron overload (Angelucci E, et al, 2000). However, liver biopsy is an invasive technique with the possibility (though low) of complications. Moreover, we should consider that the presence of hepatic fibrosis, which commonly occurs in individuals with iron overload and HCV infection, and heterogeneous liver iron distribution can lead to possible false negative results, (Villeneuve JP, et al, 1996).

In recent years, nuclear magnetic resonance imaging (MRI) techniques for assessing iron loading in the liver and heart have been introduced, (Anderson LJ, et al, 2001).

R2 and T2* parameters have been validated for liver iron concentration. Cardiac T2* is reproducible, transferable between different scanners, correlates with cardiac function, and relates to tissue iron concentration. Clinical utility of T2* in monitoring patients with siderotic cardiomyopathy has been demonstrated, (Anderson LJ, et al, 2004). Calibration of T2* in the heart will be available in the near future. Magnetic biosusceptometry (SQUID), is another option for a reliable measurement of hepatic iron concentration; [46] however, magnetic susceptometry is presently available only in a limited number of centers worldwide. As the body has no effective means for removing iron, the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool. As a general rule, patients should start iron chelation treatment once they have had 10-20 transfusions or when ferritin levels rise above 1000 ng/ml . (http://www.thalassemia.org.cy-200).

The first drug available for treatment of iron overload was deferoxamine (DFO), an exadentate iron chelator that is not orally absorbed and thus needs parenteral administration, usually as a subcutaneous 8- to 12-hour nightly infusion, 5-7 nights a week. Average dosage is 20-40 mg/kg body weight for children and 30-50 mg/kg body weight for adults, (Philadelphia, et al, 2004).

In high risk cases, continuous administration of DFO via an implanted delivery system (Port-a-cath) or subcutaneously, at doses between 50 and 60 mg/kg per day, were the only options to intensify the chelation treatment before the advent of the combined therapy with DFO and deferiprone, (Anderson LJ, et al, 2004).

Implanted delivery systems are associated with risk of thrombosis and infection.

With DFO, iron is excreted both in faeces (about 40%) and in urine. The most frequent adverse effects of DFO are local reactions at the site of infusion, such as pain, swelling, induration, erythema, burning, pruritus, wheals and rash, occasionally accompanied by fever, chills and malaise. Other complications, mainly associated with high doses of DFO in young patients and low ferritin values are
- Sensorineural hypoacusia, particularly at high frequencies
- Ocular toxicity (night-blindness, blurred vision, decreased visual acuity, impairment of colour vision, cataract and other disturbances of the eye)
- Retarded growth and skeletal changes with a disproportionately short trunk and dysplasia of the long bones
- Infections by yersinia enterocolitica, and other pathogens (klebsiella pneumoniae).

It is therefore important to monitor patients receiving DFO regularly with audiometric and ophthalmologic tests and with regular evaluation of growth and bone changes.

The use of DFO decreases morbidity and mortality among those who are able to comply with regular prolonged infusions, (Gabutti V, et al, 1996).

However, because of the side effects and the inconvenient parenteral administration, a consistent proportion of patients is non-compliant, limiting the usefulness of this chelator (Cunningham MJ, et al, 2004).

The orphan drug deferiprone (DFP) is an orally active iron chelator which has emerged from an extensive search for new treatment of iron overload. Comparative studies have shown that this chelator, at doses of 75-100 mg/kg/day may be as effective as DFO in removing body iron, (Galanello R, et al, 2003).

Retrospective and prospective studies have shown that DFP monotherapy is significantly more effective than deferoxamine in decreasing myocardial siderosis in thalassemia major (Anderson LJ, et al, 2002).

Agranulocytosis is the most serious side effect associated with the use of DFP, occurring in about 1% of the patients. (Galanello R, et al, 2003).

More common but less severe side effects are gastrointestinal symptoms, arthralgia, zinc deficiency, and fluctuating liver enzymes. Retrospective studies have shown that DFP treatment is associated with reduced cardiac morbidity and mortality, (Piga A, et al, 2003).

DFO and DFP can be used in combination to achieve levels of iron excretion that cannot be achieved by either drug alone without increasing toxicity (Wonke B, et al, 1998).

Reversal of severe iron-related heart failure with DFO and DFP combination has been reported in many patients, (Wu KH, et al, 2004).

The effect of combined therapy versus DFO monotherapy on myocardial iron overload was evaluated in a prospective, randomized, placebo controlled trial, which
showed a statistically significant improvement in myocardial T2* with the combined treatment as compared with DFO and placebo treatment (Tanner MA, et al, 2007). Combination therapy should be considered as an alternative to continuous intravenous DFO monotherapy when an intensive chelation is required.

Deferasirox (DFX) is a once-daily, orally administered iron chelator that a large program of clinical trials has shown to be effective in adults and children. It received European Union marketing authorization as an orphan drug from the EMEA in 2002 and was authorized for marketing in most countries in 2006. The recommended starting dose of DFX for most patients is 20 mg/kg/day, although this can be modified to 10 or 30 mg/kg/day depending on the number of transfusions a patient is receiving and whether the therapeutic goal is to decrease or maintain body iron levels. The most frequent adverse events reported during treatment with DFX include transient, mild-to-moderate gastrointestinal disturbances and skin rash. These events rarely require drug discontinuation and most resolve spontaneously. Mild, usually nonprogressive increases in serum creatinine (generally within the upper limit of normal) has been observed in approximately a third of patients. Creatinine levels returned spontaneously to baseline in most of patients and data from up to 3.5 years of treatment in more than 1000 patients have confirmed that creatinine increase is non progressive, (Cappellini MD, Taher A, 2008).

However, cases of renal failure have been reported following the postmarketing use of DFX. (S)-3’-(OH)-desazadesferrithiocin-polyether, magnesium salt is an oral once a day iron chelator expected to excrete iron mainly in the stools, evaluated in experimental models. Orphan designation of this medicine has been granted in the United States of America and Europe for treatment of chronic iron overload in patients with transfusion-dependent anemias. Recently, three main practice guidelines for the management of iron overload in thalassemia major have been published and are available online, (U.K. Thalassemia Society, et al, 2008).

2.1 Treatment of Iron overload-related Complications:

a Growth Deficiency:

Studies evaluating the secretion of growth hormone (GH) in patients with thalassemia major have yielded contradictory results, limiting the therapeutic use of GH to those patients proven to have GH deficiency, who may have a satisfactory response to treatment, (Karydis, et al, 2004). In cases with signs of bone toxicity from DFO a
reduction of the dose, or its substitution with an oral chelator, can prevent progression of bone lesions and improve growth (Sanctis V, et al, 2002).

a/Delayed puberty, hypogonadism and assisted reproduction:
For delayed puberty in girls, therapy may start with the administration of ethinyl estradiol (2.5-5 μg daily) for 6 months, followed by hormonal reassessment. If spontaneous puberty does not occur within 6 months, ethinyl estradiol should be used at increasing dosages (from 5-10 μg daily) for 12 months. If breakthrough uterine bleeding does not occur, a low oestrogenprogesterone hormone replacement is recommended. For delayed puberty in males, intramuscular depot-testosterone esters at a dose of 50-100 mg twice a month should be given, until complete virilisation has been achieved. Topical testosterone gel can also be used, (De Sanctis V, et al, 1998). When there is a lack of pubertal progression over a year or longer (arrested puberty), testosterone esters in males and oestrogenprogesterone replacement therapy in females is indicated.

In males suffering from azoospermia or asthenospermia and asking for fertility treatment, spermatogenesis may be induced by combination therapy with hCG (human chorionic gonadotrophin) and hMG (human menopausal gonadotrophin) intramuscularly or subcutaneously. Moreover, the recent advent of micromanipulation techniques such as intracytoplasmatic sperm injection (ICSI) has improved conception rates. Females with thalassemia may have primary or secondary amenorrhea, which leads to failure of the reproductive axis with chronic anovulation. Despite severe hemosiderosis, ovarian function is preserved in most patients, and they are still able to increase the estradiol level following stimulation with gonadotrophins, and furthermore produce ova. Induction of ovulation must be performed under rigorous control after a global evaluation of the patient, including detailed assessment of heart, liver function, viral infections, endocrinopathies, with particular emphasis on diabetes control and thrombophilia status, (Skordis N, et al, 2004).

