Prevalence of sickle cell gene in Yemen.

Al-Nood, Hafiz Abdul Hamid

PREVALENCE OF SICKLE CELL GENE IN YEMEN

Hafiz Abdul Hamid Al-Nood

Submitted to the University of Wales in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Wales Swansea

2004

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed .... (candidate)

Date ..... 31st Jan 2005 .....

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated.

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed .... (candidate)

Date ..... 31st Jan 2005 .....

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed .. _ ..... (candidate)

Date ..... 31st Jan 2005 .....

B: *Candidates on whose behalf a bar on access has been approved by the University (see Appendix 2), should use the following version of Statement 2:*

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access approved by the University of Wales on the special recommendation of the Constituent Institution/University College concerned.**

Signed .. _ ..... (candidate)

Date ..... 31st Jan 2005 .....

ACKNOWLEDGMENTS

I am indebted to Dr. Alison May for offering me a place in the Department of Molecular Haematology to perform the laboratory genetic tests of this study, also for supervising and supporting me during that period. Thanks also to Professor Alan Kenneth (Head of Department of Haematology, University Hospital Cardiff) for allowing me to do the laboratory tests for this study in the Department.

I am grateful to my supervisor Dr. Saad Al-Ismail for his supervision during my study.
I am grateful also to Professor J.G Williams for his help.

I am grateful also to Mr. Lawrence King, Department of Haematology, Llandough Hospital Cardiff, UK for helping me in performing the High Performance Liquid Chromatography and Isoelectric Focusing tests for the study.

Thanks to Dr. Donald M. Bradley for providing the Guthrie cards and to the Leigh Douglas Memorial Fund and West Glamorgan Haematology Fund for their help in funding this research.

Thanks to Mr Waheeb Abdulla Al-Towai for his help in taking care of the scholarship paperwork in Yemen which enabled me to continue my study in Clinical School, University of Wales Swansea, UK.
Thanks to Dr Mohamed M. Al-Qubati and Mr Nasser H. Al-Hamati for their help and assistance before and during the sample collection period in Yemen.

My sincere thanks to Mr Abdulkarim K. Abdo for his continuous support and encouragement during my stay in Cardiff, UK.

Last but not least my utmost gratitude and appreciation to my wife Arwa and children Moataz, Munther and Wed for putting up with me, for their enormous sacrifice and the hardship they have endured during this period and who without their support and encouragement this research would not have been possible.

Summary

To determine the prevalence of the sickle cell gene (HbS) in Yemen and amongst people from different regions of the country living in the capital, Sana'a City, cord blood samples from 1500 consented mothers were collected from hospitals in Sana'a City between July and December 2001. The names and original homes of the parents were recorded. Cationic HPLC analysis was used for screening while isoelectric focusing (IEF) and DNA- PCR were used to confirm haemoglobin S (HbS). Thirty-three samples were found to show Hb FAS giving an overall likely Hb S gene frequency of 0.011. The Hb S gene frequency varied with the part of the country from which the parents came. Amongst people from Taiz and Haja in the west the gene frequency was more than 0.04 but less than 0.004 amongst people from Ibb, adjacent to the governorate of Taiz. Of 66 chromosomes from babies carrying HbS, only 1.5% additionally carried the presence of –158 (C→T) G-gamma globin gene *Xmn* I site compared with 16.1% of 168 chromosomes from babies without Hb S from the same regions of the sickle cell trait samples identified in this study indicated that the beta S haplotype in not that associated with a milder course found in east Saudi Arabia. In addition to the absence of both *Hind* III/$^{G}\gamma$ and *Hind* III/$^{A}\gamma$ beta globin polymorphic sites in 26 sickle cell trait samples suggesting the predominant of the African sickle cell haplotype (Benin) in Yemen. The results of this study thus show a higher Hb S gene frequency in the western coastal part of Yemen than in the central mountainous and eastern desert areas. The incidence of affected homozygous births may therefore reach 20/10,000 in the western coastal part of Yemen.

A survey to evaluate health care of sickle cell patients was performed using 86 patients attending hospitals in Sanaa City, Yemen. The results showed that the clinical services provided to the sickle cell patients in Yemen were generally very poor.

Limited health resources can best be invested in developing a program of education, screening and health care initially prioritising those communities residing in the western areas of Yemen with the highest Hb S gene frequency.

Table of content

List of Figures

List of Tables

1. HISTORICAL BACKGROUND

The first recorded characterisation of sickle cell disorder in Africa was reported by Africanus Horton, who in 1874 described fevers of crises, shifting joint pains, and abnormality of the blood (1). Before this time the sickle cell disorder must have been known for generations in West Africa.

In North America, reports also described features highly suggestive of sickle cell disorder. Lebby in 1846 (2) and Hodenpyl in 1898 (3) both described autopsies in which no spleen would be found. These were autopsies on a runaway slave executed for murder, and a 32-year-old man who died in hospital after complaining of pains all over his body, pleuritic symptoms and jaundice.

The story of the growth of knowledge of the sickle cell anaemia is fascinating. The first commonly accepted case described of sickle cell disorder in North America was in 1910 when Dr James Herrick of Chicago published an article in the Archives of Internal Medicine entitled "Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anaemia". He reported a young student from Grenada who was studying in Chicago, and who complained of coughing and fever. Herrick examined the red blood cell of the student and saw the characteristic pathognomonic elongated shape, which has become recognised with sickle cell disorder (4).

After Herrick's report, Emmel, in 1917, observed the transformation of the biconcave red blood cell to the sickle form in vitro. He also noted that sickling occurred both in persons with severe anaemia and in others who were apparently healthy, thus recognising both sickle cell anaemia and sickle cell trait (5). Hahn and Gillespie in 1927 delineated the conditions affecting sickle

cell formation in vitro including pH, temperature, fixatives, toxicity and others (6). Among the most important of their observations was that exclusion of oxygen was a prerequisite to sickling, and that the phenomenon could be reversed on re-exposure to oxygen. They postulated that similar effects of oxygen could occur in vivo, hypoxia leading to cellular distortion with consequent haemolysis. Later, Hahn applied the term sickle cell trait to the asymptomatic condition associated with in vitro sickling. He performed sickle cell studies on affected families and concluded that the trait was inherited as a dominant character (6).

In 1940, the sickling phenomenon was reinvestigated by Sherman, who confirmed the observations of Hahn and Gillespie regarding reversibility and the importance of oxygen. Sherman also found that the cells in sickle cell anaemia were birefringent. In a casual conversation, the birefringence was called to the attention of the physical chemist, Linus Pauling. In 1948, Pauling conceived of the possibility that interaction between abnormal haemoglobin molecules might explain this phenomenon. With Itano, he demonstrated electrophoretically abnormal haemoglobin in sickle cell anaemia, thus originating the concept of molecular disease (7). When Ingram in 1956 demonstrated a difference in the amino acid sequence in one small part of the polypepticle chains of sickle cell haemoglobin, the science of molecular biology took root (8).

2. THE BIOLOGY OF SICKLE CELL DISORDER

2.1. Human haemoglobin

Haemoglobin (Hb) is a hemoprotein composed of two pairs of globin chains each folded around a haem molecule that gives red blood cells their characteristic colour. The function is primarily to transport oxygen from the lungs to the body tissues and carbon dioxide from the tissues back to the lungs.

The types of haemoglobin differ between the adult, fetal and embryonic periods of life (Table 1).

Table 1 Normal haemoglobins

Haemoglobin	Globin	Notes
A	$\alpha_2\beta_2$	Major Hb in adult life (96-98%)
A_2	$\alpha_2\delta_2$	Minor Hb (1.5 - 3.5%)
F	$\alpha_2\gamma_2$	Minor Hb in adult life (<1%) but major in fetal period
Gower1	$\zeta_2\varepsilon_2$	Major Hb in embryonic life
Gower2	$\alpha_2\varepsilon_2$	Major Hb in embryonic life
Portland1	$\zeta_2\gamma_2$	Major Hb in embryonic life
Portland2	$\zeta_2\beta_2$	Major Hb in embryonic life

In the foetus, there is a minor component which forms about 20% of Hb F (9) and it is called acetylated haemoglobin F or haemoglobin F1.

2.1.1. Structure and function

At the core of the molecule is a heterocyclic ring, called a porphyrin which contains an iron atom. This iron atom is the site of oxygen binding. A haem is an iron containing porphyrin. The name haemoglobin is the combination of haem and globin, a globin being a generic term for a globular protein. In humans, haemoglobin is a tetramer, made of two alpha and two beta subunits

noncovalently bound (Fig.1). The subunits are structurally similar, each alpha chains with 141 amino acids and each beta chains with 146 amino acids. Each subunit has a molecular weight of about 16,000 daltons, for a total molecular weight in the tetramer of about 64,000 daltons. Each subunit of haemoglobin contains a single haem, so that the overall binding capacity of adult human haemoglobin for oxygen is four oxygen molecules.

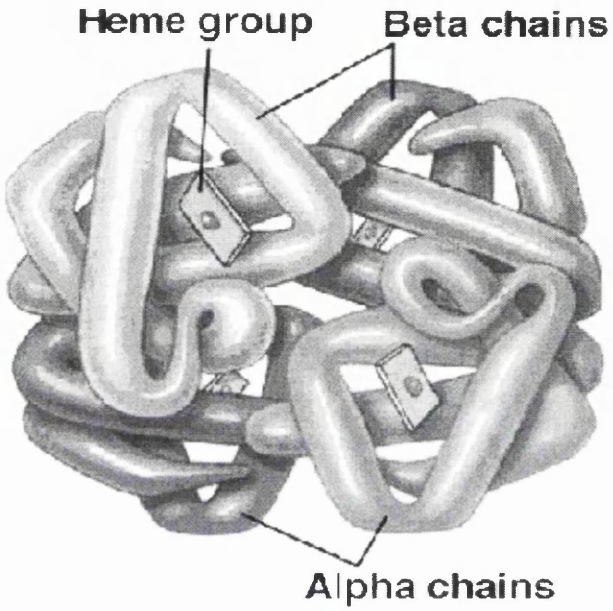


Figure 1 Haemoglobin structure

2.1.2. Haemoglobin synthesis

Synthesised polypeptide chains and the four-haem groups combine in nucleated red blood cells in the bone marrow. As the bone marrow cells mature, the nuclei are extruded and the cells start to circulate in the blood. Haem synthesis occurs in many steps involving enzymes in the mitochondrion and cytosol of the cell. In the mitochondrion, condensation of succinyl CoA

and glycine forms d-aminolevulinate, which is moved to the cytosol. A series of reactions produce the ring structure protoporphyrin IX that returns to the mitochondrion where iron is inserted to produce haem.

2.1.3. Genetic control of haemoglobin synthesis

Each of α and β globin polypeptides is encoded by multiple genes. Thus, there is an α-globin family of genes located on chromosome 16 and a β-globin family of genes located on the chromosome 11. The three genes of the α-globin family are located in a 25-kb region of DNA on chromosome 16, and the five genes of the β-globin family are located in a 65-kb region of DNA on chromosome 11. These two families of genes are expressed to synthesis haemoglobin molecules (Fig. 2).

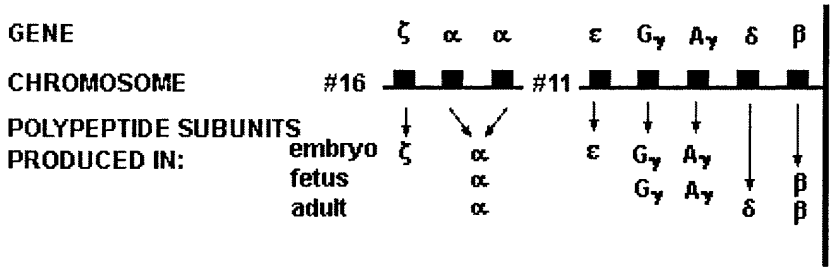


Figure 2 Genetic control of haemoglobin synthesis

Alpha globin locus

Chromosome16 has 2 identical alpha globin genes. Promoter elements exist 5' to each alpha globin gene. In addition, a powerful enhancer region called the locus control region (LCR) is required for optimal gene expression. The LCR is many kilobases upstream of the alpha globin locus.

Beta globin locus

Each cell has 2 beta globin genes, one on each of the 2 chromosomes 11 in the cell. These 2 beta globin genes express their globin protein in a quantity that precisely matches that of the four alpha globin genes (Fig.2)

2.1.4. Abnormal haemoglobins variants

Mutations in genes that determine the polypeptide structure will cause changes in the amino acid sequences and results in abnormal haemoglobin. Several hundred haemoglobin variants have been reported, the majority of them are due to the substitutions of the one amino acid (11). These substitutions lead to clinical disease only if they influence the haemoglobin stability or its function. The first recognised haemoglopinopathy was the sickle cell anaemia described by Herrick when a patient with severe anaemia was seen to have sickle shaped red blood cells (4). Sickle cell anaemia is produced by haemoglobin S and is the most frequent hereditary haemolytic anaemia in the world. Nearly 75% of affected births are in Africa. Serjeant (1997) notes that 250,000 births are affected by sickle cell disorders each year in the world (12) and Stienberg (1999) estimates that 120,000 are born with sickle cell disorders each year in Africa while 1,000 in United States of America (13). In Saudi Arabia, it is estimated that 2,500 births are annually affected by sickle cell disorders (14). In the Arabian Peninsula, the highest frequency of sickle cell gene is reported in eastern part of the Saudi Arabia where the prevalence of the carrier-state can get as high as 25%.

2.2. The molecular pathology of haemoglobin S gene

Today it is well known that the sickle cell mutation is located in chromosome 11, where the thymine substitutes adenine in the middle position of the sixth codon of the beta globin gene (GAG → GTG). This thymine substitution leads to encoding of valine (hydrophobic, uncharged, and molecular weight 99) instead of glutamic acid (hydrophilic, negative charge, and molecular weight 129) in the sixth position of the beta-chain which lies on the outside of the haemoglobin molecule (Fig.3).

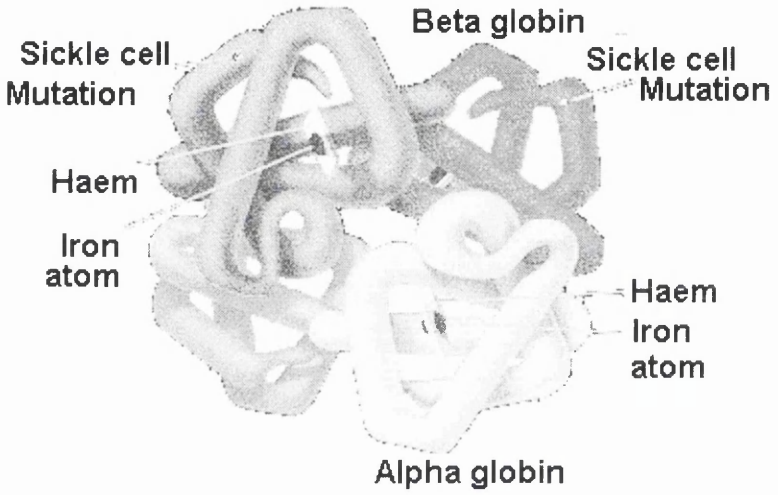


Figure 3 Haemoglobin molecule structure with sickle cell mutation

This apparently minor change in the structure is responsible for profound changes in molecular stability and solubility (15). The tendency of deoxygenated haemoglobin S to undergo polymerisation underlies the innumerable expression of the sickling syndromes. In deoxygenated state, aggregate of sickled haemoglobin molecules arrange themselves in parallel rod-like fibres forming a solid complex core which is made of 14 filaments arranged as seven pairs of double filaments. Valine substitution in the sixth position of the beta chain stabilises these molecular stacks since each valine

substitution in each molecule takes part in the polymer contact regions (16,17,18,19) (Fig.4).

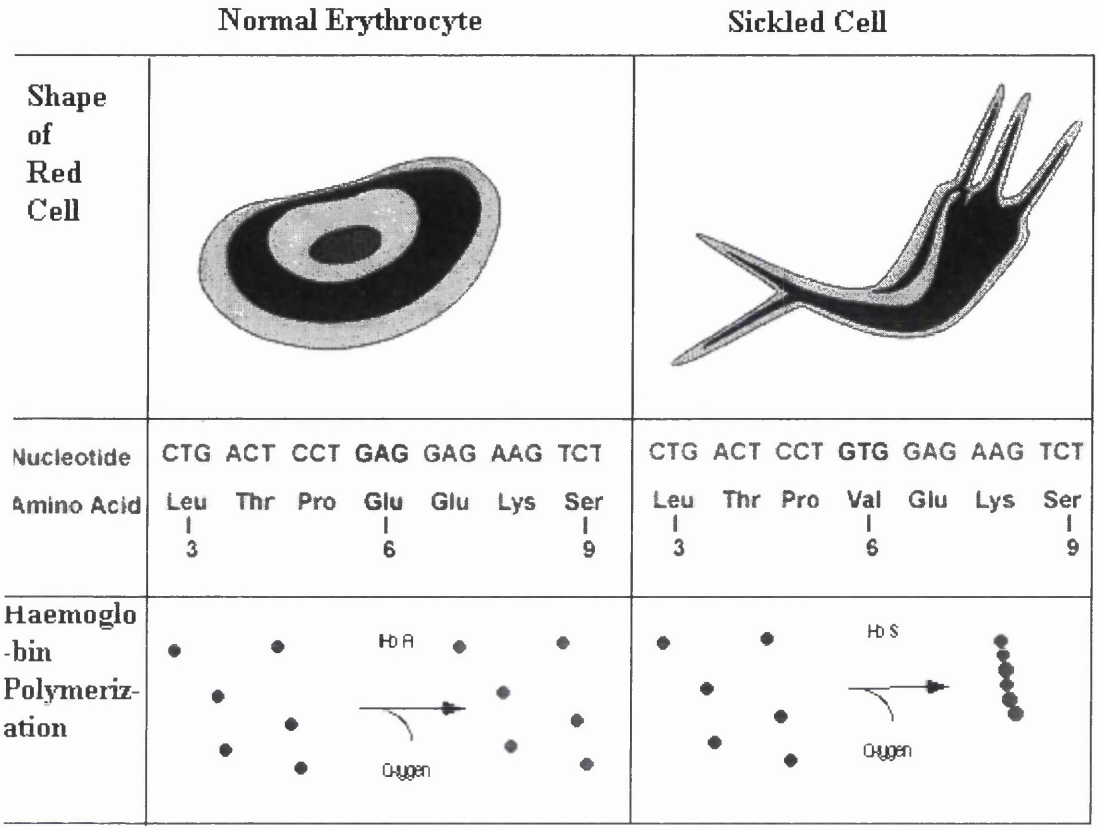


Figure 4 Comparison between normal and sickled erythrocyte

Sickle cell anaemia is due to homozygosity of a single haemoglobin S gene mutation at position six of beta-haemoglobin locus, but not all the sickle cell patients suffer the same severity of the disorder. The clinical expression of the haemoglobin S mutation is influenced by many genetic factors which are linked to the beta globin gene cluster that effect the haemoglobin F production.