Pregnant patients with thalassemia need a multidisciplinary approach involving all specialists in the medical care of thalassemia, (Origa R, et al, 2010).

c/ Hypothyroidism:
Preclinical hypothyroidism is characterized by normal thyroxine (T4) and free thyroxine (FT4), normal basal TSH and TSH slightly increased after the Thyrotropin-releasing Hormone (TRH) test. A careful follow-up with an intensification of chelation therapy is required in such cases. Subclinical hypothyroidism is defined as a
normal serum T4 and FT4 level with a slightly increased TSH level. It is debatable whether patients with subclinical hypothyroidism should be treated. If treatment is considered unnecessary, close monitoring is mandatory. Therapy can be recommended for patients with TSH levels greater than 10 U/ml, thyroid abnormalities, and vague symptoms attributable to hypothyroidism. In overt hypothyroidism, characterized by low T4 and FT4 values with signs and symptoms such as mental and physical sluggishness, weight gain, feeling of cold, sleepiness, bradycardia and constipation, treatment with increasing doses of L-thyroxine starting with 25 mg per day is indicated. Abnormal thyroid function may be reversible at an early stage through intensive combined chelation, (Farmaki K, et al, 2010).

**Hypoparathyroidism:**
Severe hypocalcemia with tetany requires intravenous administration of calcium under careful electrocardiographic monitoring, followed by oral vitamin D. In milder forms, calcitriol is the drug of choice, because of its short half-life and rapid action. A dosage of 0.25-1 μg twice daily is usually sufficient to normalize calcium and phosphate. Because of the risk of hypercalcemia and hypercalcuiuria, serum calcium level and 24-hour urinary calcium and phosphate measurements should be carefully monitored, especially at the beginning of treatment and if elevated doses of Vitamin D are administered (farmaki k, et al, 2010).

**2.1 Diabetes and Impaired Glucose Tolerance:**
Acarbose at the dose of 100 mg (orally with breakfast, lunch and evening meals) has been used with good results for impaired glucose tolerance or non-insulin dependent diabetes mellitus and hyperinsulinism, (Mangiagli A, et al, 2004). Patients with diabetes mellitus, may require daily subcutaneous injections of insulin. Since treatment of diabetes in patients with thalassemia major is an additional burden, support from doctors and psychologists is needed. Investigation of the kidney function and imaging of the fundi should be carried out to evaluate the presence and degree of diabetic complications. Intensive iron chelation therapy with DFO and DFP seems to be associated with an improvement in glucose intolerance in terms of glucose and insulin secretion, particularly in patients in early stages of glucose intolerance (Farmaki K, et al, 2006).
2.1 Osteoporosis:
Since osteoporosis is a progressive disease, prevention is the basis of the management. No smoking, a calcium-rich diet, correction of hypogonadism by sex hormone replacement therapy and regular exercise should be recommended. Oral calcium supplements should be used with caution because of the risk of renal stones. Several bisphosphonates have been used in thalassemia patients for the treatment of osteoporosis with variable results. To date, alendronate, pamidronate, and zoledronate seem to be effective in increasing bone mineral density and normalizing bone turnover, but more controlled trials are necessary to evaluate their efficacy in reducing fracture risks in larger thalassemic populations, (Gaudio A, et al, 2008).

2.13 Splenectomy:
If the annual red cell requirement exceeds 180-200 ml/Kg of RBC (assuming that the Hct of the unit of red cells is about 75%), splenectomy should be considered, provided that other reasons for increased consumption, such as hemolytic reactions, have been excluded. Other indications for splenectomy are symptoms of splenic enlargement, leukopenia and/or thrombocytopenia and increasing iron overload despite good chelation, (http://www.thalassemia.org.cy, 2008).

2.14 Bone Marrow and Cord Blood Transplantation:
Bone marrow transplantation (BMT) remains the only definitive cure currently available for patients with thalassemia. The outcome of BMT is related to the pretransplantation clinical conditions, specifically the presence of hepatomegaly, extent of liver fibrosis, history of regular chelation and hence severity of iron accumulation. In patients without the above risk factors, stem cell transplantation from an HLA identical sibling has a disease-free survival rate over 90%, (Gaziev J, Lucarelli G, et al, 2003).

The major limitation of allogenic BMT is the lack of an HLA-identical sibling donor for the majority of affected patients. In fact, approximately 25-30% of thalassemic patients could have a matched sibling donor. BMT from unrelated donors has been carried out on a limited number of individuals with beta-thalassemia. Provided that selection of the donor is based on stringent criteria of HLA compatibility and that individuals have limited iron overload, results are comparable to those obtained when the donor is a compatible sibling , (La Nasa G, et al, 2005).
However, because of the limited number of individuals enrolled, further studies are needed to confirm these preliminary findings. If BMT is successful, iron overload may be reduced by repeated phlebotomy, thus eliminating the need for iron chelation. Chronic graft-versus-host disease (GVHD of variable severity may occur in 5-8% of individuals. Cord blood transplantation from a related donor offers a good probability of a successful cure and is associated with a low risk of GVHD. For couples who have already had a child with thalassemia and who undertake prenatal diagnosis in a subsequent pregnancy, prenatal identification of HLA compatibility between the affected child and an unaffected fetus allows collection of placental blood at delivery and the option of cord blood transplantation to cure the affected child, (Locatelli F, et al, 2003).

On the other hand, in cases with an affected fetus and a previous normal child, the couple may decide to continue the pregnancy and pursue BMT later, using the normal child as the donor.

2.15 Management of Thalassemia Intermedia:

Treatment of individuals with thalassemia intermedia is symptomatic as hypersplenism may cause worsening anemia, retarded growth and mechanical disturbance from the large spleen, splenectomy is a relevant aspect of the management of thalassemia intermedia. Risks associated with splenectomy include an increased susceptibility to infections mainly from encapsulated bacteria (Streptococcus Pneumoniae, Haemophilus Influenzae and Neisseria Meningitidis) and an increase in thromboembolic events. Prevention of post-splenectomy sepsis includes immunization against the above mentioned bacteria and antibiotic prophylaxis as well as early antibiotic treatment for fever and malaise. Because of the elevated prevalence of cholelithiasis and the risks of cholecystitis in splenectomised patients, the gallbladder should be inspected during splenectomy and removed in case with or to prevent gallstones. Treatment of extramedullary erythropoietic masses, detected by magnetic resonance imaging, is based on radiotherapy, transfusions, or hydroxycarbamide. Once leg ulcer has developed, it is very difficult to manage. Regular blood transfusions, zinc supplementation and pentoxifylline, and the use of an oxygen chamber have been proposed for ulcer treatment. Hydroxycarbamide also has some benefit, either alone or with erythropoietin. Recently promising results have been obtained with platelet derived growth factor. Since patients with thalassemia
intermedia have a high risk of thrombosis, exacerbated by splenectomy, it is important to be aware of thrombotic complications. Recommended treatment options include proper anticoagulation prior to surgical or other high-risk procedures, platelet anti-aggregating agents in case of thrombocytosis (platelet count higher than 700,000/mm3) and low molecular weight heparin in patients with documented thrombosis. Because individuals with thalassemia intermedia may develop iron overload from increased gastrointestinal absorption of iron or from occasional transfusions, chelation therapy is started when the serum ferritin concentration exceeds 300 ng/ml or when iron overload is demonstrated by direct or indirect methods. Supplementary folic acid can be prescribed to patients with thalassemia intermedia to prevent deficiency from hyperactive bone marrow (Galanllo R, 2010).

2.16 Lifestyle and Diet in Beta-Thalassemia:
If the disease is fully compensated by ideal treatment, an individual with thalassemia major can enjoy a near-normal lifestyle and experience normal physical and emotional development from childhood to adulthood, including parenthood. Patients with thalassemia do not have specific dietary requirements, unless they have special prescriptions. Patients already have a heavy treatment schedule and it is counterproductive to add further restrictions without the likelihood of clear benefit. During growth, a normal energy intake with normal fat and sugar content is recommended. During adolescence and adult life, a diet low in highly refined carbohydrates may be useful in preventing or delaying the onset of impaired glucose tolerance or diabetes. There is no clear evidence that a specific diet is beneficial in preventing or managing liver disease, unless at late stages. Increased iron absorption from the intestinal tract is characteristic of thalassemia. The amount depends on the degree of erythropoiesis, the Hb level and other potential independent factors. Drinking a glass of black tea with meals reduces iron absorption from food, particularly in thalassemia intermedia, (Borgna-Pignatti C, et al, 2007).

However, there is no evidence that iron-poor diets are useful in thalassemia major; only foods very rich in iron (such as liver, many baby foods, breakfast cereals and multivitamin preparations contain added iron, along with other vitamin supplements) should be avoided. Since many factors in thalassemia promote calcium depletion, a diet containing adequate calcium (e.g. milk, cheese, dairy products and kale) is always recommended. However, nephrolithiasis is seen in some adults with thalassemia
major, and calcium supplements should not be given unless there is a clear indication; instead a low oxalate diet should be considered. Patients with thalassemia who remain untransfused or are on low transfusion regimens have increased folate consumption and may develop a relative folate deficiency. Supplements (1 mg/day) may be given if this occurs. Patients on high transfusion regimens rarely develop this condition, and usually have no need for supplements. Iron overload causes vitamin C to be oxidized at an increased rate, leading to vitamin C deficiency in some patients. Fifty mg of vitamin C in children <10 years and 100 mg >10 years at the time of DFO infusion may increase the 'chelatable iron' available in the body, thus increasing the efficacy of chelation. However there is currently no evidence supporting the use of vitamin C supplements in patients on DFP, DFX or combination treatment. Vitamin C may increase iron absorption from the gut, labile iron and hence iron toxicity and may therefore be particularly harmful to patients who are not receiving DFO, as iron mobilized by the vitamin C will remain unbound, causing tissue damage. The effectiveness and safety of vitamin E supplementation in thalassemia major has not been formally assessed and it is not possible to give recommendations about its use at this time. Patients with thalassemia should be discouraged from consuming alcohol, as it can facilitate the oxidative damage of iron and aggravates the effect of HBV and HCV on liver tissue. In general, physical activity must always be encouraged unless there is a precise secondary medical condition. Conditions requiring special attention include splenomegaly, severe heart disease and osteoporosis. There is no reason for patients with thalassemia to skip or delay standard recommended vaccinations. To prevent and minimize the risk of infection, immunization with the pneumococcal, Haemophilus Influenza and meningococcal vaccines is recommended about two weeks before splenectomy and after surgery. It is now universally recognized that thalassemia, like other chronic diseases, has important psychological implications. The way in which the family and the patient come to terms with the disease and its treatment will have a critical effect on the patient's survival and quality of life, and a general acceptance by the patient of his/her own condition constitutes the key to normal development from childhood to adulthood. A key role for treating physicians and other health care professionals is to help patients and families to face up to the difficult demands of treatment, while maintaining a positive role (Nigg Aj, 2009).
2.17 Therapies under Investigation:

Induction of HbF synthesis can reduce the severity of beta-thalassemia by improving the imbalance between alpha and non-alpha globin chains. Several compounds including 5-azacytidine, decytabine, and butyrate derivatives gave encouraging results in clinical trials (Pace BS, Zein S, et al, 2006).

These agents induce Hb F by different mechanisms not yet well defined. Their potential in the management of beta-thalassemia syndromes is under investigation. A butyrate derivative, 2,2-Dimethylbutyric acid, sodium salt has received orphan designation for betathalassemia in the United States of America and in Europe. The efficacy of hydroxycarbamide treatment in individuals with thalassemia is still unclear. Hydroxycarbamide has been used as experimental treatment in patients with thalassemia intermedia to reduce extramedullary masses, to increase Hb levels, and, in some cases, to improve leg ulcers. A good response, correlated with particular polymorphisms in the beta globin cluster (i.e., C>T at -158 G gamma), has been reported in individuals with transfusion dependence, (Bradai M, et al-2003).

However, controlled and randomized studies are needed to establish the role of hydroxycarbamide in the management of thalassemia major. The possibility of correction of the molecular defect in hematopoietic stem cells by transfer of a normal gene via a suitable vector or by homologous recombination is being actively investigated, (Sadelain M, et al, 2007).

The most promising results in the mouse model have been obtained with lentiviral vectors, (Puthenveetil G, et al, 2004).

In 2009, orphan designation was granted by the European Commission for autologous haematopoietic stem cells transduced with lentiviral vector encoding the human beta globin gene for the treatment of beta-thalassemia major and intermedia.

2.18 Types of Thalassaemia Trait:

Alpha plus thalassaemia trait. This means that you have one missing alpha haemoglobin gene. (Normally there are four of these genes.) This trait can ONLY cause a problem if your partner has alpha zero thalassaemia trait - in which case your children might inherit HbH disease. Apart from that situation, it will not affect you or your children. Alpha zero thalassaemia trait. This means you have two missing alpha haemoglobin genes (out of the normal four alpha genes). It will not make you ill, but if your partner also has alpha zero thalassaemia trait, your children might inherit a
severe condition called Hb Barts. Or, if your partner has alpha plus thalassaemia trait, then your children might inherit Hb H disease. Beta-thalassaemia trait. This means you have one abnormal beta-haemoglobin gene (out of the normal two beta genes). It will not make you ill. But, if your partner also has beta-thalassaemia trait, then your children could inherit BTM or BTI. Beta-thalassaemia trait can also interact with other abnormal haemoglobin genes which are not thalassaemias. For example, if your partner has a gene for sickle cell anaemia then your children might inherit a serious condition called sickle cell/beta thalassaemia. (www. Mirin practice. Scot. Nhs. Uk).

*BTM:
A person with BTM has two beta-thalassaemia genes. Most of their haemoglobin is abnormal and does not work. This causes severe anaemia starting around the age of 4-6 months. Before that, the baby is not affected. This is because until age 3-6 months the baby makes a different type of haemoglobin, called fetal haemoglobin, which is not affected by the thalassaemia gene. With BTM, you need regular blood transfusions, plus other treatment to prevent complications (Cappellini- Mp, 2008).

*BTI:
As the name suggests, this type is less severe than BTM. You have two beta-thalassaemia genes but can make some haemoglobin which works reasonably well. This may be because your particular combination of thalassaemia genes is (in effect) less severe, or because of some other protective factor. Although less severe than thalassaemia major, thalassaemia intermedia does need regular monitoring for life and often needs some treatment to prevent complications (Cappellini- Mp, 2008).

This can occur if one parent has a beta-thalassaemia gene, and the other parent carries a gene for a different haemoglobin disorder called sickle cell anaemia. If their child inherits one of each gene, the combination is called sickle cell/beta thalassaemia - also called sickle cell disease. This condition behaves like sickle cell anaemia (not like thalassaemia) and is treated in the same way as sickle cell anaemia. See separate leaflet called Sickle Cell Disease and Sickle Cell Anaemia for more detail.

*HbH Disease:
This is a type of alpha thalassaemia. It is due to having three missing alpha-haemoglobin genes (normally each person has four of these genes). This can happen if one parent has alpha plus thalassaemia and the other has alpha zero thalassaemia. It usually causes a mild but persistent anaemia. Sometimes HbH causes more symptoms and is similar to BTI. Some people with HbH disease need blood transfusions.
*Hb Barts:*
This is the most severe form of thalassaemia, where all the alpha-haemoglobin genes are abnormal or missing. It occurs if a baby inherits two alpha zero thalassaemia genes. In this condition, no normal haemoglobin can be made, even before birth. It is the most serious form of thalassaemia - so serious that the baby will usually die in the womb from severe anaemia. (www. Scickle- thal. Nwih. Nhs. Uk). There have been rare cases where the baby has been saved by blood transfusions being given in the womb, with the transfusions then continuing after birth.

**How is Thalassaemia Inherited?**
A child inherits haemoglobin genes from both parents. For example, if both parents have beta-thalassaemia trait, there is: a 1 in 4 chance of the child having normal haemoglobin genes; a 1 in 2 chance of the child having beta-thalassaemia trait; and a 1 in 4 chance the child will have BTM or BTI.

**2.19 Clinical Classification of the Thalassemias:**

**a\ Alpha Thalassemia:**
Alpha thalassemia has four manifestations, that correlate with the number of defective genes. Since the gene defect is almost invariably a loss of the gene, there are no "shades of function" to obscure the matter as occurs in beta thalassemia:

**b\ Silent Carrier State:**
This is the one-gene deletion alpha thalassemia condition. People with this condition are hematologically normal. They are detected only by sophisticated laboratory methods.

**c\ Mild alpha-Thalassemia:**
These patients have lost two alpha globin genes. They have small red cells and a mild anemia. These people are usually asymptomatic. Often, physicians mistakenly diagnose people with mild alpha-thalassemia as having iron deficiency anemia. Iron therapy, of course, does not correct the anemia.

**d\ Hemoglobin H Disease:**
These patients have lost three alpha globin genes. The result is a severe anemia, with small, misshapen red cells and red cell fragments. These patients typically have enlarged spleens. Bony abnormalities particularly involving the cheeks and forehead are often striking. The bone marrow works at an extraordinary pace in an attempt to compensate for the anemia. As a result, the marrow cavity within the bones is stuffed.
with red cell precursors. These cells gradually cause the bone to "mold" and flair out. Patients with hemoglobin H disease also develop large spleens. The spleen has blood forming cells, the same as the bone marrow (Latha GM, 2014). These cells become hyperactive and overexpand, just as those of the bone marrow. The result is a spleen that is often ten-times larger than normal. Patients with hemoglobin H disease often are small and appear malnourished, despite good food intake. This feature results from the tremendous amount of energy that goes into the production of new red cells at an extremely accelerated pace. The constant burning of energy by these patients mimics intense aerobic exercise; exercise that goes on for every minute of every day.

**Hydrops fetalis:**

This condition results from the loss of all four alpha globin genes. The affected individual usually succumbs to the severe anemia and complications before birth.