2.3. Pathophysiology of sickle cell

An abundance of information (20,21) indicates that the distortion of cells containing haemoglobin S is the result of haemoglobin polymerisation. The

equilibrium of haemoglobin S between its liquid and solid phases is determined by four physiologic variables: oxygen tension, haemoglobin S concentration, temperature and haemoglobins other than haemoglobin S.

2.3.1. Oxygen

The most important physiologic determinant of haemoglobin S gelation is oxygen concentration. Polymerisation occurs only with deoxygenation (Fig.5). The effect of the 2,3-diphosphoglycerate (2,3-DPG) and pH in gelation are essentially mediated through their influence on the oxygen affinity of haemoglobin S for oxygen. Decreased pH or increased 2,3-DPG leads to decrease oxygen affinity thereby increasing the amount of deoxy-haemoglobin at any given oxygen tension which enhances gelation (22).

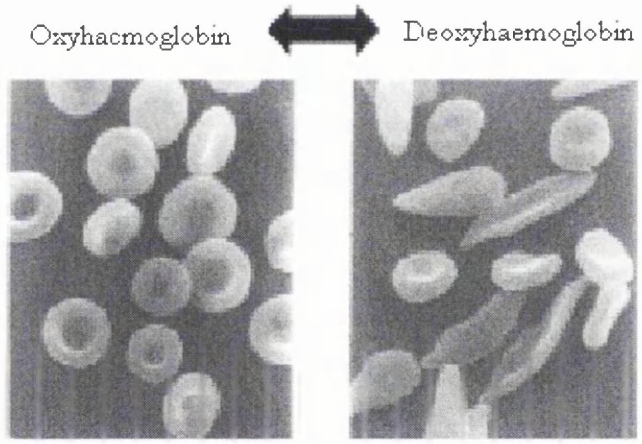


Figure 5 Deoxygenation affects on blood sickle cell morphology

2.3.2. Haemoglobin S concentration

There is a positive correlation between haemoglobin S concentration and gelation (Fig.6). Under standard laboratory conditions, gelation occurs as the concentration of deoxy-haemoglobin S is increased to more than 20.8 g/dl. Because the mean haemoglobin concentration of the red cell is normally above

30g/dl, intracellular gelation of haemoglobin S is a predictable consequence of deoxygenation (23).

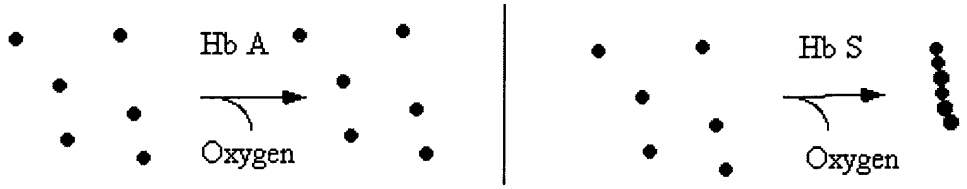


Figure 6 Haemoglobin S gelation in deoxygenation condition (right), normal haemoglobin remains soluble in deoxygenation situation (left)

2.3.3. Temperature

High temperature accelerates the sickling process. At low temperature (1- 4°C) the sickling is completely stopped. Insoluble deoxy-haemoglobin S dissolves when cooled below a critical temperature. Because the temperatures required for solubilisation of haemoglobin S are below the physiologic range, the significance of this phenomenon is limited to the laboratory study of gelation.

2.3.4. Other haemoglobins

The influence of other haemoglobins on haemoglobin S polymerisation is variable. Their addition 'dilutes' the haemoglobin S and thereby reduces polymerisation, but the degree of 'dilution' depends on the extent to which the additional haemoglobin may itself participate in the polymerisation. Haemoglobin F reduces haemoglobin S concentration in the red blood cell and also inhibits the its polymerisation while haemoglobins D-Punjab and O-Arab are involved in the formation of the sickling tubule and interact strongly with haemoglobin S causing an increase in its polymerisation. Other haemoglobins interfere with polymer formation less well. Deoxyg Hb S molecule copolymerises most effectively with other haemoglobin S molecules, and in

decreasing order with haemoglobin D Punjab, O-Arab, C, C-Harlem, D, A, J and F (23).

2.3.5. Endothelial adherence

The membrane of sickle red cell is abnormal and the sickle cell adherence to endothelium is mediated by von Willebrand's protein (24). For instance, the ratio of membrane phospholipids and cholesterol is abnormal in sickle cell disease. Therefore, sickle red cell membrane has pro-coagulant activity. As a result, a cascade could occur in which von Willebrand's protein promotes sickle red cell/endothelial cell adhesion, which then is a nidus for thrombus formation.

3. SICKLE CELL DISORDER

3.1. Nomenclature and classification

The term sickle cell disorder refers to a group of pathological conditions resulting from the presence of haemoglobin S and classified according to their genotypes (Table 2).

Table 2 Sickle cell disorder classification

Sickle cell anaemia (homozygous sickle cell)
Sickle cell/ haemoglobin C
Sickle cell / Beta thalassaemia
Sickle cell / haemoglobin D-Punjab
Sickle cell / Haemoglobin O-Arab
Sickle cell / haemoglobin Lepore
Sickle cell/ haemoglobin C-Harlem
Sickle cell/ haemoglobin S-Antilles
Haemoglobin S-Oman

Conditions excluded from the classification of sickle cell disorder: -

Sickle cell trait occurs when only one sickle cell gene is inherited from one parent and a normal beta chain gene inherited from the other parent. Clinical manifestations occur when sickle cell trait is associated with independent pathology such as spherocytosis and sickle cell/ hereditary persistence of fetal haemoglobin, causing haemolysis.

3.2. Interaction of Hb S with thalassaemia and other haemoglobinpathies

3.2.1. Sickle cell and haemoglobin C

Haemoglobin C results from a mutation at codon 6 of beta-globin gene (beta 6; GAG→AAG) leading to the substitution of glutamic acid by lysine on the sixth position on the beta globin. The severity of haemoglobin SC disorder is similar

to sickle cell anaemia. Haemoglobin SC disorder is one of the most prevalent sickle-cell disorders. It is found in West Africa, west of the Niger River, and in some West Indians and black Americans. It is occasionally seen in North Africa, Italians, and Spaniards.

On alkaline cellulose acetate electrophoresis, haemoglobin C migrates at the same position of haemoglobin A2, E and O-Arab, but it can be distinguished from both haemoglobin E and O-Arab by acid citrate agar electrophoresis.

In haemoglobin SC, blood film shows abundant target cells, folded cells, dense cell and rarely sickled or crystaled shaped cell (26).

On alkaline electrophoresis, haemoglobin SC-Harlem migrates as haemoglobin SC while on acid acetate agar electrophoresis separates on the same position of haemoglobin S (27). The compound heterozygous of both haemoglobin S and C-Harlem causes less severe sickle cell disorder than sickle cell anaemia

3.2.2. Sickle cell and beta-thalassaemia

The beta thalassaemia syndromes are recognised by low or absent production of beta globin because of the mutations in the beta globin gene. The combination of beta thalassaemia with the sickle cell mutation leads to a group of compound heterozygous condition known as haemoglobin S/beta thalassaemia. The production of haemoglobin A is the most important determining factor for the clinical severity of the disorder. No haemoglobin A is produced in haemoglobin S/ $beta^0$ thalassaemia while in haemoglobin S/ $beta^+$ thalassaemia, the haemoglobin A vary from 3 to 45%. Heterozygous patients for haemoglobin S and $beta^0$ thalassaemia have similar clinical severity to sickle cell anaemia. Those individuals heterozygous for haemoglobin S and $beta^+$ thalassaemia tend to have milder symptoms. In comparison with sickle

cell trait, the percentage of haemoglobin A is more than haemoglobin S, while in sickle cell and beta-thalassaemia, haemoglobin S is more than 50% of total haemoglobin. In sickle cell and beta-thalassaemia, the haemoglobin F is usually between 5 – 15%.

The laboratory differential diagnoses between the sickle cell anaemia and haemoglobin S/beta^0 thalassaemia in a blood sample from a newborn is difficult. Since the level of haemoglobin A in blood of the newborn is very low, high performance liquid chromatography, isoelectric focusing and alkaline cellulose acetate or acidic citrate agar electrophoresis may not be adequately sensitive to detect the level of haemoglobin A. When accurate diagnosis is required, DNA analysis should be performed (20,28).

3.2.3. Sickle cell and alpha-thalassaemia

Alpha thalassaemia is a globin gene disorder that leads to diminished rate of one or more of the alpha globin chains resulting in a reduce rate of production of haemoglobin. The most common types of alpha thalassaemia seen with sickle cell are - $\alpha^{3.7}$ and -$\alpha^{4.2}$ mutations. The -$\alpha^{4.2}$ mutation indicates to the deletion of α_2 gene while the - $\alpha^{3.7}$ mutation indicates to the deletion of part of both α genes with formation of an $\alpha_2\alpha_1$ fusion gene.

In sickle cell anaemia, alpha thalassaemia reduces the mean corpuscular haemoglobin concentration (MCHC) inhibiting polymerisation of Hb S and improve flow in capillaries and small vessel, but the elvated blood viscosity effects due to increased total haemoglobin concentration may retard flow in large vessels causing an increase in episodes of bone pain (147).

El-Hazmi et al. (1999) studied the coexistence of alpha thalassaemia in sickle cell anaemia among Yemeni patients. They examined the pattern for alpha thalassaemia in 26 Yemeni children with sickle cell anaemia and 18 normal haemoglobin Yemeni individuals (control group) living in Riyadh, Saudi Arabia. In the sickle cell patients, the frequency of one alpha gene (-α/αα) deletion was 0.346, where in the control group the frequency was 0.263. The frequency of two-gene (-α/-α) deletion was 0.231 in the sickle cell patients, compared to zero in the control group. Therefore, the overall frequency of alpha thalassaemia in the Yemeni children with sickle cell anaemia living in Riyadh is 0.576 (Table 3) (29).

Table 3 Frequency of alpha thalassaemia gene in Yemen in children with sickle cell anaemia living in Riyadh

	αα/αα		-α/αα		–α/–α	
Group	No	%	No	%	No	%
Hb SS	11	42.3	9	34.6	6	23.1
Hb AA	13	68.4	5	26.3	0	0
Total	24	53.3	14	31.1	6	13.3

The haematological parameters of these sickle cell patients with alpha thalassaemia showed lower mean cell volume and mean cell haemoglobin and higher red blood cell count than sickle cell patients without alpha thalassaemia. Haemoglobin F concentration was higher in the sickle cell patients with alpha thalassaemia (7.5-10.1%) than in sickle cell patients without alpha thalassaemia (5.7%) (29).

The origin of the Yemeni children examined in this study had not been reported, but the high prevalence of alpha thalassaemia among Yemeni sickle

cell patients living in Riyadh may suggest high prevalence of alpha thalassaemia in the Yemen population.

The clinical picture of alpha thalassaemia with sickle cell anaemia linked with Arab-Indian haplotype in a study in India reported that the painful crises, infections and episodes of hospitalisation significantly reduced (148).
In other a study, alpha thalassaemia trait was appeared to ameliorate the red blood haemolysis in sickle cell anaemia without reducing the painful crises frequency (140). In second study, alpha thalassaemia trait was reported to decrease the incidence of soft-tissue end-organ failure in sickle cell patients but the incidence of osteonecrosis was expanded (141). In another study, deletion of two alpha genes was linked with an increased prevalence of avascular necrosis, splenomegaly and retinopathy in sickle cell patients (142). In fourth study, reported that the life expectancy was not changed by the coexisting alpha thalassaemia with sickle cell anaemia (113).

In south western part of Saudi Arabia, alpha thalassaemia frequency is 0.55 and haemoglobin S gene is 0.0765, while in eastern region of Saudi Arabia, the frequency of alpha thalassamia is 0.45 and the haemoglobin S gene is 0.1446 (Table 4) (30). These figures suggest the coincidences of haemoglobin S gene and alpha thalassemia in eastern region of Saudi Arabia is high. They also suggest high frequency of coincidences of haemoglobin S and alpha thalassaemia in the south western region of Saudi Arabia which is the geographic extension of the west coastal strip of Yemen.

Table 4 Frequency of α and β thalassamia and Hb S in Saudi Arabia

Region	α– thalassaemia	β–thalassaemia	HbS gene frequency
Northern	0.010	0.010	0.0065
North-western	0.190	0.076	0.0465
South-western	0.550	0.101	0.0765
Central	0.010	0.030	0.005
Eastern	0.450	0.130	0.1446

3.2.4. Sickle cell and haemoglobin D^{Punjab}

Haemoglobin D results from a mutation occurring at the 121 codon of the beta globin leading to substitution of GAA→ CAA, that causes the normal glutamic acid to be replaced by the glutamine on the beta globin chain at position 121. On alkaline cellulose acetate electrophoresis, haemoglobin D separates in the haemoglobin S position while on acid citrate agar electrophoresis it separates in the same haemoglobin A position (31).

The compound heterozygous haemoglobin S and haemoglobin D^{Punjab} cause a disorder that is generally less severe the sickle cell anaemia. This has been seen in Sikhs, people of Afro-Caribbean, Afro-American and South and Central American. Compound haemoglobins S and D variants other than D-Punjab or D-Los Angeles do not show the same severity as compound haemoglobins S and D-Punjab.

3.2.5. Sickle cell and haemoglobin O-Arab

The GAA→AAA mutation at codon 121 found in haemoglobin O-Arab results in substitution of lysine for glutamic acid at position 121of the beta globin chain. This compound is a combination of the haemoglobin S and haemoglobin O-Arab. On alkaline cellulose acetate electrophoresis, it has the same mobility of haemoglobin E, C and A2. On acid citrate agar

electrophoresis, it separates between haemoglobin A and S bands. For confirmation, haemoglobin O-Arab can be diagnosed by using DNA analysis or immunologic technique (32).

Usually heterozygosity for haemoglobin S and O-Arab causes severe sickle cell disorder. Its highest prevalence is seen in Bulgaria, which suggests that it was carried from Bulgaria to the Arab area during the Ottoman Empire. This compound sickle cell disorder has been seen in Arab, African, Afro-American, and Afro-Caribbean populations. The proportion of the haemoglobin S is slightly more than haemoglobin O-Arab (33).

3.2.6. Sickle cell and haemoglobin Oman

This compound is a combination of haemoglobin S mutation and another haemoglobin that is identical to haemoglobin O-Arab mutation (GAA→AAA at codon 121). It has two mutations in the beta globin; besides the beta S mutation in the globin, an additional mutation occurs in the same chain (beta 121 Glu →Lys). Because it has an additional positive charge, it migrates slower than haemoglobin C (34). The pathology of heterozygous haemoglobin S-Oman is the result of both sickling properties and the haemolytic anaemia enhanced by the mutation at the position 121 in the beta globin chain (35). It has been linked either to heterozygous or homozygous alpha thalassaemia. With haemoglobin S-Oman in the heterozygous alpha thalassaemia -α/αα, patients have 20 – 27 % of the variant haemoglobin and show severe anaemia, while with coinheritance of –α/–α thalassaemia patients have 13 – 15% haemoglobin S-Oman, and no clinical symptoms (36).

3.2.7. Sickle cell and haemoglobin E

Haemoglobin E results from a mutation at codon 6 of the beta-globin gene at position 26 GAG→GAA leading to the substitution of lysine for glutamic acid on the beta globin chain at position 26. Also in this mutation, activation of a cryptic splice donor site in exon 1 occurs leading to incorrectly spliced mRNA transcripts, reduced haemoglobin beta gene mRNA accumulation, and a mild beta-thalassaemia phenotype (37).

On alkaline cellulose acetate electrophoresis, haemoglobin E separates at the same position of haemoglobin A_2. On acid citrate agar, it migrates in the same position of haemoglobin A.

This heterozygous state of haemoglobin S and E leads to an asymptomatic or mild symptomatic condition. This has been reported in Saudi Arabians, Pakistani, Turks, Afro-Caribbeans and Afro-Americans (28).

3.2.8. Sickle cell and haemoglobin Lepore

The Lepore haemoglobin results from unequal cross over during meiosis with deletion of 3' part of the delta gene and the 5' part of the beta gene. The resultant haemoglobin Lepore has 2 normal alpha chains and 2 delta beta fusion chains (38).

Three types of haemoglobin Lepore have been recognised and differ in the delta-beta fusion point. They are haemoglobin Lepore Hollandia (delta22/beta50), haemoglobin Lepore Baltimore (detla59/beta86) and Haemoglobin Lepore Boston (delta87/beta116).

This combination of haemoglobin S and haemoglobin Lepore has been observed in Mediterraneans, Afro-Americans and Afro-Caribbeans.