### 2.20 Relationship of the Genetic and Clinical Classifications of Thalassemia:

The advent of modern molecular biology permits the genetic classification of thalassemias, outlined earlier in this document. A rough correlation exists between the clinical and genetic classifications. The relationship between genetics and clinical state is not absolute, however, thalassemia trait (minor)- normal beta gene/ thalassemia gene (beta zero or (+ thalassemia intermedia- often two beta-(+)-genes thalassemia major- two beta-(+)-genes (where the plus is not substantial); beta-(+)-gene/ beta-0-gene; beta-0-gene/ beta-0-gene (Refman, 2006).

### 2.21 Prevalence of Beta Thalassemia Among Arab:

The economies of Arab countries vary considerably with GNI per capita ranging from a high of over US$40,000 in Kuwait, Qatar, and UAE, to around US$1,000 in Comoros, Mauritania, Sudan, and Yemen (World Bank, 2012). Health deficits impede human development in many Arab countries and limit scientific research and hence the paucity of data from some Arab countries in this review. Incidence and prevalence rates of recessive genetic diseases, such as haemoglobinopathies could be influenced by the demographic and cultural characteristics of the population studied. The populations of many Arab countries are characterised by marriage at a young age, large family sizes and advanced maternal and paternal ages. Consanguineous marriage is customary in most if not all Arab communities and intra-familial unions currently account for 20–50 % of all marriages. First cousin unions are especially popular and
constitute almost one quarter of all marriages in many Arab countries, particularly the paternal parallel subtype (Bittles 2012; Hamamy and Bittles, 2008). Moreover, Arab populations exhibit significant levels of genetic diversity resulting from the admixture with other populations extending from east and south Asia to Europe and Africa (Teebi and Teebi, 2005) and resulting in the present day molecular profile of the haemoglobinopathies which are known to be common genetic diseases among Arabs with reported carrier rates of 1–11 % for β-thalassaemia, 1–58 % for α-thalassaemia and 0.3–30 % for sickle cell trait (Al-Gazali et al. 2006). This high frequency could be related to the selective advantage of carriers against falciparum malaria, to the large family size with multiple affected children, to the high consanguinity rates, to the general low availability of public health measures directed at the care and prevention of these disorders and maybe to other as yet unknown factors. Despite the high rates of haemoglobinopathies, many Arab countries face major challenges in providing comprehensive and up-to-date care and prevention services due to paucity of resources, presence of other competing priorities of communicable and non-communicable disorders, such as cardiovascular diseases, cancer and diabetes, due to the insufficient number of trained health professionals in this field, and to the inadequate data on the real magnitude, health and economic burden of haemoglobinopathies. Currently, community genetic services, such as newborn screening (NBS), premarital carrier screening, prenatal screening, genetic counselling, education and registries are available in some Arab countries, though, in most, these services remain patchy and selective. Further impediments facing prevention and care initiatives include the low genetic literacy among the health sector and the public with lack of awareness about genetic risks and possibilities for prevention of these disorders coupled by certain cultural, legal and religious limitations, such as the cultural fear of families with genetic diseases to be stigmatised within their community and the legal and religious restrictions to selective abortion of an affected fetus. To highlight the need for defining priorities to implement care and prevention programs on a community level, this review aims at portraying the epidemiological profile of haemoglobinopathies in Arab countries and the currently available services. Knowledge of the molecular defects allows the development and improvement of diagnostic tests and management protocols for these disorders. The considerable challenge posed by the high prevalence rates of haemoglobinopathies in Arab countries calls for the development of care and prevention national programmes.
through the establishment of public health services that do not require sophisticated technical facilities but are primarily based on strengthening the training of health professionals and on public education (Hamamy H, 1997). The strategies adopted should, however, be carefully selected to match the unique demographic, cultural and religious characteristics of each population and take into consideration the priorities set and the resources available. Community services that have proven their efficacy in lowering the prevalence rate of β-thalassemia and SCD while at the same time offering timely management include NBS and premarital screening to detect carriers of these disorders coupled with genetic counselling. However, experience in some countries had shown that if options are not made available to carrier couples, such programmes will not be effective in reducing the burden of haemoglobinopathies. In particular, the issue of selective termination of an affected fetus remains fraught, with marked attitudinal differences between countries involving both the religious and legal authorities and families who are directly affected. Some studies have recommended screening for carriers of haemoglobinopathies among high school students, ahead of marriage arrangements. Prerequisites for considering such options would be to raise awareness through targeted education of the public. Given the knowledge barrier experienced by many health care providers in the implementation of health interventions at community level, structured courses and educational campaigns to train and educate primary health care workers in premarital, preconceptional and prescreening counselling should be considered essential components of any national strategy for the prevention of haemoglobinopathies. Knowledge of the molecular defects in each country allows the development and improvement of diagnostic tests and management protocols for these disorders (Borgna-Pignatti C, et al, 2003).

2.22 The Prevalence of Beta Thalassemia in Yemen:
Yemen is a poor country with a population of 24 million and limited health resources. The prevalence of sickle cell trait (Hb AS) in Yemen is 2.2%, with a higher frequency in the western coastal and mid-western parts of the country where the incidence of affected homozygous births (Hb SS) may reach up 20/10,000. Also the prevalence of β-thalassemia trait is 4.4% with an estimated incidence of 11.3/10,000 of homozygous β-thalassemia births in the western coastal and mountainous regions of Yemen. The prevalence of thalassemia in SCD found to be high (19.4%) in Taiz region in mid-
western area of the country, the incidences of affected birth of either homozygous sickle cell anemia or β-thalassemia could be higher, depending on the proportion of consanguineous marriages and the frequency of heterozygous Hb S/β-thalassemia disease, the most effective factor for high prevalence and incidence of sickle cell and β-thalassemia diseases in Yemen is the consanguineous marriages, which was reported to be high (44.7%) and traditional. To reduce the number of affected birth and their social, emotional and economical burden on the family and health system in Yemen, it is essential to apply PMSCG program. There is no any premarital screening preventive practice in Yemen yet. Therefore, this simple and low-cost premarital screening program is proposed to be suitable for the limited health resources of the country. Such proposal is essential to evaluate how a premarital screening program can be implemented in Yemen (Al – Nood H, 2009).

2.23 Carrier Screening:

Preventing beta thalassemia disease at high-risk population by carrier screening and genetic counseling is proved to be an acceptable and effective process to reduce the number of affected birth. This program consists of premarital screening and genetic counseling that is designed to produce a general infrastructure for accessible prevention of thalassemia before completing marriages proposal. Screening programs with genetic counseling can be implemented initially at high-risk populations in local communities in different parts of Yemen. To begin with, optional premarital screening using inexpensive and simple blood tests. Only couples at risk will receive information and genetic counseling about the consequences effects on the health of their children (Al - Hamdan NAR, 2007).

2.24 Prenatal Test:

If a woman is pregnant, the best time to have a thalassaemia blood test for herself is before 10 weeks pregnant. This allows more time to test partner or baby, if needed. can ask your doctor for a test early in pregnancy, if it is not already offered to that time. However, tests can still be done at a later stage.

A prenatal test (on the unborn baby) can be done from 10 weeks of pregnancy onwards, depending on the type of test used. The usual tests offered are chorionic villus sampling (CVS) or amniocentesis, should and partner have a thalassaemia test before starting a family, women or couples may want to have tests for thalassaemia before starting a family, especially if their family origins make thalassaemia more
likely. The UK Thalassaemia Society and many health professionals encourage awareness of thalassaemia and early testing. The test can be arranged by doctor. The advantage of having tests before become pregnant, is that you will know whether or not there is a possibility that baby could inherit a severe form of thalassaemia. This may be helpful when making decisions about pregnancy. For example, may want to have a prenatal test during pregnancy if there is a risk of a severe condition for the baby (www. Mirin practice scot. Nhs. UK).

2.25 Tests for newborn babies:
In the UK, all newborn babies are offered a bloodspot test at 5-8 days after birth. This tests for a number of medical conditions, which are considered important because early treatment makes a difference. The test is done by taking a small spot of blood from the baby's heel. Throughout the UK, the bloodspot test now includes testing for thalassaemia and other haemoglobin disorders such as sickle cell disease. Policies for screening newborn babies vary throughout the UK - see the UK Screening Portal link below, the bloodspot test will diagnose most types of thalassaemia. You will be given the results about six weeks later. If the baby has thalassaemia trait, no action or treatment is needed. If the baby has a more severe type of thalassaemia which needs treatment, the result will be explained. will be given a clinic appointment so that the diagnosis can be checked, and treatment can be started if necessary (Health information, 2015).