Haemoglobin S/haemoglobin Lepore causes sickle cell disorder similar to sickle cell/beta^{+} thalassaemia (28). It has the same mobility as haemoglobin S on alkaline electrophoresis. The only bands apparently present are haemoglobin S, F and A_2 and diagnosis can be misinterpreted as sickle cell anaemia or sickle cell/beta^{0} thalassaemia. However, High Performance Liquid Chromatography (HPLC) shows that haemoglobin Lepore is present. Usually the amount of haemoglobin Lepore is about 10 – 12 % of total haemoglobin while the amount of the haemoglobin S is about 63- 90% in sickle cell anaemia.

3.2.9. Hb S and hereditary persistence of fetal haemoglobin (HPFH)

Hereditary persistence of fetal haemoglobin describes inherited states in which heterozgyotes show an increased proportion of haemoglobin F, persisting after infancy into adult life with little or no imbalance of chain synthesis and normal haematological values.

Haemoglobin F concentration is increased relatively in HPFH according to the type of HPFH mutation as either deletional or nondeletional. Individuals with combination of nondeletional HPFH and sickle cell trait usually show no symptoms and may show very minimal increases in haemoglobin F level. Carriers with compound heterozygosity for haemoglobin S and gene deletion HPFH have milder disorder than in sickle cell anaemia with 20-30% haemoglobin F. The severity of the combination of haemoglobin S with HPFH depends also on the concentration and cellular distribution of haemoglobin F. The pancellular distribution of haemoglobin F is more efficient in inhibiting clinical symptoms than the heterocellular distribution (20). Some patients with sickle cell anaemia or haemoglobin S/beta^{0} thalassaemia have haemoglobin F

levels close to the levels of gene deletion haemoglobin S/HPFH. This has been reported in African, Afro-Caribbean and Afro-American population (28).

3.2.10. Sickle cell and G6PD deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is commonly found among sickle cell anaemia patients (39,40). This is not universal as in the Congo. Bouanga et al. (1998) investigated the prevalence of G6PD deficiency, using PCR analysis, in 188 sickle anaemia Congolese patients (109 females and 79 males) and 210 normal controls (115 females and 95 males). They found the prevalence of G6PD genotype among the sickle cell anaemia patients did not differ from that in the non-sickle cell patients (41).

The technical difficulty of G6PD deficiency diagnosis in sickle cell anaemia is, in part, due to the high reticulocyte population characterised by higher G6PD enzyme levels even in deficient patients. Small sample size studies or the use of different methods with various ranges of accuracy, such as semiquantitative methaematology reduction test, G6PD electrophoresis or cytochemical techniques, has confused the interpretation of the effect of G6PD deficiency in sickle cell anaemia. Different studies have suggested that G6PD deficiency effects on sickle cell anaemia can be beneficial, deleterious, or neutral.

Piomelli et al. (1972) studied the G6PD deficiency effects in 15 male sickle cell anaemia unrelated patients and 26 normal males (control group) at the Sickle-Cell Anaemia Clinic, Sydenham Hospital, New York. They found that the preponderance of G6PD deficiency with sickle cell anaemia supported the hypothesis that the simultaneous inheritance of G6PD deficiency and sickle

cell anaemia may be beneficial (42). This simultaneous inheritance of G6PD deficiency and sickle cell anaemia may ameliorate each other. This is because in sickle cell anaemia, the number of reticulocytes increases leading to high level of G6PD enzyme that masks G6PD deficiency. On the other hand, G6PD deficiency also increases the young red blood cell population leading to reduction in the number of the old red blood cells and irreversible red blood cells that may modify the clinical severity of sickle cell anaemia (42). Because of the small number of sickle cell anaemia patients involved in this study, the beneficial effect due to the simultaneous inheritance of G6PD deficiency and sickle cell anaemia may be limited and may not be generalised.

The clinical reports of Konotey-Ahulu (1972) in Ghana, that included more than 1500 sickle cell anaemia patients, concluded that G6PD deficiency could have a deleterious influence upon sickle cell anaemia because it might have an additional haemolysis effect in sickle cell anaemia cases (43).
This deleterious effect of G6PD deficiency could be due to the high coincidence of G6PD deficiency with sickle cell anaemia in hospital based reports.

Steinberg et al. (1988) studied 801 sickle cell anaemia male patients from The Cooperative Study of Sickle Cell Disease in the USA, where 10.4 % of them had G6PD deficiency. Based on the laboratory results, they failed to find haematological differences between sickle cell anaemia patients with or without G6PD deficiency. According to the clinical observations in this study, the G6PD deficiency was not associated with differential survival, increased

haemolysis, reduced haemoglobin concentrations, more pain crisis or higher incidence of acute anaemia crisis (44).

This study could show valid results because the high number of sickle cell anaemia patients involved and the reliable method of G6PD enzyme determination (G6PD electrophoresis) used. In addition, the patients were enrolled in The Cooperative Study of Sickle Cell Disease which studied the natural history of the severe sickle cell disorders.

3.2.11. Sickle cell and pyruvate kinase deficiency

Pyruvate kinase deficiency is characterised by high concentrations of erythrocyte 2,3-diphosphoglycerate. Usually patients with compound heterozygosity for haemoglobin S and pyruvate kinase deficiency manifests a severe form of sickle cell anaemia (45).

3.3. Sickle cell trait

Sickle cell trait indicates compound heterozygosity for Hb S and A. It is the most benign form of the sickle cell disorder. The concentration of haemoglobin A is always more than the haemoglobin S. Therefore, haemoglobin A dilutes haemoglobin S leading to a reduction in the clinical significance of haemoglobin S polymerisation at the oxygen saturation and physiologic situations in most tissues. At oxygen saturation lower than 60%, the blood is totally deoxygenated and polymerisation of haemoglobin S occurs, but the haemoglobin S polymerisation is about 40% of the total haemoglobin and the health of the carrier usually is not affected.

Sickle cell trait reduces morbidity and mortality due to malaria infection.

Sickle cell trait is of genetic importance and its diagnosis is usually needed for

genetic counselling of couples that are carrying the haemoglobin S gene, or individuals who are at risk of inheriting the haemoglobin S gene, or for a population studies to determine the prevalence of haemoglobin S gene.

3.4. Genetics of sickle cell disorder

3.4.1. Inheritance of sickle cell gene

Sickle cell anaemia is an autosomal recessive genetic disorder caused by a defect in the haemoglobin S gene, which codes for haemoglobin. The inheritance of sickle cell disorder occurs according to the principle of Mendelian inheritance. If both parents have sickle cell trait (haemoglobin AS), they can each form germ cells containing genes either for haemoglobin A or S (Fig.7). Therefore, the presence of two defective genes (SS) is needed for sickle cell anaemia. If each parent carries one sickle haemoglobin gene (S) and one normal gene (A), each child has a 25% chance of inheriting two defective genes and having sickle cell anaemia, a 25% chance of inheriting two normal genes and not having the disease, and a 50% chance of being sickle cell trait like the parents.

The same probabilities apply for the inheritance of other abnormal forms of haemoglobin, the gene for which can be inherited in combination with those for normal haemoglobin, sickle haemoglobin and beta-thalassaemia.

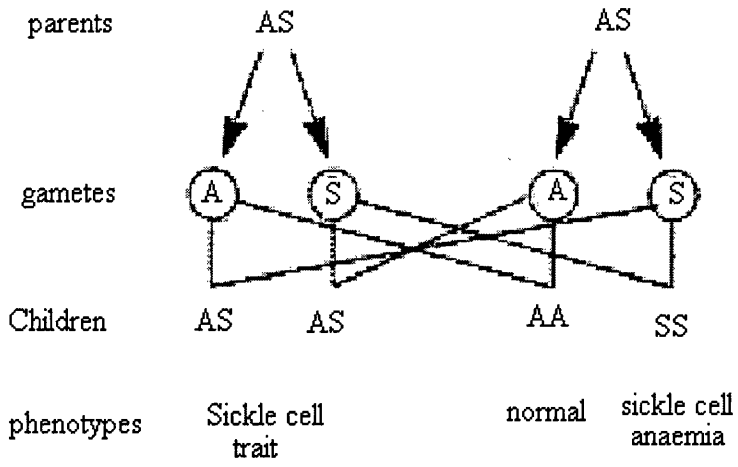


Figure 7 Probabilities of inheritance of sickle cell gene

3.4.2. Population genetics

The study of genes frequency in the population is determined by the Hardy-Weinberg principle that suggests genotype frequencies stay constant from generation to generation. The Hardy-Weinberg equation is $p^2 + 2pq + q^2 = 1$, where p is the haemoglobin A frequency, q is the haemoglobinopathy gene frequency, p^2 is the frequency of homozygotes without haemoglobinopathies, 2pq is the frequency of heterozygotes and q^2 is the frequency of homozygous with haemoglobinpathies.

The use of the Hardy-Weinberg equation to calculate the gene and genotype frequencies is limited by selection, mutation, non-random mating (consanguineous marriage), drift and gene flow due to population movements (25).

The frequency of the haemoglobin S gene is calculated by gene counting and includes also the haemoglobin S gene in the AS genotype and other haemoglobin S gene containing genotypes (25).

3.4.3. Incidence of sickle cell genotypes

With the knowledge of the haemoglobin S gene frequency, the Hardy-Weibery formula can be used to predict the incidence of the sickle cell genotype at birth. The predicted incidence of homozygous SS sickle cell disorder in a population at birth can be approximated from the prevalence of the sickle cell trait. Therefore, in a population where 10% have sickle cell trait, the chance of any parent having sickle cell trait will be 1 in 10. Assuming random mating, therefore, 1 in 100 mates will be among parents with the sickle cell trait. Since 1 in 4 of their children will have sickle cell anaemia (HbSS). Therefore, the incidence can be expected approximately as 1 in 400 or 2.5 sickle cell anaemia per1000 births (25).

4. DISTRIBUTION OF POPULATION GENETICS OF SICKLE CELL

4.1. Origins of the sickle cell gene

The high prevalences of erythrocyte genetic mutations in human populations has resulted form the selective pressure of malaria, which causes 2 to 4 million deaths per year especially among children (46). The precise age of the haemoglobin S gene is not known, but it probably emerged when malaria was endemic and agriculture was established as a major resource for human living. The appearance of the sickle cell mutation at high frequency needed a selection factor such as malaria which arose about 3000 years ago (47). Agriculture favoured large sedentary populations and the requirement for irrigation made suitable conditions for the *Anopheles* mosquito reproduction by making still-water ponds. The Malaysian agricultural system that reached Africa via Madagascar from South East Asia, 2000 years ago, brought new crops that increased the population of West and East Africa, but at the same time it accelerated the spread of malaria.

It is postulated that some of the sickle cell mutations in Africa occurred between 2000-3000 years ago and the sickle cell mutation which is linked to Arab-Indian haplotype might have occurred more than 3000 years ago.

4.1.1. Unicentricity and multicentricity theories

There are two theories supporting the origin of sickle cell mutation which are described below. However, the different sickle cell haplotypes now show that the sickle cell mutation has occurred as a result of many independent events.

4.1.2. Unicentricity mutation theory

This theory postulates that the sickle cell mutation occurred in the Neolithic times when climatic changes caused the desertion of the fertile Arabian Peninsula leading to migration of haemoglobin S gene carries to India and Equatorial Africa. This theory was supported by the geographical distribution of the sickle cell gene in Africa which showed a decline in sickle cell gene frequency from East to West Africa (48,49).

This theory does not explain the DNA polymorphic sites have coinherit with sickle cell gene.

4.1.3. Multicentricity mutation theory

This theory is supported by the coinheritance of polymorphic DNA sites with haemoglobin S gene. If the sickle cell mutation occurred only once as proposed by the unicentricity theory, all haemoglobin S gene carriers would be associated with the same beta globin polymorphic sites. The multicentricity theory suggests that the haemoglobin S gene could be linked to a different haplotype every time a new mutation occurred.

It is now known that there are five sickle cell haplotypes - Benin, Senegal, Bunta, Carmeroon and Arab-Indian (Fig. 8). Each region has a characteristic haplotype which indicates that sickle cell mutation has occurred as a result of several independent events (50,51).

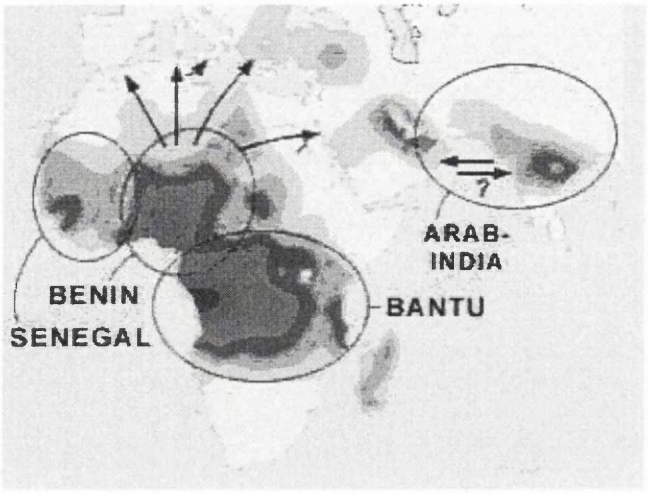


Figure 8 Sickle cell haplotypes and their spreading over time

The arrows indicating spread of Benin sickle cell haplotypes spreading to North Africa, Mediterranean and Arab peninsula. It shows also the Arab-Indian haplotype spreading between East of Saudi Arabia and India (139).

4.1.4. Sickle cell haplotypes

Polymorphic DNA sites within the beta gene cluster between the ε gene and β gene (Fig.9) have very high probability of coinheritance with mutations of haemoglobin S gene. These polymorphic sites as a set are called haplotypes. Results of studies of DNA polymorphisms linked to the beta S-gene suggest that it arose from at least five independent mutations: four in Africa and another in Asia (50,51).

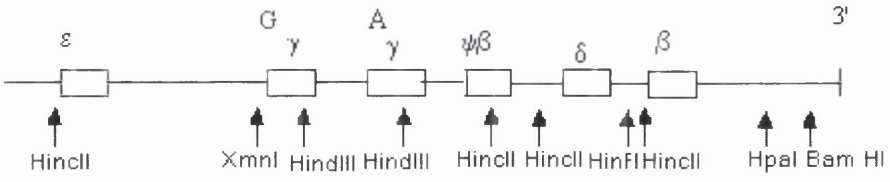
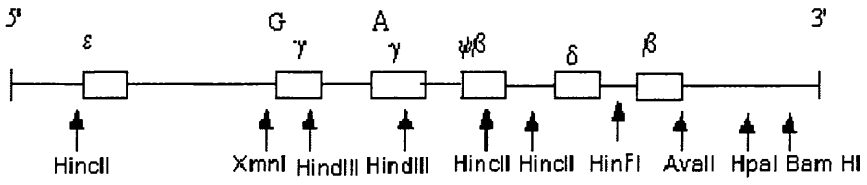


Figure 9 DNA polymorphic sites on the beta globin cluster

The chromosomal backgrounds or haplotypes of these mutations (Fig.10) are known as the Senegal haplotype, Bantu haplotype, Benin haplotype, Cameroon haplotype and Arab-Indian (Asian) haplotype.



Benin	-	-	-	-	-	+	-	+	-	+
Bantu	-	-	+	-	-	-	-	+	+	+
Senegal	-	+	+	-	+	+	+	+	+	+
Cameroon	-	-	+	+	-	+	+	-	+	+
Arab-Indian	+	+	+	+	-	+	−	+	+	-

Figure 10 DNA polymorphisms on the β globin in sickle cell haplotype

1) **The Senegal haplotype**

It is common in the Atlantic West Africa region including Senegal, Gambia, Guinea, Sierra Leone and parts of Ivory Coast. It is characterised by polymorphisms in the 5'flanking region of the G gamma globin gene at the positions –1280, -1225, -1067, –807, and specific polymorphisms at positions –535 and –534 (52). It shows absence of *Hind* II/ε and *Hind* III/$^{A}\gamma$ and presence of *Xmn* I, *Hind* III/$^{G}\gamma$, *Hinc* II/3'φβ and *Hinf* I/5'β polymorphic sites.

2) **The Bantu haplotype**

It is common in the Bantu-speaking region of equatorial and southern Africa, and is also seen in Kenya, Zimbabwe, Namibia, Zambia and Malawi. It shows a gene conversion 5' to the A gamma-globin gene of a beta chromosome starting between bases –307 and –271 and extending to between 25 and 1107.

It is characterised by absence of *Hind* II/ε, *Xmn* I, *Hind* III/$^{A}\gamma$, *Hinc* II/3'φβ and *Hinf*I /5'β and presence of *Hind* III/$^{G}\gamma$ polymorphic sites (53).

3) **The Benin haplotype**

It is common in the central west of Africa including Mali, Niger, Burkina-Faso, Ghana, Togo, Benin, and Nigeria. It is also found in Central, West and North Africa, Spain, Portugal, Sicily and southern mainland Italy, Greece (Macedonian), Turkey and north-western Saudi Arabia. It is also common in the Arabian Peninsula, except the eastern part of Saudi Arabia, and this reflects the past history of the Arab slave trade. It shows that the 5' flanking region of the G gamma-gene has two specific polymorphisms at positions –369 and –309 linked to the beta S gene, also another one at –657 (54). The Benin haplotypes is recognised by absence of *Hind* II/ε, *Xmn* I, *Hind* III/$^{G}\gamma$, *Hind* III/$^{A}\gamma$ and *Hinf*I/5'β and lack of *Hinc* II/3'φβ polymorphic sites (53).

4) **The Cameroon haplotype**

It is found among Eton people in southern Cameroon. It is linked to the A and T gamma globin. It shows absence of *Hind* II/ε and *Xmn* I, and presence of *Hind* III/$^{G}\gamma$, *Hind* III/$^{A}\gamma$, *Hinf*I/5'β and *Hinc* II/3'φβ polymorphic sites (53).

5) **The Arab-Indian (Asian) haplotype**

It is associated with other foci in the eastern Saudi Arabia and some parts of southern and central India. This haplotype is characterised by the presence of the –158 (C→T) polymorphism 5' to the G gamma-globin gene and this explains the higher G gamma levels seen among carries. It is recognised by

presence of *Hind* II/ε, *Xmn* I, *Hind* III/$^{G}\gamma$, *Hinc* II/3'φβ and *Hinf*I/5'β but absence of and *Hind* III/$^{A}\gamma$ polymorphic sites (53).

6) **Atypical sickle cell haplotypes**

Atypical sickle cell haplotypes often come from recombination between sickle cell haplotypes common in the sickle cell anaemia and haplotypes unusually linked to the β^{s}-globin gene.

It is seen in less than 10% of Bantu speaking African and Senegalese.

4.1.5. Flow of the African HbS gene to North Africa and Arabian Peninsula

About 4000 year ago, the people of Africa comprised three main racial groups living in sub-Saharan Africa: Negroes in West Africa, Pygmies in equatorial Africa and Bushmen in southern Africa. Semitic and Hamitic groups lived in North Africa. About 2000 years ago, the negroids of West Africa became the main power group. The expansion required for agricultural land and development of weapons led to invasion and migration of populations living in the margins of the Benur River in eastern Nigeria. By using the rivers they expanded in all directions (55). Slavery was common in Africa and the slavery structure facilitated the flow of genes in West Africa since the slaves were mainly prisoners of war and the descendants were free and accepted in new societies.

The haemoglobin S gene linked to the Benin haplotype is found in North Africa (Morocco, Algeria, Tunis, Egypt) (56). It arrived from central West Africa through the ancient north-south trans-Saharan routes. The Benin haplotype is also found in the western part of Saudi Arabia. The flow of this

gene from central West Africa to the western part of the Arabian Peninsula was probably through North Africa during the Arab slave trade. Except for the eastern region of Saudi Arabia, the haemoglobin S gene, which is associated with the Benin haplotype, is found in the rest of the Arabian Peninsula.

4.1.6. Flow of the Hb S gene linked to the Arab-Indian haplotype

In India, the haemoglobin S gene is almost restricted to tribal groups living in the central and southern India. All these tribal population have the same sickle cell haplotype. The ancestral home of these tribes might have been the margins of the Indus River, which is close to other population that have the same sickle cell haplotype such as the population in of the eastern part of Saudi Arabia (56).

4.2. Balanced polymorphism of the haemoglobin S gene

Homozygous sickle cell anaemia patients often die during childhood reducing the prevalence of haemoglobin S gene, while in sickle cell trait the haemoglobin S gene is preserved and propagated. This results in balanced haemoglobin S gene frequency.

4.3. Haemoglobin S and malaria

People vary in their susceptibility to malaria infection depending on genetic factors and acquired immunity. Genetic resistance to Plasmodium falciparum occurs by impairment of merozoite invasion or intracellular growth or erythrocytic lysis preventing merozoite maturation. There are four species of Plasmodia causing malaria in humans. These are Plasmodium falciparum, Plasmodium ovalae, Plasmodium vivax and Plasmodium malariae. The deadly

species is Plasmodium falciparum, which often kills people in childhood due to cerebral malaria. Sickle cell trait has some protection against malaria in early childhood.

The Bantus who lives north of the Zambezi River, an endemic malaria area, have a significant incidence of sickle cell disorder, while the Bantus in the free malaria area in South Africa have no significant incidence of sickle cell disorder. Sickle cell trait carriers have a partial resistance against malaria. This is believed to be due to:

1. High sickling rates of infected cells that are then removed by the spleen reducing their life span (57).
2. In the deep vascular schizogony, infected sickle cells become static by adhering to venule endothelium and become highly deoxygenated which decreases the malaria parasite growth (58).
3. Increased haemoglobin F concentration for a longer time in childhood, since the growth of Plasmodium falciparum is minimised in red cells containing haemoglobin F in a relatively deficient medium of reduced glutathiond (58).

4.4. Geographical distribution of sickle cell disorder

4.4.1. World-wide distribution of sickle cell

Estimates made for the World Health Organisation in 1995 showed that 6% of the world's population carry a haemoglobin disorder, and 7% of children born today are carriers. The world wide figure for sickle cell and thalassaemia showed that about 2.5 million patients would have a thalassaemia syndrome, and about 10.5 million patients would have a sickle cell disorder (Table 5) (25).

Table 5 Proportion of the world population carrying Hb S

Region	Population (million)	% Hb S
Africa	664	10
Americas	734.2	1.4
Asia	3,143	0.6
Europe	633	0.12
Oceania	27	0
World	5,202	1.86

The frequency of Hb S differs largely with geographic places and racial groups. Its prevalence is influenced by the occurrence of the sickle cell mutation and its selection by falciparum malaria. Haemoglobin S reaches its highest incidence in equatorial Africa particularly, in a broad zone extending from coast to coast reaching a carrier state prevalence in some places as high as 40 - 50% (59,60).

Haemoglobin S is also found in non-African populations such as in parts of Sicily and Southern Italy, Northern Greece, Middle East, especially in Southern Turkey, eastern part of Saudi Arabia and much in Central and South India (59,61).

Haemoglobin S is frequent amongst any black population with roots in Africa such as the Caribbean, North and South America and many inner city areas of Northern Europe.

The literature resources show different figures of the prevalences of Hb S gene in the developing countries and this maybe due to either small sample studies, hospital based research (certain groups), or estimated figures.

Table 6 shows the prevalence of Hb S in different countries of the world (12,59,62, 25) including Yemen (1-2%) (Fig11) (63). The prevalence of the Hb S in Yemen is an estimated value, which may not precisely reflect the accurate prevalence of Hb S in the country and does not show the distribution of Hb S across the country. This is lower than the prevalences of Hb S in Syria, Jordan, Arabs in Palestine, Iraq, Saudi Arabia, Kuwait, Bahrain, Oman and United Arab Emirates but it is higher than prevalences of Hb S in Lebanon, Iran and Jews in Israel. The prevalence of Hb S in Yemen is much lower than that in West Africa but slightly higher than the prevalences in the Horn of Africa countries (Ethiopia, Djibouti and Somalia).

Table 6 Prevalence of Hb S in countries of the world

(12,59,62, 25)

	% Prevalence of Hb S (%)	Country	Prevalence of Hb S (%)
Senegal	3 – 15	Ethiopia	0 – 1
Gambia	6 – 28	Djibouti	0
Guinea Bissau	<1 – 25	Somalia	0
Guinea	13 – 33	Jamaica	3.5 – 12
Sierra Leone	22 – 30	Bahamas	14
Liberia	<1 – 29	Cuba	0 – 23
Ivory Coast	2 – 26	Haiti	7 – 17
Mali	5 – 17	Dominican	6 – 12
Burkina Faso	2 – 34	Puerto Rica	<1 – 8
Ghana	3 – 25	Lesser Antilles	1 – 14
Togo	6 – 28	Guadeloupe	4.4
Benin	5 – 31	Mexico	<1 – 9
Niger	5 – 23	Guatemala	<1 – 17
Nigeria	10 – 41	Belize	0 – 25
Gabon	8 – 32	El Salvador	<1 – 2
Cameroon	<1 – 31	Honduras	<1 – 16
Central African Republic	2 – 24	Nicaragua	0
Congo	7 – 32	Costa Rica	<1 – 8
Democratic Republic of Congo	1 – 46	Panama	0 – 21
Kenya	<1 – 34	Colombia	0 – 15
Uganda	1 – 39	Venezuela	0 – 9
Tanzania	1 – 38	Guyana	<1
Rwanda Tutsi Hutu	 <1 – 5 5 – 15	Surinam	0 – 22
Burundi	1.5- 26	French Guyana	0 – 18
Angola	8 – 40	Ecuador	0
Zambia	<1 – 30	Peru	<1
Zimbabwe	<1 – 11	Bolivia	0
Malawi	3 – 18	Brazil	0 – 16
Mozambique	<1 – 40	Paraguay	0
Madagascar	<1 – 23	Argentina	<1
Botswana	<1	Uruguay	0
Namibia	0 – 15	Chile	<1
South Africa Bantu Indian Cape Coloured	 <1 – 4 2 – 10 <1	Greece	0 – 32
Morocco	<1 – 7	Turkey	<1 – 34
Algeria	<1 – 15	Cyprus	<1
Tunisia	< 1 – 2	Italy	

		Sicily Sardinia Mainland southern	<1 – 13 0 0.5 – 1
Libya	<1 – 70	Portugal	<1 – 5
Egypt	<1	India	0 – 35 (in some tribes)
Sudan	<1 – 17	Pakistan	< 1
Countries of Arabian Peninsula			
Syria	<5 – 25	Emirates	2
Lebanon	<1	Saudi Arabia	<1 – 36 (Eastern region)
Jordan	4 – 6	Kuwait	2
Arabs in Palestine	1 – 38	Bahrain	2.5
Jews in Israel	0	Oman	5
Iraq	0 – 25	Yemen	1 – 2

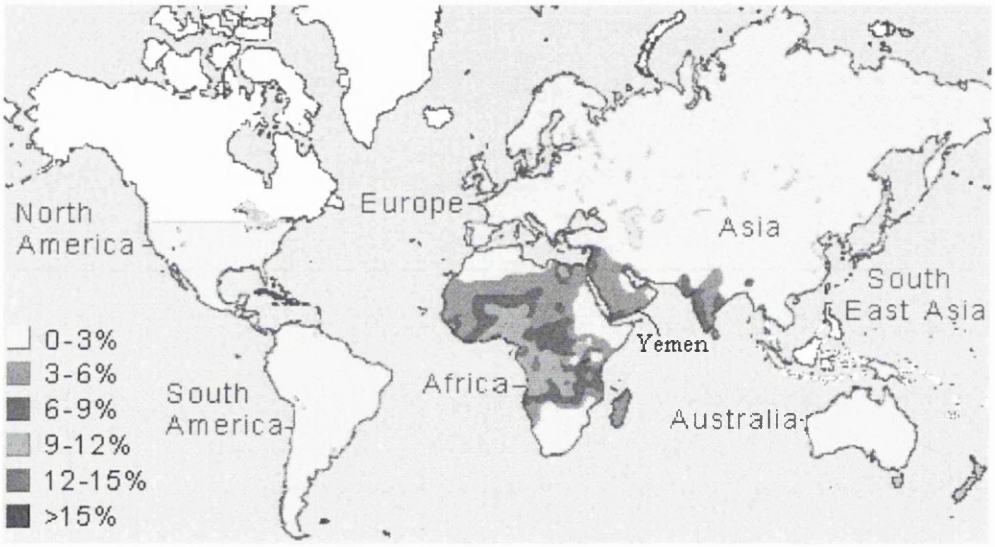


Figure 11 Geographic distribution of Hb S

4.4.2. Haemoglobin S in Yemen, Arabian Peninsula and Horn of Africa

4.4.2.1.a. Haemoglobin S in Yemen

Published studies addressing the prevalence of the sickle cell gene in Yemen are very few and have been done on Yemeni immigrant populations living in other countries such as the United Arab Emirate. So far no indigenous Yemen population study discussing the prevalence of sickle cell gene has been published. Therefore, the information resources regarding the prevalence of

sickle cell gene in Yemen are very limited and the prevalence of the Hb S in Yemen is an estimated value which may not be accurate. As seen in Table 7 (62), it is higher than the prevalences in Horn Africa countries (Ethiopia, Djibouti and Somalia), but lower than in many West Africa countries (Table 6).

White (1983) reported the percentage of Hb S in Yemen is 1.3 which indicates the Hb S gene frequency was 0.0065. In his study, the blood samples were obtained from 651 Yemeni patients attending hospitals in United Arab Emirates. Solubility test was used to detect Hb S. In this study, the author did not indicate the origin of the population studied, but the prevalence of sickle cell gene in the eastern part of Saudi Arabia is higher than the western part. Table 7 shows the percentage of Hb S in Saudi Arabia, Yemen, Oman, and Abu Dhabi nationals found in White study (62).

Table 7 The percentage of Hb S in some Arabian Peninsula countries

Country	Number of the Samples	Number of the positive	%
Saudi	230	13	5.8
Yemen	651	9	1.3
Oman	724	37	5.1
Abu Dhabi nationals	1291	29	2.3

In a study using 5060 blood samples obtained from individuals routinely attending the Corniche Hospital, Abu Dhabi, United Arab Emirates, White et al. (1986) found that alpha thalassaemia was the most common disorder followed by beta thalassaemia and Hb AS in Oman, Yemen and United Arab Emirates. The sickle cell trait frequencies were in Oman: 0.038; the United

Arab Emirates: 0.019; Yemen: 0.0095 (Table 8.a). Therefore the Hb S gene frequency in Yemen is 0.0048 (Table 8.b) (64).

Table 8.a Frequency of α, β thalassaemia, G6PD and sickle cell trait in Oman, UAE and Yemen

	Oman	UAE	Yemen
Alpha thalassaemia	0.389	0.165	0.065
G6PD	0.328	0.087	0.062
Beta thalassaemia	0.024	0.017	0.024
Sickle cell trait	0.038	0.019	0.0095

Table 8.b Frequencies of sickle cell gene in Yemen, UAE and Oman

Countries	Total Number	Positive Samples	Hb S gene frequency
Yemen	1260	12	0.0048
UAE	2750	52	0.0095
Oman	1050	40	0.019

Both studies by White (1983 and 1986) show two slightly different rates of prevalence of the sickle cell gene in Yemen. White's (1983) study showed that among 651 individuals from Yemen, 9 had sickle cell trait and this indicated the frequency of sickle cell gene in Yemen was 0.0065 while the White et al. (1986) study among 1260 Yemeni individuals, showed 12 had sickle cell trait and this indicated the frequency of sickle cell gene was 0.0048.

In 1994, the World Health Organisation published an estimate of haemoglobin disorder (Table 9). The prevalence of heterozygotes haemoglobin disorder including Hb S in the Democratic Republic of Yemen (former South Yemen) was 7% and 2.45 homozygous births/1000. Regarding to former North Yemen, Arab Republic of Yemen, the prevalence of heterozygous haemoglobin disorder including Hb S was 6%, and 1.8 homozygous births/1000. The

percentage of annual births of homozygotes with Hb SS was 55%, and Hb S /thalassaemia was 17% in both former South and North Yemen (25).

Table 9 WHO estimates of Hb S in Yemen

Country	Population (Million)	Birth rate /1000	Homozygous Birth/1000	Heterozygotes %	% Annual Births of homozygotes	
					SS	S/Th
South Yemen	2.76	47.3	2.45	7.0	55	17
North Yemen	9.55	53.6	1.8	6.0	55	17

In a WHO report of the estimates of frequency of haemoglobin disorders in countries of the Eastern Mediterranean Region (World Health Organisation, 1997), the prevalence of Hb S in Yemen is 4%, annual number born with homozygous Hb SS is 864, prevalence of beta thalassaemia is 2%, and the annual number born with compound of Hb S and beta thalassaemia is 267 (14). This report shows the prevalence of Hb S as being higher than that reported by Serjeant (1992), Livingstone (1985), and White (1983, 1986) but lower the estimated prevalence by WHO (1994) (25) (Table10).

Table 10 Summary of studies of Hb S prevalence in Yemen

Studies	Method of analysis	Samples No	% Hb AS
White (1983). The approximate gene frequency of sickle haemoglobins in the Arab peninsula (62).	Solubility Test	651	1.3
White (1986). Red cell genetics abnormalities in Peninsular Arabs: sickle haemoglobin, G6PD deficiency, and alpha and beta thalassaemia (64).	Sickling Cell Test, Haemoglobin-electrophoresis	1260	0.95
WHO (1994). Guideline for the control of haemoglobin disorders (25)		Estimate	7 North 6 South
The Regional Office of the Eastern Mediterranean Countries of the World Health Organisation (1997) (14).		Estimate	4

4.4.2.1.b. Sickle cell haplotyte in Yemen

El-Hazmi and Warsy (1999) studied.the *Xmn* I polymorphism among 30 Yemeni patients with severe sickle cell anaemia resident in Riyadh, Saudi Arabia. The *Xmn* I polymorphic site (C→T) at –158 in the G gamma globin gene promoter is one of several restriction endonuclease polymorphic sites occurring in the beta globin gene cluster on chromosome 11, which is believed to contribute to high level of Hb F in carries. They examined a total of 60 chromosomes carrying the sickle cell gene and 14 chromosomes carrying normal Hb AA (control group). *Xmn* I polymorphism among the control group was positive in 42.9% and negative in 57.1%. The 60 chromosomes from the sickle cell patients showed only one homozygous case for the presence of the *Xmn* I polymorphism while the rest of the chromosomes showed absence of the

Xmn I polymorphism. Thus *Xmn* I polymorphism is present in 3.3% and absent in 96.7% of Yemeni sickle cell patients in the study. The authors noted that this finding resembled the very low prevalence of *Xmn* I polymorphism in the severe sickle cell patients from Saudi Arabia's south-western region, which is the geographical extension of western region of Yemen (65). In the same study, the *Xmn* I polymorphic site was high (frequency of 0.932) in mild sickle cell patients from the eastern region of the Saudi Arabia. The Hb F levels among these severe sickle cell anaemia Yemeni patients varied between 2 to 20%, but was unrelated to the *Xmn* I polymorphic site or the severity of sickle cell anaemia. There was however a relationship between the severity of sickle cell anaemia and the existence of *Xmn* I polymorphic site (65).

This study investigated the prevalence of the *Xmn* I polymorphic site amongst severe sickle cell anaemia Yemeni patients living in Riyadh, Saudi Arabia, and showed the low prevalence of the *Xmn* I polymorphic site in the severe type of sickle cell anaemia in Yemeni patients, but it did not report the regional origin of the Yemeni patients.