2.26 Methods and Instrument of Preventing Beta Thalassemia:
Relevant instruments, methods, trained health workers, target groups and genetic counseling are required to carry out the preventive program. Hematological analyzer machines are required for CBC analysis to determine the microcytosis and/or hypochromia in red blood cells. Methods of sickle cell using either sickling test or solubility test also are required for detection of Hb S. Genetic counselors consist of trained doctors or professionals with Bachelor of Science degrees in health studies that will provide advices to carrier couples about the genetic condition, which may affect them, so that the couples have to make the appropriate choices concerning marriage and reproduction. Target groups in the country are required to be prepared for premarital screening which can be preformed by giving classes about beta thalassemia for young people in high schools, universities, sports clubs and military.
In addition, the widespread of education programs through booklets, posters, TV and newspapers, Public and private laboratories equipped and licensed to screen for beta thalassemia can be the place for premarital screening. Carriers' data will be recorded for evaluation to adapt this preventive program to meet the public need (Model B, 1990).

2.27 Genetic Counseling:
Preventing beta thalassemia disease at high-risk population by carrier screening and genetic counseling genetic counseling that is designed to produce a general infrastructure for accessible prevention of beta thalassemia before completing marriages proposal. Screening programs with genetic counseling can be implemented initially at high-risk populations in local communities in different parts of Yemen. To begin with, optional premarital screening using inexpensive and simple blood tests. Only couples at risk will receive information and genetic counseling about the consequences effects on the health of their children (Al –Nood H ,2013).

2.28 Diagnosis of Thalassaemia:
The diagnosis is made by a blood test. The blood sample is analysed to see what type of haemoglobin is present in the blood. In some cases, extra tests such as DNA (genetic) tests are needed to diagnose the exact type of thalassaemia. It may help to test other family members where possible, thalassaemia trait may be suspected from the results of an ordinary blood test called a full blood count. If the result shows red blood cells that are smaller and paler than usual, this may be due to iron deficiency or to thalassaemia trait (Binns V ,2008).

2.29 Cure for Thalassaemia:
A possible cure is a stem cell transplant. This means either a bone marrow transplant, or a cord blood transplant. These treatments take normal blood-making cells from a donor, and give them to the person with thalassaemia. If the transplant is successful, these cells last for life and make normal haemoglobin - a lifelong cure. However, a stem cell transplant is not suitable for everyone. You need a suitable donor, and there are some serious risks involved. UK guidelines recommend that all BTM patients have the opportunity to discuss stem cell transplantation with a specialist. See separate leaflet called Stem Cell Transplant for more details (Yardumian A, 2008).
Chapter Three

3. Methodology

3.1 Study Area:
This study was carried out in Yemen among students who study in the High Health Institute of Sana. This area of the study is located in the centre of Sana – Al Ziraa Street.

3.2 Study Design:
Descriptive – cross sectional study One thousand (1000) students who study in The High Health Institute of Sana were randomly chosen to carry out this study.

3.3 Tools Required to Conduct the Study:
Venous blood specimen were taken from the study population and collected in EDTA tubes electronic blood analyzer (ACT diff Beckman with 18 parameters) including (Hb, WBC count, plate count, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell count (RCB). Was used to analyze the samples, also peripheral blood smears were prepared to check the morphology of the red blood cells and any abnormal CBC was done within one hour of sample collection.

3.4 Inclusion Criteria:
The study included students who study in the High Health Institute of Sana.

3.5 Exclusion Criteria:
Subject of known diagnosed beta- thalassaemia major and subject not filling the consent.

3.6 Study variables:
- dependant variables: (Hb A2, Hb F, red cell indices.
- In dependant variables: (age sex, residence).

3.7 Procedures Used to Conduct the Study:
A survey was done in 1000 students who study in The High Institute of Sana to find out the prevalence of beta thalassaemia carrier among them.
Pipette 35µl of the sample into the appropriate well of the sample tray or disposable sample cups.

i) SAS-1 & SAS-1 Plus users: Use SAS-1 sample tray. Carefully place the sample tray onto the
1. Applicator drawer. Ensure that the tray is pushed firmly down into position.

ii) SAS-3 users: Use SPIFE / SAS-3 sample tray. Carefully locate the sample tray using the sample base locating pins. Ensure that the tray is positioned securely.

2. Remove the gel from the packaging and:
   i. SAS-1 users: place the gel in the SAS-1, agarose side up, aligning the positive and negative sides with the corresponding electrode posts.
   ii. SAS-1 Plus users: dispense 400µL of REP Prep onto the heat sink. Place the gel onto the heat sink, agarose side up, aligning the positive and negative sides with the corresponding electrode posts, taking care to avoid air bubbles under the gel.
   iii. SAS-3 users: place the alignment guide onto the pins and dispense 400µL of REP Prep onto the centre of the chamber. Place the gel into the chamber agarose side up, using the guide, align the positive and negative sides with the corresponding electrode posts, taking care to avoid air bubbles under the gel.

3. Blot the surface of the gel with a blotter C, discard the blotter.
   i. SAS-1 users: attach the electrodes onto the top side of the electrode posts so that they are in contact with the gel blocks.
   ii. SAS-1 Plus users: (as above). Place the cover over the gel and electrodes and press firmly for 5 seconds to ensure contact.
   iii. SAS-3 users: attach the electrodes onto the electrode posts so that they are in contact with the gel blocks.

5. Place 1 applicator blade assembly into the top position on the instrument, (SAS-3 users: slot 8).

6. Perform the Alkaline Haemoglobin electrophoresis:
   i. SAS-1 users: 120 volts, 40 mins, 1 applications
   ii. SAS-1 Plus users: Electrophoresis: 200 volts, 30 mins, 25°C, 1 applications
   iii. SAS-3 users: Step Time (mm:ss) Temperature (°C) Voltage Other

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mm:ss)</th>
<th>Temperature (°C)</th>
<th>Voltage</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Sample</td>
<td>00:10</td>
<td>21</td>
<td></td>
<td>Speed 1</td>
</tr>
<tr>
<td>Apply Sample</td>
<td>00:10</td>
<td>21</td>
<td></td>
<td>Speed 1</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>30:00</td>
<td>25</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

* Use Location 1
7. Following electrophoresis, (SAS-1 Plus users: remove the cover), remove the electrodes and both gel blocks using the Gel Block Remover.

8. Attach the gel to the staining chamber holder.

Select the Alkaline Haemoglobin test program on the staining unit and, following the prompts,
Stain, Destain and Dry the gel.

a) SAS-2 (Auto-Stainer)

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mm:ss)</th>
<th>Port</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain</td>
<td>Acid Blue Stain</td>
<td>05:00</td>
<td>6</td>
</tr>
<tr>
<td>Destain</td>
<td>Destain solution</td>
<td>00:30</td>
<td>2</td>
</tr>
<tr>
<td>Dry</td>
<td>——</td>
<td>15:00</td>
<td>65</td>
</tr>
<tr>
<td>Destain</td>
<td>Destain solution</td>
<td>02:00</td>
<td>2</td>
</tr>
<tr>
<td>Destain</td>
<td>Destain solution</td>
<td>02:00</td>
<td>2</td>
</tr>
<tr>
<td>Wash</td>
<td>Purified water</td>
<td>01:00</td>
<td>1</td>
</tr>
<tr>
<td>Dry</td>
<td>——</td>
<td>15:00</td>
<td>65</td>
</tr>
</tbody>
</table>

b) SAS-4 (Auto-Stainer)

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mm:ss)</th>
<th>Temperature (°C)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain</td>
<td>05:00</td>
<td></td>
<td>Recirculate ON</td>
</tr>
<tr>
<td>Destain</td>
<td>00:30</td>
<td></td>
<td>Recirculate ON</td>
</tr>
<tr>
<td>Dry</td>
<td>15:00</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Destain</td>
<td>02:00</td>
<td></td>
<td>Recirculate ON</td>
</tr>
<tr>
<td>Destain</td>
<td>02:00</td>
<td></td>
<td>Recirculate ON</td>
</tr>
<tr>
<td>Dry</td>
<td>12:00</td>
<td>63</td>
<td>Recirculate ON</td>
</tr>
</tbody>
</table>

c) Manual

Follow the sequence listed for the SAS-2 Auto-Stainer, using a staining bath for the Stain, Destain and Wash steps, and a Drying Oven with forced air at 60...70°C for the Dry steps.

10. At the end of the staining cycle, remove the gel from the staining chamber. The gel is now ready for examination. (Schneider, et al., 1978).
3.8 Sample Size:
The thousand volunteer institute students was screened for the evidence of thalassemia minor.

3.9 Experimental Design:
a/ After analyzing the samples for complete blood count Those with low red cell indices (MCV, MCH and MCHC) and high RBC count were further investigated for determination of hemoglobin A2 (HbA2) and hemoglobin F (HbF) levels.
b/ chromatographically using commercial kit (Beta-Thal HbA2 Quik Column) from (Helena Laboratories). Hemoglobin F estimation was done by alkali denaturation method.