4.4.2.2. Sickle cell haemoglobin in Arabian Peninsula

The countries of the Arabian Peninsula (Fig.12) historically share the language, religion, culture and history. Tribes of this region moved freely and mixed together in the past, but this has become limited since the Second World War.

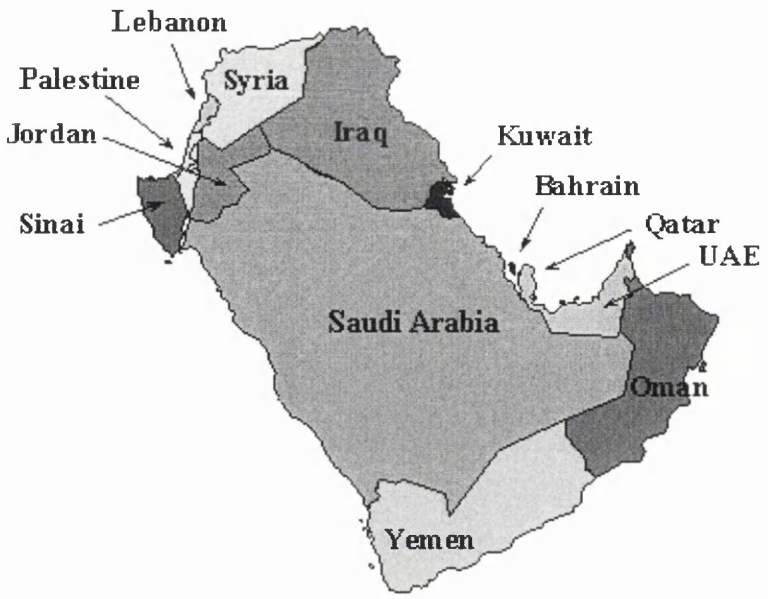


Figure 12 Map of Arabian Peninsula

4.4.2.2.1. Saudi Arabia

Saudi Arabia is located to north of Yemen with a population of 24 million which includes 5.6 million non-nationals in 2003 (138).

The frequency of the sickle cell gene in Saudi Arabia has been investigated using 30,055 blood samples from the different provinces (66). The frequency of the of the sickle cell gene was 0.1446 in the eastern province, 0.0050 in central province, 0.0465 in north-western province, 0.0765 in south-western province, and 0.0065 in northern province. The frequency of Hb S gene was 0.0474 in the country as a whole.

This study showed that the prevalence of Hb S gene frequency was highest in eastern part of Saudi Arabia and the lowest was in the central part of the country. It also showed the prevalence of sickle cell gene in the south west, which is the nearest populated neighbour region and the geographic extension of the west coastal strip of Yemen, which was the second highest (0.0765).

El-Hazmi (1990) studied the beta globin gene haplotypes among the sickle cell anaemia patients from the eastern and western parts of Saudi Arabia. A total of 93 sickle cell anaemia patients were investigated using restriction endonucleases *Hinc* II and *Hind* III. The number of the sickle cell anaemia patients from the eastern and western parts of Saudi Arabia was 22 and 71 respectively. The results of this study showed that all the sickle cell anaemia patients from eastern Saudi Arabia had Arab-Indian haplotype while the Benin haplotype was common (more than 88%) among the sickle cell patients from western parts of the country (67) (which is historically part of the western region of Yemen).

A collaborative group of Arab researchers from Saudi Arabia, Egypt, Syria Arab Republic and Jordan studied 126 sickle cell patients from their countries, and from different provinces of Saudi Arabia (14 Egyptians, 9 Syrians, 10 Jordanians and 22, 67 and 4 Saudis from eastern, western and northern parts of Saudi Arabia respectively). The aim of the study was to examine the effect of genetics on the clinical features of the sickle cell disorder, also to identify the origin of the sickle cell gene. This study showed the Benin haplotype was found in 98.5% of sickle cell patients from south western part of Saudi Arabia, 9% from eastern part of Saudi Arabia, 100% from north western of Saudi Arabia, 100% in patients from Egypt, 66.7% in patients from Syria, and 80% in patients from Jordan. The results of the study suggest that the clinical features of the sickle cell disorder are influenced by the haplotypes of the beta globin gene and there were at least two common foci of the origin of the sickle cell gene, one in the eastern province of Saudi Arabia and another common in North Africa and north-western part of the Arabia Peninsula (68,69). This

confirmed previous findings that the sickle cell haplotype in eastern part of Saudi Arabia is different from the sickle cell haplotype in the western part of the Arabian Peninsula, where Yemen is located, and may be affected by the Benin haplotype since the south western part of Saudi Arabia was until 1934 part of the western of Yemen.

4.4.2.2.2. Oman

Oman lies to the east of Yemen and has a population of 2.8 million in 2003 (138). A national register of haemoglobinpathies has been built to develop the national program for the control of genetic blood disorders. The data was first gathered from hospital records, and then filtered prospectively with information collected by a survey of paediatricians. This national register was planned to determine the distribution of the haemoglobinpathies in different ethnic groups and different parts of the country; incidence of births with haemoglobinpathies; age pattern of patients and carriers of haemoglobinpathies; and determining factors influencing the prevalence of haemoglobinpathies in Oman. In 1995 the register reported 1757 cases of sickle cell anaemia and 243 cases of beta-thalassaemia major in a population of about 1.5 million. It revealed that the prevalence of sickle cell trait in Oman was about 10% while the prevalence of the beta thalassaemia carriers was about 4%. The study also showed that sickle cell anaemia is prevalent in 40% of Omani tribes. Haemoglobin S was mainly found in the north part of Oman where the prevalence was 6-14% while in the middle, and west regions of the country only one case was reported (70).

Both the Dhofar and Wusta regions showed absences of beta thalassaemia and only one case of sickle cell disorder was reported. According to this study the

incidence of live births for homozygous sickle cell anaemia was 2.7 in 1000, and 0.4 in 1000 for beta thalassaemia. Genetic studies for determination of the sickle cell haplotype in 23 tribes in Oman showed 46 Benin, 8 Bantu, 6 Bantu A4 and 3 Arab-Indian. The results of the DNA analysis suggested the sickle cell haplotype in Oman mainly belongs to the African haplotypes (35). Historically, the people of Dhofar (West of Oman) and people of Al-Mahrah (East of Yemen) are descendants from the same tribes and shared the same local language (Al-Amhari) and this may suggest the reason for the same prevalences of both Hb S and beta thalassaemia in both areas.

Baysal (2001) also studied the sickle cell haplotype among 50 Omani sickle cell anaemia patients in his laboratory in the Emirates. The results showed 34% of the cases were Benin haplotype, 24% were Bantu haplotype and 22% were Arab-Indian haplotype (71). These finding support the finding of the national register of haemoglobinpathies in Oman (70).

4.4.2.2.3. United Arab Emirates

United Arab Emirates is part of the Arabian Peninsula, bordering the Gulf of Oman and the Arabian Gulf, between Oman and Saudi Arabia with a population of 2.5 million which includes 1.6 million non-nationals in 2003 (138),

Baysal (2001) reported that prevalence of sickle cell chromosome in United Arab Emirates was as high as 11.4%. The common sickle cell haplotype was the Arab-Indian haplotype with a prevalence of 68% among sickle cell anaemia patients (71).

The high percentage of prevalence of Arab-Indian sickle cell haplotype in Emirates suggests that it has been influenced by the very high prevalence of Arab-Indian sickle cell haplotype in the eastern region of Saudi Arabia.

4.4.2.2.4. Bahrain

Bahrain is an island in the Arabian Gulf, east of Saudi Arabia with a population of 667,238 which includes 235,108 non-nationals in 2003 (138).

A total of 5503 neonatal samples of Bahraini individuals were investigated for haemoglobinpathies disorders between 1982-1987 (72). Abnormal haemoglobin was identified in 44.35%. Sickle cell trait was identified in 18.1%, sickle cell anaemia in 2.1% and alpha thalassaemia in 24.2%.

Molecular studies to identify the sickle cell haplotype in Bahrain had been carried out among 19 families, which showed the Arab-Indian haplotype was common to all the families.

The predominance of the Arab-Indian sickle cell haplotype in Bahrain may be due to the influence of the Arab-Indian haplotype that is present in eastern part of Saudi Arabia.

4.4.2.2.5. Kuwait

Kuwait is bordering the Arabian Gulf, between Iraq and Saudi Arabia with a population of 2.2 million which included 1.3 million non-nationals in 2003 (138).

Adekile et al. (1996) studied the sickle cell haplotype in Kuwait. Of 125 sickle cell homozygote patients, the Arab-Indian haplotype was found in 80.8%, the Benin haplotype in 11.2%, the Bantu haplotype in 5.7%, and the atypical haplotype in 2.4% (73).

The high prevalence of the Arab-Indian haplotype suggests the influence of Arab-Indian haplotype that exists in the eastern part of the Saudi Arabia.

4.4.2.2.6. Lebanon

Lebanon is bordering the Mediterranean Sea, between Palestine and Syria with a population of 3.7 million in 2003 (138).

Inati et al. (2003) studied the sickle cell haplotype in Lebanon. Fifty sickle cell anaemia Lebanese patients were studied using the restriction sites (*HincII*, *Xmn* I, *Hind* III, *Hinc* II, *Hinf* I, *Ras* I, *Ava*ll). The results identified the Benin haplotype in 60%, a mixture of both the Benin and Cameroon haplotype in 22%, a mixture of both the Benin and Senegal haplotype in 4%, the Cameroon haplotype in 4% and the Arab-Indian haplotype in 10% (74).

Thus the Benin haplotype is a common haplotype in Lebanon while the Arab-Indian haplotype is uncommon. The location of Lebanon suggests that the Benin sickle cell haplotype arrived in the western region of the Arabian Peninsula through North Africa.

4.4.2.2.7. Iraq

Iraq borders the Arabian Gulf, between Iran and Kuwait and to the north east of Saudi Arabia with a population of 24.7 million in 2003 (138).

The prevalence of the Hb S gene in the Abu-Al-Khasib, district of southern Iraq, was investigated using the sickling test and cellulose acetate electrophoresis for haemoglobin identification. Two groups were involved in this study. The investigated groups were 706 children (age 10–12 years) randomly selected from a primary school in Abu-Al-Khasib, and a group of 525 school children from Basrah. The results of this study showed that the

overall prevalence of sickle cell disorder was 16% in Abu-Al-Khasib and 2.5% in the Basrah (75). The high prevalence of Hb S among the children of Abu-Al-Khasib may have been influenced by the high prevalence of Hb S in the eastern part of Saudi Arabia, which also has similar effect on Bahrain and Kuwait. The sickle cell haplotype was not investigated in this study.

4.4.2.2.8. Jordan

Jordan lies to the northwest of Saudi Arabia, between Syria to the north and Palestine to the west, with a population of 5.5 million in 2003 (138). The population in Jordan is divided geographically into three distinct sections, all sharing the same Arabic heritage. These are the urban area of the capital, the Bedouin southern and eastern desert provinces and the agriculturally oriented northern sector.

The prevalence of Hb S and beta thalassaemia has been investigated to determine the incidence of the Hb S and beta thalassaemia in three areas of north Jordan (Al-Gor, Ajloun, Irbid). In this study, 2290 individuals and 568 cord blood samples were analysed. Haemoglobin electrophoresis was used for analysis. The overall prevalence of Hb S was 4.45% while beta thalassaemia was found in 5.93%. Among the cord blood samples the prevalence of sickle cell trait was 3.17%. Haemoglobin S and beta thalassaemia showed a higher prevalence in Al-Gor area than Ajloun and Irbid (76).

This study did not investigate the sickle cell haplotype in Jordan but provided sickle cell prevalence in a region linked to the old Arab slave trade road which suggested its responsibility for the movement of the sickle cell gene to the western part of the Arabian Peninsula.

The prevalence of Hb S in Jordanian newborns has been studied (68). A total of 181 cord blood samples from neonates born at Princess Basma Teaching Hospital, Irbid, Jordan were analysed using haemoglobin electrophoresis. The frequency of sickle cell trait was 4% among females and 6% among males (68). The result of this study supported the findings of Sunaa et al. (1996) (77).

4.4.2.2.9. Israel

A survey of the various haemoglobinpathies in Israel in 1983 concluded that the patients with thalassaemia often originated from Kurdistan, Yemen and Iraq, while the sickle cell gene came mainly from Arabic Muslims (mostly from northern Israel) or Bedouins who originally immigrated from southern Sudan, northern Iraq or Libya (78). This study did not report the number of investigated samples and prevalence of the sickle cell gene among the Yemeni immigrants to Israel.

All these studies from the Arabian Peninsula countries strongly suggest that the sickle cell gene is present in all the countries but in varying proportions. There is a very high prevalence in the eastern region of Saudi Arabia with a predominance of the Arab-Indian haplotype. The African sickle cell haplotype is predominant in the western region of the Arabian Peninsula.

4.4.2.3. Haemoglobin S in the Horn of Africa (Ethiopia, Somalia)

Countries of Horn of Africa are separated from the west of Yemen by the Red Sea. These countries are Ethiopia, Eritrea, Djibouti and Somalia. Very limited resources can be cited regarding the sickle cell haemoglobin in these countries. The WHO, Country Estimates of Prevalence of Haemoglobin Disorders (1994)

indicates the percentage of population heterozygous for Hb S in Ethiopia and Somalia as zero (25). The absence of the sickle cell gene suggests that the Hb S gene has not been transported to Yemen from these countries.

5. CLINICAL COURSE OF SICKLE CELL DISORDER

5.1. Clinical features of sickle cell disorder

Haemoglobin SS usually produces a severe anaemia with a wide spectrum of manifestations. It is characterised by acute, recurrent, and chronic complications affecting many organs and tissues. It is distinguished from other haemolytic anaemia by the vasculitic features.

Clinical features may be divided into those that are acute and episodic (crises) and those that are chronic and unremitting (20) (Table 11).

Table 11 Sickle crisis and organ damage

Vaso-occlusive crises	Haematological crises	Chronic organ damage
Hand and foot syndrome	Aplastic crises	Growth and development disorders
Bone and joint crises	Sequestration crises	Bone and joint disease
Abdominal crises	Megaloblastic crises	Cardiovascular disorders
Central nervous crises	Haemolytic crises	Pulmonary disorders
Acute chest syndrome	Infections crises	Hepatobiliary disorders
		Genitourinary disorders
		Ocular disorders
		Leg ulcers

Some features of the natural history of sickle cell anaemia

1. Haemolytic anaemia is apparent in infants by 12 weeks of age.

2. Splenomegaly is first noted after six months of age.

3. The first vaso-occlusive crisis is seen between 6 – 12 months in about half the subjects and before 6 years by the vast majority (79).

4. Dactylitis and acute splenic sequestration account for more than 80% of initial symptoms in the first year of life (80).

5. Stroke is a catastrophic complication that affects 6 – 17% of children and young adults (81).

6. Infection is the major presenting manifestation of sickle cell anaemia in early childhood, and the most common complication requiring hospitalisation. It is the most frequent cause of death at all ages (20).

7. Although normal at birth the heights and weights of children are significantly delayed by 3 – 6 years of age.

Recently Berchel and his colleagues had proposed four periods for the natural history of sickle cell anaemia (82):

1. The neonatal period which is asymptomatic.

2. The first five years of life, recognised by a high risk of mortality, a high level of morbidity due to severe infectious crises of acute anaemia, and painful crises typical to that age-group.

3. Older children and adolescents, whose life is dotted with painful crises. It is in this period that degenerative tissue pathology begins.

4. In adulthood, the acute crises are less frequent but multiple complications develop which affect the prognosis.

5.2. Laboratory features of sickle cell anaemia

5.2.1. Blood count

At birth, blood count is normal. As Hb F decreases, the total haemoglobin concentration decreases and the reticulocyte number increases. Usually the haemoglobin level in adults is between 5 to 11 g/dl. With high Hb F percentage, the mean corpuscular volume (MCV) appears to be increased and the mean corpuscular haemoglobin concentration (MCHC) may be mildly increased. The red cell distribution width (RDW) is usually high and correlates with the severity of the disease. The number of nucleated cells is increased due to the high number of circulating erythroblasts. The number of white blood cells is usually high especially during crises. Increased platelet count and large platelets are frequently seen. Both of these platelets abnormalities are due to hyposplenism (83,84).

5.2.2. Blood film

At birth, the blood film is normal. At 6 months of age, a few sickle cells and target cells are generally seen. After the first year, sickle cell, nucleated cells and Howell-Jolly bodies are commonly seen due to hyposplenism. In adults, the blood smear shows a variable number and shape of sickle cells. Patients with high Hb F show much less blood film abnormalities (83) (Fig. 13).

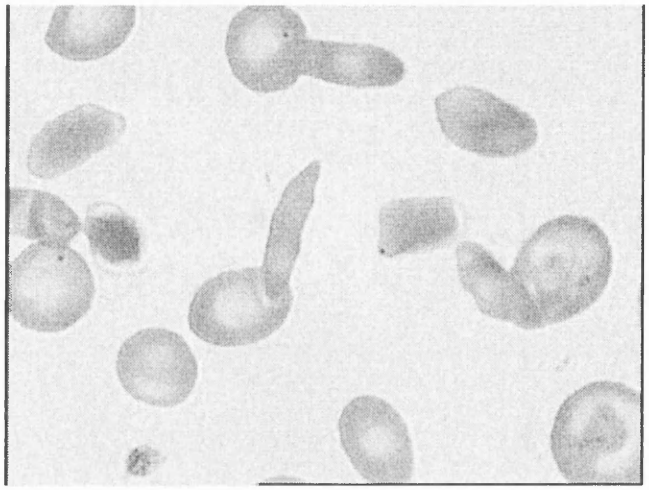


Figure 13 Sickle cell picture

5.2.3. Haemoglobin F percentage

Haemoglobin F in sickle cell anaemia is generally between 5 and 10%. In some cases may rise to 40 %. The level is higher in infancy, and women tend to have higher levels of Hb F than men (84).

5.3. Risk factors for early death of sickle cell patients

The high number of white blood cells has been recognised to be a risk factor for stroke, adverse outcomes and early death (85). A history of acute chest syndrome, renal failure, seizures, dactylitis and haemoglobin concentration less than 7g/dl are also prognostic factors of adverse outcomes and early death.

5.4. Sickle cell anaemia and pregnancy

In pregnancy, sickle cell anaemia increases the incidence of pyelonephritis, pulmonary infarction, pneumonia, acute chest syndrome, antepartum haemorrhage, prematurity and fetal death. The cause of neonatal death is unclear, but it may result from vaso-occlusion of the placenta. A high maternal

mortality rate in sickle cell anaemia (33%) has been reported in some parts of the world (86).

Among 297 Jamaican pregnant women with sickle cell anaemia, the rate of spontaneous abortion was 11.8%, stillbirth was 12.8% and prenatal mortality was 17.1% (87). It was reported that in 61 pregnant women with sickle cell anaemia in Al-Hassa in the eastern part of Saudi Arabia, only one abortion occurred suggesting a mild clinical expression of sickle cell disorder in east of Saudi Arabia (88). The maternal mortality rates reported in West Africa and black America was 11.5% (89), which was much higher than in Al-Hassa.

5.5. Heterogeneity of sickle cell anaemia

The expression of sickle cell disorder severity depends on the inheritance of other beta globin genes with Hb S gene (haplotypes), HPFH and alpha thalassaemia, but it is also influenced by many environmental factors such as climate, infections, nutrition, medical care and age.

5.5.1. Genetic factors

5.5.1.1. Sickle cell haplotypes

The increase of Hb F levels not only reduces the Hb S concentrations in the red blood cell but also inhibits its polymerisation due to its high affinity to oxygen. There is a relationship between the sickle cell haplotype of the chromosome containing the beta^S globin gene and Hb F level. The Arab-Indian haplotype is often combined with Hb F levels of 10-25%. The Senegal haplotype is combined with high Hb F at around 7-10% in adults. Bantu and Benin

haplotypes have lower levels of Hb F, around 6-7%. The Cameroon halpotype shows the lowest level of Hb F (90).

The relation between the haplotype and Hb F level seems to result from a link between haplotype and determinants of non-deletional hereditary persistence of fetal haemoglobin.

The percentage of Hb F depends on factors related and unrelated to the beta globin gene cluster, age, and sex (mildly higher in females). DNA sequences influencing the Hb F level include (91,143,144,145): the *Xmn*I polymorphism at –158 (C→T) G-gamma globin gene (being linked with a higher Hb F); difference of the number of repeats of particular motif in a sequence determined hypersensitive site (HS2) within the locus control region (LCR) of the beta gene [$(AT)_xN_{12}GT(AT)_y$]; a *trans*-acting locus at 6q23: a *trans*-acting locus at Xp22.2-22.3.

The method by which the polymorphism in the LCR at –530 bp to the G-gamma gene control gamma chain synthesis seems to be that, in comparison with $(AT)_7$ T_7, the $(AT)_9$ T_5 sequence appears elevated binding of Bp-1 (negative trans-acting factor (146).

The Arab-Indian and Senegal haplotypes are associated with the common –158 (C→T) G-gamma globin gene *Xmn* I polymorphism whereas other haplotypes are not associated with this polymorphism. The *Xmn* I polymorphic site (-158) 5' to G gamma is one of several restriction endonuclease polymorphic sites occurring in the beta globin gene cluster on chromosome 11, which is believed to contribute to high level of Hb F in carriers (91). Another polymorphism at A-gamma globin IVSII, which is associated with high fetal haemoglobin is seen when the β^S gene is linked with the Senegal and Arab-Indian haplotypes

(92). The IVSII sequences seem to be important in the gamma globin expression in stably transfected human erythroleukaemia cell (K562). This region was investigated in the five sickle cell haplotypes. The $^{A}\gamma$ IVSII gene has many differences among haplotypes and the Benin haplotype. Between bases 514 and 1159, there is a mixture of polymorphisms, but the four bases after position 743 are unique. The existence of the sequence 10(TG) 4(CG) 7(TG) in the repetitive sequences beginning at position 1062, which is specific for Bantu haplotype, but different for the sequence 13(TG) shared by the Senegal and Arab-Indian haplotypes. Also four specific polymophic sites at 1272, 1203, 1207 and 1208 (TGGG→GCAA) also recognize the Benin haplotype.

The Arab-Indian haplotype is also linked to a polymorphism at –530 bp resulting in high affinity for BP-1 (a negative trans-acting factor) and lowering of β^{S} production. In the Arab-Indian haplotype sickle cell anaemia, the fetal haemoglobin level is higher than that in sickle cell anaemia with the Senegal haplotype and this may be due to the effect of –158 C→T G-gamma globin gene, A-gamma globin IVSII and –530 bp polymorphisms.

5.5.1.2. Persistence of fetal haemoglobin

Individuals with a combination of non-deletion hereditary persistence of high fetal haemoglobin (HPFH) and sickle cell trait usually show no symptoms and may show very minimal increases in Hb F level. Carriers with compound heterozygosity for Hb S and gene deletion HPFH have a milder disorder than in sickle cell anaemia with 20-30% Hb F.

The level of Hb F that modifies the complications of sickle cell disorder may depend on the population group. Among North American blacks, Hb F levels of 10% or more modify the severity of the sickle cell disorder while a higher level of Hb F (22-26.8%) could contribute to the mild sickle cell disorder in eastern part of Saudi Arabia and India.

Inati et al (2003) in Lebanon reported that 4 patients with a haemoglobin F concentration greater than 15% had severe sickle anaemia (74). Seltzer et al. (1992) found that in 5 black families with high levels of Hb F (19-45%), 8 people had sickle cell anaemia and 2 of them had moderately severe anaemia. These 2 individuals had Hb F levels of 25% and 31%. Two other patients had Hb F levels of 19%. One of these patients had mild disease while the other had severe symptoms (93).

The haemoglobin F levels required to modify sickle cell disorders is a key question as to why different studies supply varying answers. The average level of Hb F in the blood is important as well as the distribution of Hb F between the cells. Uneven Hb F distribution indicates that some cells will have none of the protective Hb F. These cells would be prone to sickling, and could occlude the microcirculation.

5.5.1.3. Alpha thalassaemia

Alpha thalassaemia affects the cellular changes, haematological parameters and subsequently the clinical picture of sickle cell anaemia as summarised in Table 12 (109).

Table 12 Cellular, clinical and haematological effects of alpha thalassaemia in sickle cell anaemia

Cellular effects	Clinical effects	Haematological values
Reduced Hb S polymer	Increased osteonecrosis	High Hb A_2
Lowered cation exchange	Increased splenic sequestration	Microcytosis
Decreased red cell density	Painful episode	Low reticulocyte
Increased red cell deformability	Fewer leg ulcers	Increase of total Hb
	Fewer cerebrovascular accidents	

5.5.2. Environmental factors

5.5.2.1. Infections

a. Malaria

Although, sickle cell trait does offer a relative resistance to malaria the infection is a major cause of morbidity and mortality in patients with sickle cell anaemia. Malaria is a usual precipitating factor for painful crises (94) and also in causing an additional severe haemolytic anaemia in sickle cell anaemia patients (95).

b. Encapsulated organisms

The early lack of splenic function renders sickle cell patients more vulnerable to be infected with encapsulated organisms such as Streptococcus pneumoniae, Salmonella spp. and Haemophilus influenzae type b. These are common childhood infections which are likely to cause a higher morbidity when combined with sickle cell anaemia. However, immunisation programs should

minimise the infection and improve the outcome of the disorder. The morbidity and mortality from pneumococcal septicaemia may be lowered by prophylactic penicillin during the early childhood. Pneumococcal vaccine can be used at a later age (96,97). Osteomyelitis and salmonella septicaemia can cause death in sickle cell anaemia patients, the incidence of which reflect the frequency of Salmonella carriage in the general population and can be minimised by good hygiene (95,98).

c. Viral infections

Human parvovirus infection can cause aplastic crises (99), however proper observation and intervention may lower mortality.

Protocols for the control of many of these infections are available and the effective implementation of these depends on the efficacy of the health care system.

5.5.2.2. Nutrition

The failure to meet the high nutritional demand of the bone marrow and the high cardiovascular work may increase the morbidity and mortality rate among sickle cell patients.

5.5.2.3. Climate

Exposure of sickle cell patients to extreme weather plays a role may cause painful crises especially in cold wet weather. Hot dry climates may cause dehydration as a risk factor for painful crises. The painful crises are largely a determinant of morbidity in young adults patients.

5.5.2.4. Medical Care

Sickle cell clinics provide special medical services for sickle cell patients and have lowered the morbidity rate of the sickle cell disorder. An important role of these clinics is the advice and education provided to the patients and families on the disorder and how to manage complications (100).

5.5.2.5. Age and secular changes

Prognosis and survival in sickle cell disorders are related to the patient's age. The highest mortality rate occurs in the second six months of life, and then it declines for each individual succeeding year.

5.6. Ameliorating factors of sickle cell disorder in Arabian Peninsula

It has been reported that the severity of sickle cell anaemia in the Arab population of the Arabian Peninsula, especially in the eastern part of Saudi Arabia, is milder than that in the other population groups. It has been reported when Hb F is greater than or equal to 20% of the total haemoglobin, the sickle cell disorder is commonly associated with a milder clinical course (101). This particular association has been seen uncommonely in eastern region of Saudi Arabia and is linked to a haplotype containing the –158 *Xmn* I polymorphic site in the G-gamma globin gene promoter, which is often associated with raised Hb F levels.

In the eastern region of Saudi Arabia, the frequency of alpha thalassaemia is 0.45 (30). The coincidence of alpha thalassaemia trait with sickle cell anaemia reduces most of the clinical symptoms of sickle cell anaemia and is associated with longer survival (102), since alpha thalassaemia affects the haematological

parameters (microcytosis) and cellular changes where it decreases Hb S polymerisation, cation exchange, erythrocyte density and increases erythrocyte deformability.

El-Hazmi (1992) suggested a minor role for Hb F as a modifier of sickle cell disorder severity. In his study, 264 Saudis from different regions with a variety of symptoms associated with sickle cell disorder were evaluated to make a "severity" index. El-Hazmi concluded that among these patients, no correlation existed between Hb F levels and the severity index (103). This questions the correlation between Hb F levels and mild forms of sickle cell course.

5.7. Epidemiology of the clinical picture of sickle cell anaemia

5.7.1. Arabian Peninsula

The haematological features of sickle cell anaemia were studied in 264 children from different parts of Saudi Arabia. The control group comprised normal children from the same parts of the country. Children from eastern Saudi Arabia showed the highest levels of haemoglobin, red blood cells and haematocrit, whilst children in the western regions showed the lowest levels of these haematological parameters. Children from eastern and western regions showed no significant difference in red cell indices and HbA_2 levels. The sickle cell anaemia children showed higher Hb F than the normal control group. Fetal haemoglobin also showed differences in the same region (104). This study clearly reported the two differing clinical courses of sickle cell anaemia in the eastern (mild form) and western (severe form) parts of Saudi Arabia confirming previous studies (105,106).

A total of 71 sickle cell anaemia patients (36 patients from the western Saudi Arabia and 35 patients from Yemen) were studied to characterise sickle cell anaemia in the western Saudi Arabia region. Patients were aged between 1 ½ and 42 years. Mean haemoglobin level was 8.1 g/dl, and 44 of the patients had Hb F less than or equal to 10%. Among them, the incidence of hepatomegaly was 69% and splenomegaly was 54.9%. Most of the sickle cell patients suffered from severe anaemia, respiratory and urinary tracts infections, bone pains and infarcts. The picture of sickle cell anaemia among these patients indicates that sickle cell anaemia in the western part of Saudi Arabia is as severe as in African patients (107).

The authors analysed the patient population as a homogenous group and did not separate them into Saudi and Yemeni patient groups, nor did they report the origin of the Yemeni patients, but the results suggest that the clinical presentation of the sickle cell anaemia in the Yemeni patients may have a severe course.

In Bahrain, 100 children and their parents completed a survey to identify the characteristics of sickle cell disease (72). This survey showed that patients suffered from fever (69%), pain in the hands (59%), pain in the limbs (58%), abdominal pain (56%), pain in the knee (55%), chest pain (36%) and urinary problems (18%). Haematological parameters were studied using 50 sickle cell patients aged between 15 to 50 years. The haematological picture showed 60% of the patients had haemoglobin levels less than 10 g/dl, 57% had packed cell volume less than 30, 64% had mean cell haemoglobin less than 25 pg and 62%

had mean cell volume less than 76 fl. This may suggest microcytosis due to coexistence of the alpha thalassaemia gene. Generally, the clinical presentation of sickle cell anaemia among Bahrainis was mild and the haematological values resemble those seen in sickle cell patients in the eastern province of Saudi Arabia.

Characterisation of sickle cell anaemia among patients from Irbid in Jordan was examined in Princess Badi'a Teaching Hospital, Irbid, Jordan. Forty-one sickle cell anaemia patients were included in this study, 28 boys and 13 girls aged between 1.5 and 21 years. The main clinical feature among them was pallor (62%). A palpable spleen was found in 44% of patients older than 8 years. The clinical presentation of the sickle cell anaemia showed different patterns. The fetal haemoglobin level did not correlate with the severity of the sickle cell anaemia (77).

5.7.2. Africa

The clinical presentation of sickle cell anaemia in Africa is due to a combination of the pathology of the disorder, accelerated by malnutrition, vitamin deficiencies, parasitic infection and extreme dehydration. It has been confirmed that in Africa there is a high frequency of acute anaemia episodes, painful crises and acute chest syndrome (108).

In a screening study in northern Burkina-Faso, no patients with sickle cell anaemia (Hb SS) were identified although the high frequency of the sickle cell trait (110) may indicate the high severity of sickle cell course in Africa.

5.7.3. India versus Jamaica

Kar et al. (1986) compared sickle cell patients from India and Jamaica. The patients from India has a less symptomatic course when compared with Jamaican patients; also their average Hb F levels were more than twice the Jamaican patients (111).

5.8. Survival of sickle cell disorder

The life span of patients with sickle cell anaemia depends on early diagnosis, environmental, educational, social, medical and economical factors. Prognosis and survival are also affected by the age of first presentation. When the condition remains unrecognised, life expectancy is very poor.

Most mortality occurs early in childhood, and declines in subsequent years. Infant mortality in sickle cell patients is high in developing countries in particular and peaks between 1 and 3 years of age (112). In developed countries, the average survival improves as prophylactic and medical care improves.

In a study performed between 1978 and 1988 in the USA, 2542 patients with sickle cell anaemia (age ranging from birth to 66 years at enrolment) were recruited to determine the life expectancy and calculate the median survival. Median survival was 42 years for males and 48 years for female (Fig14) and 50% patients survived beyond the fifth decade (113). In Jamaica, median survival was 53 years for male and 58.5 for female in 3301 sickle-cell patients attending the Jamaican sickle-cell clinic between 1987 and 1996 (102).

The lower survival age of sickle cell anaemia patients in the USA when compared with Jamaica is probably due to the fact that, the effect of defaulted

sickle cell patients on mortality rate was not investigated and precision of survival estimates were not presented in the USA study.

In the eastern part of Saudi Arabia the majority of the patients with sickle cell disorders grow to adult life (112). Padmos, el at (1995) investigated 61 sickle cell anaemia patients from the eastern province of Saudi Arabia with median survival of 24 years. They also investigated 55 sickle cell anaemia patients from the Southwest of Saudi Arabia with median survival of 15 years (54). In Northern Jordan, the median survival was 9 years among the studied group of 41 sickle cell anaemia patients (77) which suggested that the pattern of sickle cell anaemia in this region may have severe presentation.

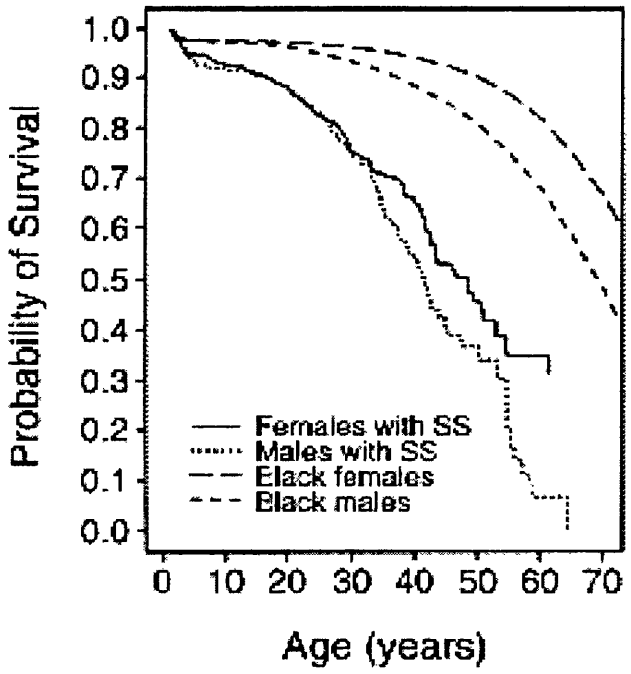


Figure 14 Survival of sickle cell patients in USA

6. SICKLE CELL DISORDER DIAGNOSIS

Sickle cell disorder diagnosis depends on detecting the presence of haemoglobin S.

6.1. Sickling test

This test depends on the morphological changes of Hb S inside the red blood cell when deoxygenated. In this test, one drop of a 2% sodium metabisulphate is mixed with one drop of blood on a microscope slide, then covered and sealed.

6.2. Solubility test

Deoxygenated Hb S is insoluble in the presence of high molarity solution such as a concentrated phosphate buffer and forms a turbid suspension that can be visualised (114).