3.10 Sampling Techniques:
A sample of 2 ml venous blood was obtained by venipuncture under aseptic conditions from each participant and collected into EDTA tube until use. Freshly collected EDTA or heparin anticoagulated blood is the specimen of choice. Samples can be stored refrigerated at 2...6°C for up to 1 week. For optimal results, saline washed red cells should be used to prepare lysates. This removes possible interference from plasma proteins.
a) Mix 200μL of well mixed whole blood with 1000μL of saline solution.
b) Centrifuge to sediment the red cells.
c) Remove 1000μL of the supernatant and discard.
d) Add a further 1000μL of saline solution and mix well.
e) Repeat steps b-d x2.

3.11 Data Analysis:
According to the affected results of the CBC and hemoglobin fractions pattern; subjects were divided into three groups:
And the data were analyzed by using spss program using 16.
Chapter Four

4. RESULTS

Figure 4-1: Distribution of students in different groups:

This figure shows the distribution of students in different groups according to the test results it was found that the holding of 4.8%, (Table 1 and Fig. 1).
Figure 4-2: Percentage of males and females in the age groups:

Note through the figure that the highest percentage of males in the age group (22-25), 25.4% for females by 9.8%, followed by the age group (18-21) increased by 20.6% for males and 8.3% for females, followed by age group (26-29), which means that any of the three age groups of (18-29) represent a higher proportion of fertility period adolescent youth who can work tests them during their part of the marriage.
Figure 4-3: The relationship between disease holders for distribution to the provinces:

This figure shows the distribution of students in different groups according to the test results it was found that the holding of 4%

Through Figure note that the highest percentage of holders of the disease in Sana'a Governorate and argument which reached by (16.76%), followed by Hodeidah (12.50) and then Dhamar and Taiz by (10.42%), followed by the rest of the provinces as in Fig, (Table 2 and Fig. 2).
### Table 4-1:

Distribution of students in high health institute:

<table>
<thead>
<tr>
<th>Dep sex</th>
<th>emergency</th>
<th>Med.stat</th>
<th>Nor.treatment</th>
<th>Med instr</th>
<th>Public health</th>
<th>x-ray</th>
<th>Pre g careg</th>
<th>nurs e</th>
<th>lab</th>
<th>pharmacy</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>51</td>
<td>62</td>
<td>87</td>
<td>19</td>
<td>79</td>
<td>65</td>
<td>0</td>
<td>60</td>
<td>172</td>
<td>131</td>
<td>726</td>
<td>72.6%</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>7</td>
<td>22</td>
<td>0</td>
<td>30</td>
<td>18</td>
<td>67</td>
<td>35</td>
<td>71</td>
<td>14</td>
<td>274</td>
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<tr>
<td>Total</td>
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<td>19</td>
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<td>83</td>
<td>67</td>
<td>95</td>
<td>243</td>
<td>145</td>
<td>1000</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table 4-2:

Statistical summary of hematological and clinical parameter of studied subjects:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Number</th>
<th>Reference value</th>
<th>Mean</th>
<th>standard deviation</th>
<th>p value</th>
<th>The level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Hb g/dl</td>
<td>Normal</td>
<td>913</td>
<td>12.0 ± 14.1</td>
<td>13.2</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tha. Minor</td>
<td>48</td>
<td>11.5 ± 0.64</td>
<td></td>
<td>0.001</td>
<td>Statistically significant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H.cx.Anemia</td>
<td>39</td>
<td>11.3 ± 0.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pcv%</td>
<td>Normal</td>
<td>913</td>
<td>41.3 ± 0.33</td>
<td>41.5</td>
<td>0.52</td>
<td></td>
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<tr>
<td></td>
<td>Tha. Minor</td>
<td>48</td>
<td>38.7 ± 0.69</td>
<td></td>
<td>0.001</td>
<td>Statistically significant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H.cx.Anemia</td>
<td>39</td>
<td>37.5 ± 0.89</td>
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<tr>
<td>MCV fl</td>
<td>Normal</td>
<td>913</td>
<td>86.2 ± 0.38</td>
<td>86.7</td>
<td>0.93</td>
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<td></td>
<td>Tha. Minor</td>
<td>48</td>
<td>67.9 ± 0.87</td>
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<td>0.001</td>
<td>Statistically significant</td>
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<td>H.cx.Anemia</td>
<td>39</td>
<td>74.1 ± 6.29</td>
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<td></td>
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<tr>
<td></td>
<td>Total</td>
<td>1000</td>
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<td></td>
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<td></td>
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<tr>
<td>MCH pcg</td>
<td>Normal</td>
<td>913</td>
<td>28.7 ± 0.14</td>
<td>28.5</td>
<td>0.76</td>
<td></td>
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<td>Tha. Minor</td>
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<td>20.9 ± 0.66</td>
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<td>0.005</td>
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<td>H.cx.Anemia</td>
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<td>23.2 ± 0.40</td>
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<tr>
<td>MCHC g/dl</td>
<td>Normal</td>
<td>913</td>
<td>32.6 ± 0.76</td>
<td>32.9</td>
<td>0.76</td>
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<td>Tha. Minor</td>
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<td>0.005</td>
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<td>H.cx.Anemia</td>
<td>39</td>
<td>30.7 ± 0.70</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xBC×10^6/ul</td>
<td>Normal</td>
<td>913</td>
<td>4.2 ± 3.04</td>
<td>4.3</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tha. Minor</td>
<td>48</td>
<td>5.2 ± 0.60</td>
<td></td>
<td>0.003</td>
<td>Statistically significant</td>
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<tr>
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<td>H.cx.Anemia</td>
<td>39</td>
<td>4.5 ± 0.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1000</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
According to the results of the CBC and hemoglobin fractions pattern; subjects were divided into three groups:

- Group I: Individuals with normal CBC. This group included 913 students.
- Group II: Individuals with low red cell indices and high HbA2 levels with or without elevation of HbF levels, these individuals were considered to be β-thalassemia carriers. This group included 48 students; this figure makes the frequency of β-thalassemia trait in this sample (4.8%).
- Group III: Individuals with low red cell indices but normal hemoglobin fractions, these were labeled as having anemia with low indices. This group included 39 students.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal</th>
<th>Tha. Minor</th>
<th>H.c.x.Anemia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>913</td>
<td>48</td>
<td>39</td>
<td>1000</td>
</tr>
<tr>
<td>Percentage</td>
<td>91.3</td>
<td>4.8</td>
<td>3.9</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4-3: Distribution of students in different groups:

Through the table note that carriers of the disease in all tests by Valio coefficient turns out that there is a high statistically significant relationship, which means that all tests show that the 48 people are carriers of a disease and there is a significant relationship statistic for non-persons carrying but they have anemia and there are no statistically significant relationship for the rest of the sample, which means that members of the (913) a person not in possession of the disease (normal).
Table 4-4:
The relationship between age and sex of the sample studied for students:

<table>
<thead>
<tr>
<th>Age group</th>
<th>18-21</th>
<th>22-25</th>
<th>26-29</th>
<th>30-33</th>
<th>34-37</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>206</td>
<td>254</td>
<td>160</td>
<td>90</td>
<td>16</td>
<td>726</td>
</tr>
<tr>
<td>The proportion of males</td>
<td>20.6%</td>
<td>25.4%</td>
<td>16%</td>
<td>9%</td>
<td>1.6%</td>
<td>72.6%</td>
</tr>
<tr>
<td>Females</td>
<td>83</td>
<td>98</td>
<td>67</td>
<td>16</td>
<td>10</td>
<td>274</td>
</tr>
<tr>
<td>Female ratio</td>
<td>8.3%</td>
<td>9.8%</td>
<td>6.7%</td>
<td>1.6%</td>
<td>1%</td>
<td>27.4%</td>
</tr>
</tbody>
</table>

Note through the table(4) that the highest percentage of males in the age group (22-25), 25.4% for females by 9.8%, followed by the age group (18-21) increased by 20.6% for males and 8.3% for females, followed by age group (26-29), which means that any of the three age groups of (18-29) represent a higher proportion of fertility period adolescent youth who can work tests them during their part of the marriage.
Table 4-5:
Shows the results of Point Beiseraal link to the relationship between sex variable coefficient.

<table>
<thead>
<tr>
<th>The level of significance</th>
<th>T value tabular</th>
<th>T calculated value</th>
<th>Point Beiseraal correlation coefficient</th>
<th>The standard deviation of the sample as a whole</th>
<th>Numbe r</th>
<th>SMA</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05(*)</td>
<td>1.960</td>
<td>0.653</td>
<td>0.025</td>
<td>32.655</td>
<td>726</td>
<td>124.062</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>274</td>
<td>125.716</td>
<td>Females</td>
</tr>
</tbody>
</table>

The results indicated that the lack of a variable relationship sex and tests of the sample reaching their correlation coefficient (0.025) and for the correlation function test resorted researcher into the equation Altaúahvohart the results of the use of test samples t to that link is D at the level (0.05), as was T calculated value equal to (.653), a degree lower than the value of T tabular of (1.960) at the level of (0.05) and the degree of freedom (682) this means that the function is the relationship between Gansomen variable here is clear to us that the test is not related to the sex variable results.
Table 4-6:
Shows the results of the Pearson correlation coefficient of the relationship between test * Hbgm / dl and test Pcv%.

<table>
<thead>
<tr>
<th>The level of significance</th>
<th>T value tabular</th>
<th>T calculated value</th>
<th>Pearson correlation coefficient</th>
<th>The standard deviation</th>
<th>SMA</th>
<th>Number</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05(*)</td>
<td>25.61667</td>
<td>27.275</td>
<td>0.076-</td>
<td></td>
<td></td>
<td></td>
<td>Natural persons</td>
</tr>
<tr>
<td></td>
<td>0.755</td>
<td>27.35</td>
<td>913</td>
<td></td>
<td></td>
<td></td>
<td>People living</td>
</tr>
<tr>
<td></td>
<td>0.665</td>
<td>25.1</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td>Persons who are not carriers and non-natural</td>
</tr>
<tr>
<td></td>
<td>0.825</td>
<td>24.4</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This can be explained this result to test and test relationship correlation results that appeared in the first examination shows that people living with the disease are the same who appeared in the second test and the number (48) and also other persons not in possession and non-natural and number (37) appeared in the first test and the second and this shows that the test result showed people living with the disease and this shows that there is a relationship between the first and second test.
Chapter Five

5. Discussion

Among our studied subjects male predominated. Out of a thousand people we studied, 726 were male and 274 were female, with a male to female ratio of 2.6 to 1. 48 subjects were β-thalassemia carriers, giving it a prevalence arte of 4.8%. Of the carriers, 30 (4.1%) were male and 18 (6.5%) were females. The mean age of the normal subjects was 22.7 years, while mean age of the thalassemia carriers was 23.1 years, this result is not significant. The geographical distribution of the studied students showed that 686 were from Sana'a center, 92 subjects from Hajjah, 60 subjects from Taiz, 58 from Hodidah and 38 subjects from Damar, and 30 subjects from Ammran, and 16 subjects from Mahweet, and 16 subjects from Ibb, and subjects from bidah, and 2 subjects from Mareeb, shown in figure 1. The bulk of carriers were from Sana'a Center (eight) which means that 16.6% of carriers were from Sana'a center. The second population city studied was Hajjah; there were 92 subjects tested and 8 of them were carrier, that is (16.6%) of carriers are from this town. According to the number of subjects tested, the third town was Taiz, with 60 persons tested and 5 carrier = (10.4 %) of all carriers. Then Hodidah from which we have 58 subjects, 6 of them were carriers (12.5%) of all the carriers. Then Dammar from which we have 38 student and 5 of them (10.4%) were carriers. Then Ammran from which we have 30 students and 4 of them (8.3%) were carriers. Then mahwaat and Ibb we have 16 students and 4 of them (8.3%) were carriers. Then Bidah and Mareeb we have 2 students and 2 of them (4.1%) Malaria was endemic throughout Yemen. It would be expected to find thalassemia genes prevalent in Yemen and we are now in the control phase of eradication of malaria in Yemen. Regarding the countries near or neighbouring Yemen, the estimated prevalence of β-thalassemia minor was, in Qatar 28%, Saudia Arabia 3% 12, Lebanon 2 to 3% 13,14, Jordan 3-3.5% 15, prevalence in Turkey is ranging between 3.4 in East Anatolia to 11% in Western Thrace and Antalya 16,17, these results are comparable to our results.

Mean difference between the means of MCV for the three groups were 86.7, 67.9 and 74.1 fl respectively; here there is an obvious difference of 18.8 fl between the MCV of the normal students in comparison with the carriers. The MCH values were 28.5, 20.9, 23.2 pg respectively with a p value of less than 0.001 which is highly significant
difference. 7.6 pcg was the difference between the MCH values of the carriers and the normal students (table 4-2). Mean cell hemoglobin concentration (MCHC) values were 32.9, 30.3 and 30.7 g/dl respectively; there is a significant difference of 2.6 g/dl between the normal subjects and carriers with a p value of < 0.001. Finally RBC counts were notably elevated among β-thalassemia carriers as compared to the normal. RBC counts were 4.3, 5.2, 4.5 ×10^6 /ul with 0.9 ×10^6/ul difference between carriers and normal subjects and p value was < 0.001(Tabe 1). During this study Complete Blood Count (CBC) was performed on a total of one thousand Institute students. It was the cornerstone to determine the subjects on whom HbA2 had to be estimated. Reduced MCV or MCH values in the majority of heterozygous βthalassemia has been used as a basis for population screening for these disorders (18,,22), and although cut-off values for the MCV and MCH of 80 fl and 27 pcg respectively may involve a relatively large number of confirmatory HbA2 estimation it would detect virtually all affected cases. Hemoglobin A2 estimation was done for 87 students with hypochromic microcytic parameters. 48 of these were β-thalassemia carriers based on elevated HbA2 levels. No association could be noticed between the severity of the anemia and the HbA2 level. It was noted that increasing level of HbA2 above 6% was negatively associated with MCV. , when MCV values were less than 72 fl, among 11 carriers with HbA2 more than 6% we have 9 individual (81.8%) whom MCV values were less than 72 fl. Same thing when applied to MCH we have 81.8% of carriers having MCH values of (22 pcg) and less, no such relation could be found between HbA2 level and RBC count.
Chapter Sex

6-Conclusions

6-1 Conclusions

1- This study revealed that the prevalence of thalassemia minor or thalassemia carrier state in our community is 4.8 %

2- Thalassemia carriers can be detected through clinical examination and complete blood count the cardinal feature of thalassemia carrier state is elevated HbA2 level.

3- Preventive measures such as health education carrier screening and pre-marital counseling remain the best ways for lowering the incidence of these diseases which might be reflected in financial saving, social benefits, and health benefits.

4- The project also raised community awareness of the need to reduce the prevalence of this disorder among future generation.
6-2 Recommendation:

1- To raise awareness among young people about the thalassemia and thus to empower them to make informed choices to reduce the risk of this disease in their future families.

2- Intensify awareness campaigns, media and the establishment of scientific seminars to introduce the risk of thalassemia on the individual and society, and how to prevent them.

3- The establishment of educational activities in schools, colleges, universities and civil society organizations to illustrate the seriousness of the problem of thalassemia to reduce them and Mitigation.

4- The claim of the Ministry of Health to provide full health care for patients with thalassemia and to provide the necessary diagnostic tests to alleviate the suffering of patients.

5- Pass a law to check the pre-marriage and work tests before marriage and attaching a marriage contract to limit the spread of this disease.

6- Conduct such field surveys to determine the size of the problem Thalassemia to limit the spread and aggravation society.

7- Future molecular studies on selected cases to aid greater understanding of genotypes of this disease.
References


3- Al – Nood H,( 2009), thalassemia trait in out patient clinics of Sana’a city.


91- Webthal [http://www.talassemia.it/sito_eng/THAL_intro_eng.html]


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<tr>
<th>المحافظة</th>
<th>حالات مرضية غير مشخصة</th>
<th>حالات أخرى اتت من التاليسما</th>
<th>اتت من التاليسما</th>
<th>اتت من الثلاسما مع الحالات الأخرى</th>
<th>اتت من الثلاسما من غير الحالات الأخرى</th>
<th>الإجمالي</th>
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<td>664</td>
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<td>تعز</td>
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<td>22</td>
<td>15</td>
<td>12</td>
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الملاحظات: الأرقام في البداية تشير إلى عدد الحالات، في حين الأرقام في النهاية تشير إلى كبيرة الحالات.
**Intended Purpose:**

The SAS-1 Alkaline Hb-12 kit is intended for the separation of human haemoglobins by agarose gel electrophoresis.

Haemoglobins (Hb) are a group of proteins whose chief functions are to transport oxygen from the lungs to the tissues and carbon dioxide in the reverse direction. They are composed of polypeptide chains called globin, and iron protoporphyrin haem groups. A specific sequence of amino acids constitutes each of the four polypeptide chains. Each normal haemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. In normal adult haemoglobin (HbA), the non-alpha chains are called beta. The non-alpha chains of foetal haemoglobin are called gamma. A minor (3%) haemoglobin fraction called HbA2 contains alpha and delta chains. Two other chains are formed in the embryo. The major haemoglobin in the erythrocytes of the normal adult is HbA and there are small amounts of HbA2 and HbF. In addition, over 400 mutant haemoglobins are now known, some of which may cause serious clinical effects, especially in the homozygous state or in combination with another abnormal haemoglobin. Wintrobe1 divides the abnormalities of haemoglobin synthesis into three groups.