Both sickling and solubility tests do not differentiate between homozygous sickle cell (SS) and heterozygous haemoglobin S conditions such as sickle cell trait or Hb S/β^+ thalassaemia.

The solubility test is not useful for neonates who synthesis large amounts of fetal haemoglobin, and fails to detect the low concentrations of Hb S that might be present in some varieties of Hb S/β^+ thalassaemia or sickle cell trait with α thalassaemia. It is useful for distinguishing Hb S form other haemoglobin variants that have the same electrophoresis migration.

6.3. Haemoglobin electrophoresis

The alkaline cellulose electrophoresis method is the initial method used. It is an inexpensive method, can be prepared quickly and easily, and produces sharp resolution of haemoglobin bands. Electrophoresis is typically carried out at a pH of 8.6 using cellulose acetate or agar as the support medium. At this pH, the overall haemoglobin molecule is negatively charged and, when placed in an electric field, will move towards the positive terminal (anode). The test is based on the charge change in a haemoglobin molecule due to the replacement of the neutral valine of the negatively charged glutamic acid that results in one net positive charge per chain and two net positive charges per molecule relative to Hb A. Therefore, when Hb S is placed in an electric field it moves more slowly than Hb A towards the positive terminal (anode). In this method, relative percentages of the major haemoglobin bands can be quantified by densitometry or elution and spectroscopy. The cellulose acetate membranes can be fixed and cleared and used as a permanent record of the procedure. In acid agar electrophoresis, most haemoglobin variants move toward the cathode from their point of origin and display different relative mobilities compared with alkaline electrophoresis. Citrate agar electrophoresis can separate some haemoglobin variants that do not separate on cellulose acetate electrophoresis, and can resolve confusion in the detection of many common haemoglobin variants such as Hb G-Philadelphia, Hb D, and Hb Lepore (115)

6.4. Iso-Electric focusing (IEF)

This electrophoretic method utilizes carrier ampholytes, small proteins that are able to carry both current and pH. The ampholytes are incorporated into the

support medium (usually agarose). When a current is applied to the support medium, these ampholytes will gradually establish a pH gradient throughout the gel. When samples are placed on the gel, they will travel to their isoelectric point where migration stops (116). Haemoglobin S tetramers have two more positive charges than Hb A tetramers and migrate more slowly towards the anode. The high resolution of the IEF has made it the method of choice for screening, particularly for neonatal screening. Gels have to be evaluated visually but great skill is needed because many extraneous minor haemoglobin bands are usually present. The quantitation of haemoglobin fractions is difficult.

6.5. High performance liquid chromatography (HPLC)

High Performance Liquid Chromatography generally utilizes a weak cation exchange column. As the ionic strength of the eluting solution is increased, haemoglobin variants will come off of the column at a particular retention time. Amino acid substitutions that are present in the haemoglobin variant will alter the retention time relative to haemoglobin A. HPLC has the advantages that many types of haemoglobin can be isolated, the procedure can often be automated by a microcomputer interface, that can give a reliable interpretation of the chromatogram, and the various haemoglobin fractions are quantifiable. The disadvantages of HPLC are that it cannot always resolve Hb S or Hb C from other variants with the same charge, and the equipment is costly to purchase and maintain (117).

6.6. DNA analysis

The use of molecular biology techniques to study genetic red cell disorders has furthered understanding regarding their molecular basis. The information has been applied to produce prenatal diagnosis programmes by fetal DNA analysis. The discovery of restriction fragment length polymorphisms allowed sickle cell anaemia and beta thalassaemia to be diagnosed in amniocyte DNA and blood. The introduction of PCR in 1985 allowed precise diagnosis of sickle cell anaemia on small amount of DNA (80), but it has the disadvantage of costliness and limited availability.

6.7. Choice of suitable method

Suitable and sensitive methods for analysing small blood samples such as a dry blood spot are high performance liquid chromatography (HPLC) and iso-electric focusing (IEF) (Table 13) (118). Electrophoresis with cellulose acetate can be used but the high dilution of the elute of a dried blood spot may not form clear visible bands. The use of haemoglobin-electrophoresis is less sensitive than HPLC and IEF for detection of low concentrations of Hb A and other haemoglobins especially when their concentration is lower than 4% (118).

In neonates, the sickling solubility test should be avoided because low Hb S concentration may give false negative results.

All abnormal haemoglobins detected by these screening methods should be confirmed by other reliable methods such as IEF and molecular genetic studies.

Table 13 Comparison of laboratory techniques for haemoglobinopathies screening (118,119)

Technique	Disadvantages	Sensitivity	Specificity
Alkaline Cellulose Electrophoresis	Manual	93.1 %	95.2 %
Iso-Electric Focusing	Visual inspection, prone to human error	100 %	100 %
High Performance Liquid Chromatography	HbA_2 level may be inaccurate if Hb S present and less resolution than IEF	99.9 %	99 %

Determination of haemoglobin S and F in a neonate blood sample is usually intended to detect sickle cell anaemia but heterozygosity for Hb S and HPFH gives the same pattern. Follow-up and proper management programmes for affected children are an important part of neonatal screening programme.

6.7.1. Postnatal diagnosis

The diagnosis of sickle cell disorder in children and adults depends on a combination of electrophoretic methods and sickling or solubility tests. Differentiation of homozygous sickle cell and heterozygous Hb S/beta^0 thalassaemia is by quantifying the HbA_2 which is increased in the S/beta^0 thalassaemia trait.

6.7.2. Neonatal diagnosis

During the neonatal period, the diagnosis is complicated by the need to identify small amounts of haemoglobin A, S and C. Haemoglobin electrophoresis can separate haemoglobin S from F but small quantities of Hb A may not be separated as easily in the presence of large amounts of Hb F. Isoelectric focusing is superior to cellulose acetate electrophoresis in detecting smaller

amounts of Hb S. High Performance Liquid Chromatography has been used successfully in the screening programmes of haemoglobinpathies in newborns.

6.7.3. Antenatal diagnosis

For prenatal diagnosis of sickle cell disorders, amniotic fluid and chorionic villi samples are used for detection of Hb S. DNA amplification by polymerase chain reaction, using the synthetic oligonucleotides is specific and can identify Hb S in amniotic fluid and chorionic villi. DNA analysis can also be used to confirm the presence of Hb S which is detected by other methods.

7. MANAGEMENT OF SICKLE CELL

As sickle cell anaemia is a chronic disorder, it is recommended that the patient maintains a good nutritional intake, undergoes immunisations and avoids extremes of temperature, dehydration and activity. Prophylactic penicillin has to be given to children starting from the age of 3-4 months.

Balanced nutrition is essential to replenish the energy required by the rapid turnover rate in sickle cell anaemia. The increased need for folic acid may be achieved by either increasing dietary intake or by supplementation.

Other therapeutic approaches for treating sickle cell anaemia patient are:

7.1. Increasing oxygen affinity

Compounds such as tucaresol (589C80, 4[2-fromyl-3-hydroxphenoxymethyl] benzoic acid) increases the affinity of haemoglobin S for oxygen by binding the N-terminus of the alpha chain and inhibiting oxygen release (120).

7.2. Reducing haemoglobin S concentration

Compounds such as hydroxyurea increase haemoglobin F concentration by increasing the synthesis of gamma chains and hence haemoglobin F formation which reduces the haemoglobin S content of the red cell.

7.3. Decreasing adhesiveness of haemoglobin S red blood cells

Compounds such as hydroxyurea and sulphasalazine have anti-adhesive effects thereby decreasing adhesion of sickle cells (121).

7.4. Bone marrow transplantation

Bone marrow transplantation (BMT) needs considerable experience and resources but it has the potential to change the phenotype of SS patients to AA or AS phenotypes. The major problem of BMT is the inability to predict a severe clinical course.

8. PREVENTION

8.1. Antenatal screening

This aims to allow informed reproductive choice by identifying couples at risk of having an affected infant at an early stage in pregnancy. Options include prenatal diagnosis and termination of affected pregnancies if warranted.

8.1.1. Antenatal screening for sickle cell gene

Antenatal diagnosis of sickle cell disorders is useful for the detection of inherited abnormalities in the foetus when the parents are known to be carriers of the sickle cell genes, other interacted haemoglobinpathies genes, or thalassaemia. In severe cases, the foetus may be aborted. Antenatal diagnosis is influenced by many factors such as gestational age, social consideration and religious beliefs.

8.2. Neonatal screening

This aims to identify infants with sickle cell disorder who are at risk of presenting for the first time with severe overwhelming infections and splenic sequestration crises. Early diagnosis allows prophylactic management with penicillin and vaccines, and parent education to identify any complications thereby initiating earlier treatment, reducing complications and death.

8.2.1. Neonatal screening for sickle cell gene

Neonatal screening programmes differ according to the affected population, level of the service needed, technology involved and availability of resources. The benefits of the neonatal screening can be improved if combined with a counselling programme, proper education and sufficient primary health care resources for the affected patients.

Screening of neonates may be used selectively or as part of a universal programme. Selective neonatal screening can be used for people who are known to be at risk of a sickle cell disorder or where the prevalence of haemoglobin S is relatively low. Screening all the neonates is useful in high-risk populations of the sickle cell disorder.

Cord blood can be used for neonatal screening for the sickle cell gene either as a dry blood spot using Guthrie Cards or with anticoagulant. However, there is a risk of contamination with maternal blood. Blood samples taken by heel-prick can be used to eliminate such a risk.

In order to determine a health problem in any population, epidemiological studies are an essential tool to figure out the magnitude of the problem. Neonatal screening can provide the researchers with the data required to produce epidemiological information especially in inherited disorders such as

haemoglobinopathies and thalassaemia, and inform the configuration of haemoglobinopathy services. Neonatal screening programmes can also monitor the quality control of prenatal diagnosis.

9. RESEARCH SETTING

9.1. Yemen location, population and demographic indicators

Yemen is located in the south west of Asia on the southwest corner of the Arabian Peninsula (Fig.12). It has an area of 527,970 sq km. Saudi Arabia borders the country to the north while the Arabian Sea and Gulf of Aden are to south. The Sultanate of Oman borders Yemen from the east and the Red Sea from the west.

Yemen is one of the world's most ancient civilisations and played an important part in Middle Eastern trade, supplying the ancient world with exotic items such as frankincense, myrrh, spices, condiments and other luxury items. The south -western coastal strait known as Bab El-Mandab (gate of tears), which links the Indian Ocean with the Red Sea, was an important trade corridor for about 3,000 years. Yemen is an Arab country in both language and culture. There are many tribal distinctions with regard to location.

Yemen consists of 19 governorates in addition to the capital Sana'a City (Table 14), as shown in figure 15.

Table 14 Yemen governorates

North	East	West	South
Sana'a City	Al-Jawf	Haja	Al-Daleh
Sana'a	Mareb	Al-Hodeidah	Lahge
Saadah	Shabwah	Taiz	Aden
Amran	Hadramout		Abyan
Al-Mahweet	Al-Mahrah		
Dhammar			
Ibb			
Al-Baida			

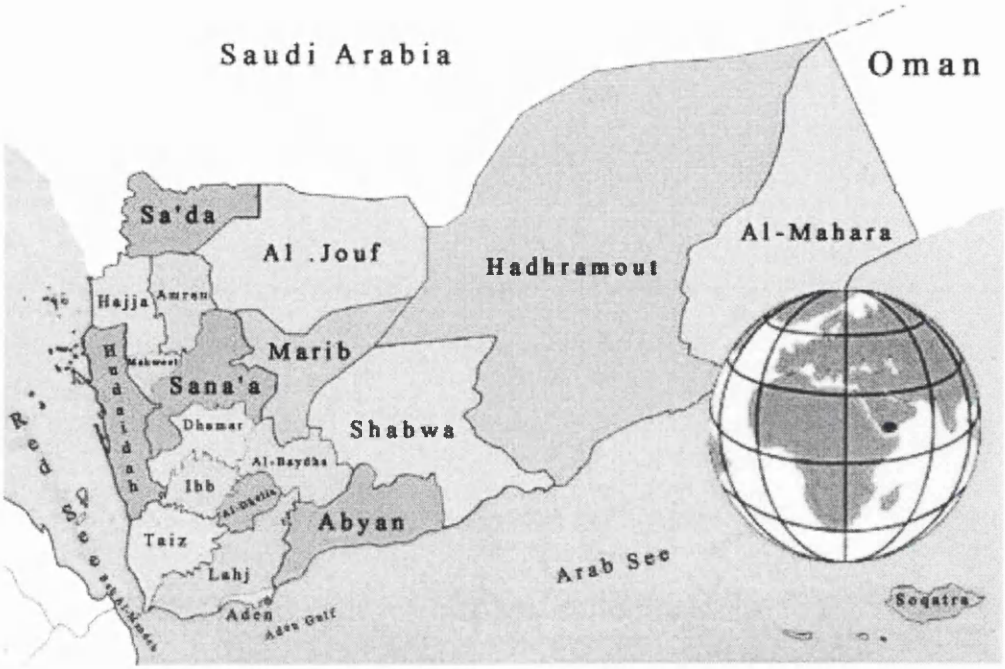


Figure 15 Yemen governorates

Yemen is geographically divided into five different regions: Mountainous, Plateau's, Coastal, Empty Quarter (AR-Rub-Alkali) and Islands.

The climate is hot and humid along the west coast. The climate in the western mountains is affected by the seasonal monsoon, but is very hot and dry (desert weather) in the east.

Yemen is a developing country. It has a population of 18.26 million with a homogenous population. This population is distributed among the 19 governorates and Sana'a City as shown in Table 15 (122).

Table 15 Distribution of resident population by governorates (Year 2000)

Governorate	Total of Population
Sana'a and Sana'a City	2,840,497
Aden	503,794
Taiz	2,295,990
Al-Hodeidah	1,942,251
Laheg	650,044
Ibb	2,018,878
Abyan	420,243
Dhamar	1,199,998
Shabwah	454,786
Haja	1,361,084
Al-Baida	562,851
Hadramout	873,119
Saadah	596,334
Al-Mahweet	451,624
Al-Mahrah	70,318
Mareb	226,488
Al-Jawf	433,235
Amran	955,978
Al-Daleh	403,488
Total	18,261,000

Health care is limited, as shown in Table 16 (122).

Table 16 Health data in Yemen (Year 2000)

Heath data	Number
The physician number	3491
Nurses number	5437
Hospital number	106
Beds number	8631
Population number per physician	5231
Population number per bed	1916
The total Fertility Rate	5.8 children/women
Crude Birth Rate	11.2/1000
Infant Motility Rate	71.5/1000 live births
Birth Rate	45.1/1000
Death Rate	10.3/1000
Growth Rate	3.48
Health expenditure/person/year	6.3 $

Table 17 a, b, and c, show many demographical figure of Yemen in year 2000 (122).

Table 17.a Proportion of population in broad age group

Year	Age 0 – 14	Age 15 – 64	Age > 65
2001	46.3	50.7	3

Table 17.b Rate of population illiteracy (10 years and above)

Male	27.7 %
Female	67.5 %

Table 17.c Population demographic indicators (Year 2000)

	Total	Rural	Urban
Total Population	18,261,000	13,459,000	4,802,000
Family Average Size	7.4	7.5	7.2
Male life Expectancy	58.8	57.3	59.9
Female life Expectancy	62.7	60.3	64.0

9.2. Health services in Yemen

Fever, diarrhoea, vomiting and cough with difficulty in breathing are the common features preceding the death of children under the age of five (123). Many children with the sickle cell disorder die due to its combination with other common infectious diseases. Affected children in Yemen often die before they are diagnosed. The patients involved in this study are from the Capital City of Sana'a with population of 1,488,108. Health services in Sana'a City also serves Sana'a governorate (population of 1,352,389), a combined of 2,840,497. The residents of Sana'a City include people from all governorates of the country in varying proportion with a relatively low proportion of people from the south and east governorates. Sana'a City is the base of almost all the main health facilities in the country.

10. AIMS AND OBJECTIVES

10.1. Aim

This study aims to determine the prevalence of the sickle cell gene and the other related haemoglobinpathies in Yemen.

10.2. Objectives

These were to determine

1. The prevalence of the sickle cell gene among the residents of Sana'a City.
2. The prevalence of the sickle cell gene among different groups of people who came from different parts of the country (Yemen), but are living in the Sana'a City.
3. The interaction of thalassaemia and the other haemoglobinpathies with sickle cell gene in Yemen.
4. Health care issues in relation to management of patients with sickle cell disorder.

11. METHODS

11.1. Objective 1 and 3

These were addressed by identifying the haemoglobin A, S, F and A_2 in a cord blood obtained following childbirth in the maternity wards in four main hospitals in the Sana'a city, namely the Al-Sabain Maternity and Children Hospital, Al-Thawra General Hospital, Al-Kuwait University Hospital and the Mother Hospital, for the period from 15 July 2001 to 28 October 2001.
Only Yemeni mothers admitted into the labour room who gave consent were considered for sample collection, regardless of the method of delivery.

Objective 2

It was addressed by analysing data related to the origin of the family of the neonate.

Objective 4

It was addressed by analysing a questionnaire about the health care management which was completed by 86 sickle cell patients in the main hospitals in the Sana'a City.

11.2. Statistical Information

Statistical information about the Republic of Yemen relevant to this project was sought from a variety of sources such as Central Statistical Organization Ministry of Planning and Development, Sana'a, Yemen, World Health Organization (WHO), World Wild Web (www), and medline (Pubmed).

11.3. Pilot study

A system for collecting neonatal blood onto Guthrie cards (Fig.16) was tested for its feasibility by carrying out a pilot study involving 400 babies. This pilot study was undertaken to ensure the accuracy of the chosen method of sample collection and the storage, transportation, sample preparation and analysis methods, and those were found to be satisfactory.