1. Production of an abnormal protein molecule (e.g. sickle cell anaemia).
2. Reduction in the amount of normal protein synthesis (e.g. thalassaemia).
3. Developmental anomalies (e.g. hereditary persistence of foetal haemoglobin (HPFH)).

The two mutant haemoglobins most commonly seen are HbS and HbC. Hb Lepore, HbE, HbGPhiladelphia, HbD-Los Angeles, and HbO-Arab may be seen less frequently2.

Electrophoresis is generally considered the best method for separating and identifying haemoglobinopathies. The protocol for haemoglobin electrophoresis involves step-wise use of two systems3-8. Initial electrophoresis is performed in alkaline buffers. However, because of the electrophoretic similarity of many structurally different haemoglobins, the evaluation must be supplemented by acid buffer electrophoresis which measures a property other than electrical charge.

This method is based on the complex interactions of the haemoglobin with an alkaline electrophoretic buffer and the agarose support. The SAS-1 Alkaline Hb-12 procedure
is a simple procedure requiring minute quantities of haemolysate to provide complementary evidence (along with the results from SAS-1 Acid Hb-12 analysis) of the presence of HbS, HbC and HbF as well as several other abnormal hemoglobins.

Warnings and Precautions:
All reagents are for in-vitro diagnostic use only. Do not ingest or pipette by mouth any kit component.
Wear gloves when handling all kit components. Refer to the product safety data sheet for risk and safety phrases and disposal information.

Composition:
1. SAS-1 Alkaline Hb Gel Contains agarose in a Tris / EDTA / Glycine buffer with sodium azide as preservative. The gel is ready for use as packaged.
2. Acid Blue Stain Concentrate:
3. Contains concentrated Acid Blue stain. Dilute the contents of the bottle to 700ml with purified water. Stir overnight and filter before use. Store in a tightly stoppered bottle.
4. Haemoglobin Lysing Agent Contains Triton X-100 in purified water with potassium cyanide, and thiomersal as preservative. The Lysing Agent is ready for use as packaged.
5. Destain Solution Concentrate: Dilute the contents of Destain A to 1 litre with purified water. Then add the contents of Destain B and add a further 1 litre of purified water, slowly.
6. Other Kit Components: Each kit contains Instructions For Use and sufficient Blotter C to complete 10 gels.

Storage and Shelf Life
1. SAS-1 Alkaline Hb Gel Gels should be stored at 15...30°C and are stable until the expiry date indicated on the package. DO NOT REFRIGERATE OR FREEZE. Deterioration of the gel may be indicated by 1) crystalline appearance indicating the gel has been frozen, 2) cracking and peeling indicating drying of the gel or 3) visible contamination of the agarose from bacterial or fungal sources.
2. Acid Blue Stain The stain concentrate should be stored at 15...30°C and is stable until the expiry date indicated on the label. Diluted stain solution is stable for 6 months at 15...30°C. It is recommended to discard used stain
immediately to prevent depletion of staining capability. Poor staining performance may indicate deterioration of the stain solution.

3. Haemoglobin Lysing Agent: The Lysing Agent should be stored at 15...30°C and is stable until the expiry date indicated on the label. Particulate contamination or cloudiness may indicate deterioration.

4. Destain Solution: The destain concentrate should be stored at 15...30°C and is stable until the expiry date indicated on the label. The diluted destain solution is stable for 6 months at 15...30°C.

**Items Required But Not Provided**

- Cat. No. 210200 Sample Applicator Blades (1 x 10)
- Cat. No. 210300 Sample Applicator Blades (5 x 10)
- Cat. No. 210100 Disposable sample cups (100)
- Cat. No. 3100 REP Prep
- Drying oven with forced air capable of 60...70°C
- Saline solution (0.85% NaCl)
- Purified water

**SAMPLE COLLECTION AND PREPARATION**

Freshly collected EDTA or heparin anticoagulated blood is the specimen of choice. Samples can be stored refrigerated at 2...6°C for up to 1 week. For optimal results, saline washed red cells should be used to prepare lysates. This removes possible interference from plasma proteins.

a) Mix 200µL of well mixed whole blood with 1000µL of saline solution.

b) Centrifuge to sediment the red cells.

c) Remove 1000µL of the supernatant and discard.

d) Add a further 1000µL of saline solution and mix well.

e) Repeat steps b-d x2.

f) Following the final centrifugation, remove 1000µL of supernatant and treat the remaining sample as whole blood, or remove all of the supernatant and treat the remaining sample as washed packed cells.

For patient samples with a total haemoglobin of 12-15 g/dL dilute 1+4 in Lysing Agent. For controls and samples not in this range, dilute to a haemoglobin concentration of 2.0-3.0 g/dL.

**NOTE:** For A2 quantification, samples must be diluted immediately prior to electrophoresis, all
samples must be less than 24 hours old.

Step-By-Step Procedure

1. Pipette 35µl of the sample into the appropriate well of the sample tray or disposable sample cups.
   i) SAS-1 & SAS-1 Plus users: Use SAS-1 sample tray. Carefully place the sample tray onto the applicator drawer. Ensure that the tray is pushed firmly down into position.
   ii) SAS-3 users: Use SPIFE / SAS-3 sample tray. Carefully locate the sample tray using the sample base locating pins. Ensure that the tray is positioned securely.

2. Remove the gel from the packaging and:
   i) SAS-1 users: place the gel in the SAS-1, agarose side up, aligning the positive and negative sides with the corresponding electrode posts.
   ii) SAS-1 Plus users: dispense 400µL of REP Prep onto the heat sink. Place the gel onto the heat sink, agarose side up, aligning the positive and negative sides with the corresponding electrode posts, taking care to avoid air bubbles under the gel.
   iii) SAS-3 users: place the alignment guide onto the pins and dispense 400µL of REP Prep onto the centre of the chamber. Place the gel into the chamber agarose side up, using the guide, align the positive and negative sides with the corresponding electrode posts, taking care to avoid air bubbles under the gel.

3. Blot the surface of the gel with a blotter C, discard the blotter.
   i) SAS-1 users: attach the electrodes onto the top side of the electrode posts so that they are in contact with the gel blocks.
   ii) SAS-1 Plus users: (as above). Place the cover over the gel and electrodes and press firmly for 5 seconds to ensure contact.
   iii) SAS-3 users: attach the electrodes onto the the electrode posts so that they are in contact with the gel blocks.

5. Place 1 applicator blade assembly into the top position on the instrument, (SAS-3 users: slot 8)

6. Perform the Alkaline Haemoglobin electrophoresis:
   i) SAS-1 users: 120 volts, 40 mins, 1 applications
   ii) SAS-1 Plus users: Electrophoresis: 200 volts, 30 mins, 25°C, 1 appli
   iii) SAS-3 users:
<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mm:ss)</th>
<th>Temperature (°C)</th>
<th>Voltage</th>
<th>Other</th>
</tr>
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<td>Speed 1</td>
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<tr>
<td>Apply Sample</td>
<td>00:10</td>
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<td></td>
<td>Speed 1</td>
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<tr>
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<td>200</td>
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</table>

* Use Location 1

7. Following electrophoresis, (SAS-1 Plus users: remove the cover), remove the electrodes and both gel blocks using the Gel Block Remover.
8. Attach the gel to the staining chamber holder.
9. Select the Alkaline Haemoglobin test program on the staining unit and, following the prompts,

Stain, Destain and Dry the gel.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (mm:ss)</th>
<th>Port</th>
<th>Temperature (°C)</th>
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<td>——</td>
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<tr>
<td>Destain</td>
<td>Destain solution</td>
<td>02:00</td>
<td>2</td>
</tr>
<tr>
<td>Destain</td>
<td>Destain solution</td>
<td>02:00</td>
<td>2</td>
</tr>
<tr>
<td>Wash</td>
<td>Purified water</td>
<td>01:00</td>
<td>1</td>
</tr>
<tr>
<td>Dry</td>
<td>——</td>
<td>15:00</td>
<td>65</td>
</tr>
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</table>

b) SAS-4 (Auto-Stainer)

<table>
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<th>Other</th>
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<td></td>
<td>Recirculate ON</td>
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<tr>
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<td>00:30</td>
<td></td>
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</tr>
<tr>
<td>Dry</td>
<td>15:00</td>
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</tr>
<tr>
<td>Destain</td>
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<tr>
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</table>
c) Manual
Follow the sequence listed for the SAS-2 Auto-Stainer, using a staining bath for the Stain, Destain and Wash steps, and a Drying Oven with forced air at 60...70°C for the Dry steps.
10. At the end of the staining cycle, remove the gel from the staining chamber. The gel is now ready for examination.
الثلسيميا والأنيميا المنجلية

الثلسيميا في خطر خيار إذا لم يتم معالجتها فوراً.

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