11.4. Main study

Using the system developed, a total of 1500 cord blood samples were collected from Yemeni newborns in the maternity departments at Al-Sabain Hospital, Al-Thawra Hospital, Al-Kuwait Hospital and Mother Hospital in Sana'a City, Yemen, over 6 months from July to December 2001 (Table 19.a). The names and residential origins of parents were recorded. Soon, after the baby was delivered and during cutting the umbilical cord, cord blood samples were collected directly from the umbilical cord into the EDTA tubes avoiding as possible the contamination with the mother's blood. No blood was collected from the parents for the purpose of this study.

Due to absence of DNA-extraction facilities in Yemen, locally DNA-extraction then transportation to UK to have a good enough samples for alpha globin gene amplification to detect the alpha thalassaemia mutations among these samples was not possible.

Cord blood samples were put on Guthrie cards (Fig. 16) (Schliecher and Schuell, The Science and Art Company, batra GmbH, Traben-Trabach, Germany) within twenty-four hours of collection and these were kept at 4°C

during storage and transportation to the UK. The samples were subsequently analysed using facilities at the Cardiff Sickle Cell and Thalassaemia Laboratory located in Llandough Hospital and the University Hospital of Wales, Cardiff, Wales, UK.

Analysis of the cord blood samples carried used High Pressure Liquid Chromatography (HPLC) and both IEF and DNA techniques for confirmation of the existence of abnormal variant haemoglobin, such as haemoglobin S, C, F, E, D, G, and others.

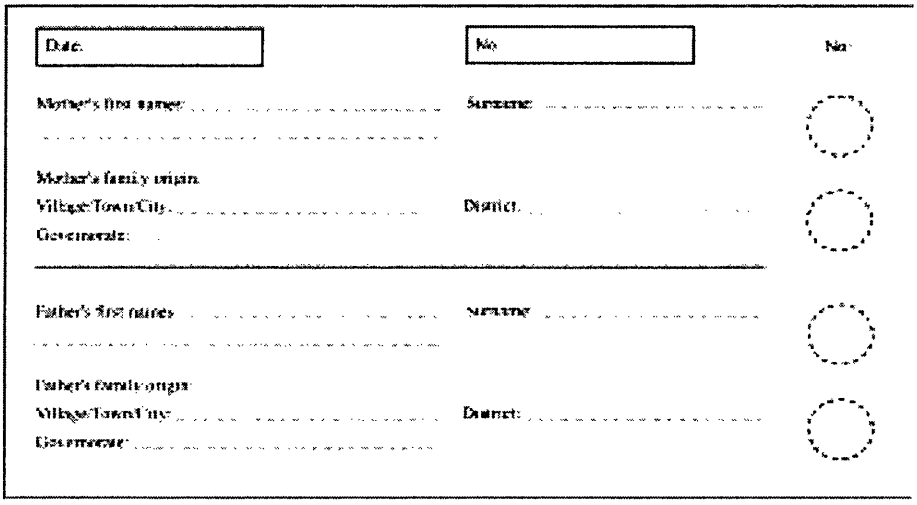


Figure 16 Guthrie card

11.5. Instrumentation

11.5.1. High performance liquid chromatography

A cation-exchange, high performance liquid chromatography (HPLC) system (Shimadzu) and an optimised gradient of a beta thalassaemia test kit (Chromsystems Instruments & Chemicals, GmbH, Munchen, Germany) were used to analyse all the samples for Hb A, F, S, C and D. Samples were

prepared for analysis by punching out a small portion of the blood spot on the Guthrie card and eluting the haemoglobin with haemolysis reagent (Chromsystems Diagnostics) (124).

A high performance liquid chromatography (HPLC) system was used for detection and quantitation of normal and abnormal haemoglobins by using an Ion exchange system, either anionic or cationic. It gives percentage of haemoglobin A, A_2, F and any abnormal haemoglobin fractions. The HPLC system comprised a high-pressure pump, a mixing unit, an in line detector, a column, a sample valve, a recorder and computer based integrator/controller unit. HPLC uses the principle of a cation exchange resin held in a cartridge in conjunction with a buffer gradient. As the ionic strength increases so certain haemoglobins are eluted from the column, and the presence of haemoglobin is detected using a spectrophotometric technique. Two buffers are pushed through the high-pressure pumps, to the mixing unit, where they are mixed in a proper proportion required for that part of the gradient. This mixture goes to the sample valve, where a certain amount of sample is entered into the buffer flow line. The sample is then pumped via the column. At the start of the gradient, the charged haemoglobin fractions attach to the charged silica packing the column, while any uncharged particles will be pumped via the column, making the breaking peak that is observed at the beginning of any chromatogram. The gradient is then initiated and the buffer mix is changed at the mixing valve. This allows the different haemoglobin fractions to elute from the column, as the charge differences on the silica and haemoglobin fraction change and the fractions are released and move via the column and detector, making the typical peaks observed on HPLC. Using the HPLC

(Chromsystems Instrument, Diagnostics by HPLC) and the Thalassemia Testing Kit (from the same company), it was possible to separate and identify clarely the different haemoglobin A, F, A, A_2, D, S, and C. The instrument settings were set so the injection volume is 10 – 20 µl; flow rate is 2.5 – 3.0 ml/min; maximum pressure is 170 bar; column temperature is room temperature; UV-VIS detector was detection wavelength was 415 nm. Known control samples were used through the system to determine the haemoglobin fractions (Table 18.b). These fractions were identified by their retention times which is the time require for them to pass through the column from the start of the gradient to the detector (Table 18.a). The area under any peak is used as the basis of integration for that peak.

Table 18.a The gradient used and run criteria

Time	% Conc.of Buffer B*
0.10	18.0
5.80	68.0
5.81	100.0
6.60	100.0
6.61	18.0
8.50	0.00 Stop

*Supplied with beta thalassaemia testing kit (Chromsystems Instruments & Chemicals, GmbH, Munchen, Germany)

Table 18.b Analysis window set up

Name of Window	Retention Time (min)	Window width (min)
? F1 (acetylated Hb F)	0.7	0.4
Hb F	0.8	0.6
Hb A (Adult Hb A)	4.6	0.8
Hb A_2	5.3	0.8
Hb S (window)	6.25	0.8
Hb C (window)	8.0	0.8

Columns to PolyCAT A, 35 x 4.6 were used for fast screens for haemoglobin variants. For mobile phase A, this buffer (01mM Bis-tris + 1mM KCN) at pH

6.8 or 6.9 was used. For mobile phase B the buffer contains 10mM Bis-tris, 1 mM KCN and 0.2 M NaCl at pH 6.55 or 6.6 was used.

For this particular study, following the analysis run, the chromatograms were analysed quantitatively for the presence of peaks not normally present in cord dried blood spot samples. The presence of any other significant peak, especially those in the haemoglobin A_2, S and C windows underwent further analysis.

To reduce carry over to a minimum, the eluting buffer should be run at 100% for a minute or more. This will remove all haemoglobins off the column. The starting buffer mixture should then be pumped through the system to re-equilibrate the column. The backpressure of a new column is about 66 bar at a flow rate of 2.5 ml/min. This can be increase with time. To protect the column, a maximum pressure of 170 bar should not be exceeded.

To evaluate the HPLC system to analyse the collected cord blood samples, dry blood spots of previously tested as EDTA blood samples were analysed using the same HPLC system. No differences were observed between the chromatographic separations.

11.5.2. Iso-Electric Focusing

Isoelectric focusing (IEF) on agarose gels (Resolve System, Haemoglobin test kit, PerkinElmer Life Sciences, Norton, Ohio, USA) was used to confirm haemoglobin variants such as Hb S detected by HPLC. A small portion from each dry blood spot sample was eluted in haemoglobin elution solution supplied by PerkinElmer. Then the samples were focused on agarose gels after

which the protein was fixed by immersion in 5% trichloracetic acid in 35% methanol and the plate was dried (116).

The principle of IEF is that the net charge of a protein relies on the pH of the buffer. When the pH of the buffer is low the carboxylic acid groups of proteins are uncharged and basic groups completely charged producing a positively net charge. But, when the pH of the buffer is high, the basic groups become uncharged where the carboxylic acid groups turn negatively charged, producing a negatively net charge. When a haemoglobin solution is applied on the Iso-electric focusing, the haemoglobin variants are separated in a gel depending on their iso-electric point that is the point at which the haemoglobin variants have no net charge.

Because the high sensitivity and specificity of IEF (119), it was used in this study.

Using Haemoglobin Test Kit (Perkin-Elmer), the preparation and separation of haemoglobin is performed by the application of the haemoglobin sample onto a recast agarose gel containing Resolve Ampholytes pH 6 to 8. The Resolve Ampholytes are made of low molecular weight amphoteric molecules with different isoelectric points. By applying electrical current to the gel, these molecules move through the gel to their isoelectric points along the gel, making a constant pH gradient. The haemoglobin variants also move through the gel till they reach the place where their individual isoelectric points equal the corresponding pH on the gel. The electrical charges of haemoglobin variants at this point are zero and movement ceases. The electric field stops

diffusion and the haemoglobin variant forms a separate thin band. The cyanide ion in the cathode solution prevents the oxidation of the iron molecule in Hb F. Haemoglobin F has a high affinity for oxygen. Partial oxidation of the iron molecules leads to a band (methaemoglobin F) focuses in the Hb S position, leading to false positive for Hb S. The presence of cyanide ions in the cathode solution ensures that adequate amounts will be existed to prevent the formation of methaemoglobin F during IEF. Trichloroacetic acid spoils the tertiary structure of haemoglobin, by sequestering internal water molecules. If this occurred, the internal hydrophobic amino acids are exposed leading haemoglobin to coagulate and fall out of solution.

11.5.3. Deoxyribonucleic acid - Polymerase chain reaction analysis

Polymerase chain reaction (PCR) has a great impact on the study of nucleic acids. By using of a thermostable DNA polymerase (Taq polymerase), PCR results in the amplification of a certain DNA fragment can be observed by ethidium bromide staining on an agarose gel.

Extraction of DNA from blood or tissue sample can be used for DNA-PCR analysis. The quality and quantity of the obtained DNA will differ according to the size, age and cell count of the sample. The DNA in the nucleus is strongly bound to many proteins as chromatin. To extract the DNA, it is essentially to remove these as well as other cellular proteins.

11.5.3.1. Principle of DNA-PCR Analysis

Two oligonucleotide primers direct multiple cycles of localised DNA replication to make an exponential increase in the number of the copies of the target sequence. After 20 to 30 cycles, amplification of the target DNA will reach to 100,000 folds. In order to amplify the DNA, four deoxynucleotide triphosphates (dATP, dTTP, dCTP, and GTP), PCR buffer, and the thermostable taq polymerase are needed

The first phase of the reaction is denaturing the DNA by heating the reaction mixture to 95 °C and followed by cooling to 50-65 °C that alloweds the annealing of the primers to the DNA. Then the mixture is heated to 72 °C in this phase the taq polymerase generates DNA, extending from the oligo in 5' to 3' direction. Repetition of the denaturing, annealing, and extension stages which occur by rising and lowering the temperature leading to exponential amplification of DNA fragment that lies between the two oligos (Fig.17).

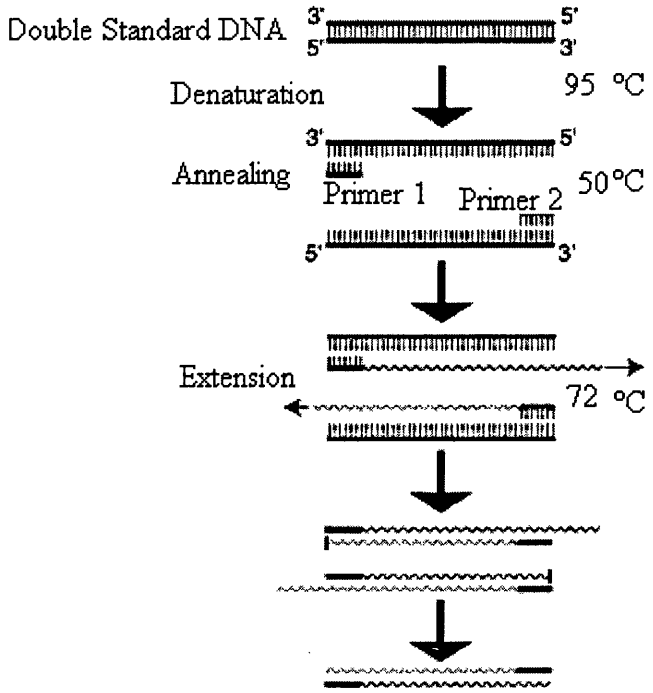


Figure 17 DNA-PCR principle

Digestion of the amplified DNA fragment can be done by a proper restriction enzyme. The producing fragments can be separated by gel electrophoresis then stained with ethidium bromide and seen under ultraviolet light.

For detection of Hb S, PCR amplification of the first two exons of the beta globin gene in order to detect the Hb S mutation was done using the primers (GGCCAATCTACTCCCAGGAG) and (ACATCAAGGGTCCCATAGAC). PCR thermal cycler instrument is used to amplify the DNA. The optimal annealing temperature used to amplify is 95 °C for 9 minutes followed by 40 cycles of 94 °C for 30 seconds, 62°C for 30 seconds and 72 °C for 1 minute. At the end of these 40 cycles the temperature was kept for 10 minute before removing the samples.

Then the enzyme *Dde*1 digests the amplified DNA samples. The undigested fragment is 597 bp (base pair) in size. 5 *Dde* I restriction sites exist normally; one of which is abolished by the Hb S mutation.

Separation of the producing fragments is carrying out using 3% agarose gel electrophoresis [1.5 gram agarose gel dissolved in 50 ml TBE buffer (0.89M Tris, 0.89M Boric acid, 0.02M Na_2 EDT, final pH 8.3) with 1.2 ethidium bromide (2.5 mg/ml)]. The gel electrophoresis is then run at 140 volts up to one hour untie full separation of the bands is completed.

The gel is placed onto an UV transilluminator and photographed.

Haemoglobin SS gives the bands of sizes 351, 88, 89, 37 and 40 bp.

Haemoglobin AS produces the bands of the sizes 351, 201, 150, 88, 89, 37 and 40 bp. Haemoglobin AA gives the bands of the sizes 201, 150, 88, 89, 37 and 40 bp.

To confirm the absence of beta S mutation in the samples that show unknown haemoglobin variants by the HPLC and IEF, they had been investigated with the other samples that contain Hb S.

11.5.3.2. DNA sequence analysis

The extracted DNA is amplified using specific primers for the required analysis in the PCR method. The PCR product is sequenced after removing of enzyme, primer and dNTPs, by a mechanism of denaturation then by primers annealing and 3'extension of a complementary strand of DNA by the enzyme AmpliTaq DNA Polymerase FS in the presence of a mixture of dNTPs and fluorescent dideoxynucleotides. The ratio of nucleotides to dideoxynucleotides is such that they are in a statistical chance of inserting a dideoxynucleotide. When this happen first the chain is stopped because 3'extension cannot

continue and second the chain 'n' nucleotides in length is tagged with a fluorescent tag indicating the nature of the 'n' nucleotide. Cycling the stages of denaturation, annealing and synthesis enables amplification of the process and an adequate yield of product for detection.

The fluorescent polynucleotides are separated from the fluorescent precursors by ethanol precipitation, redissolved, denatured, and applied to a denaturing polyacrylamid gel optimised to separate strands that differ by only a single nucleotide. Scanning each track of the gel for the four different fluorescent signals that can be assigned one to each of the nucleotides allows the nucleotide sequence to be read directly.

Samples showed unknown peaks in HPLC or bands on IEF were sequenced to identify the unknown haemoglobins.

11.6. Sample elution preparation from Guthrie cards

11.6.1. HPLC Analysis

Screw capped vials (1.5 ml) were labelled with unique identifying code. A six-mm hole punch was used to get a single disc from the centre of each blood spot on the appropriate Guthrie card which placed in the appropriately labelled screw capped vial. Then 1ml of haemolysis reagent was added to the vial. The cap was closed and sample mixed for 15 minutes. After that, the disc was removed from the vial and spun for 1minute at 13000 rpm to sediment any debris. The clear fluid containing the haemolysate of the sample was kept for 24 hours before the analysis.

11.6.2. Iso-Electric focusing

A 6 mm disc out of the dry blood spot was used to prepare the haemolysate of each blood sample. A 50 ul of haemoglobin elution solution was added on the dry blood disc in small tube. Then the tube was mixed for 15 minutes. Then the tube was spun and the clear elution of the haemolysate was used for the analysis.

11.6.3. Extraction of DNA

Guthrie cards are widely used for neonatal genetic DNA analysis. A 5 mm circle of Guthrie card containing the dry blood sample was placed into a small eppendorf tube contained 60 μl of buffer for EDTA blood (Applied BioSystem) After incubation of this mixture for 40 minutes at 97 °C, the tube centrifuged and the buffered supernatant containing the DNA was transferred to a clean tube. This separated DNA liquid was used immediately or frozen for later use.

11.7. Samples analysis

11.7.1. High performance liquid chromatography analysis

Prepared samples for HPLC were used for this analysis. The HPLC set up using a prime sample, which was a cord blood of Guthrie card sample. This primes the system ready for analytical samples, which followed by the sequence of samples for testing. The run was set afternoon and continue overnight, the results were obtained next day. Known sample also were run with each batch to ensure the accuracy of the obtained results.

If any abnormal fraction was seen in the peaks of the curves of the result, the sample preparation and analysis for HPLC was repeated using a new blood

spot of the same Guthrie card. To ascertain that the peak was valid and there had been no mix-up or contamination of the original blood spot on the card. If the abnormal fraction was confirmed, then Iso-electric focusing was carried out using suitable controls to mark the positions of common abnormal haemoglobins on the gel.

11.7.2. Iso-Electric focusing analysis

Prepared samples for IEF analysis and a control mixture of Hb A, S and C were used in this test. The samples were run according to the method and manufacture instruction at 1500 volts (18mA) for 90 minutes. The running was resumed after the 15 minutes of removing the template. Trichloroacetic acid in concentration of 5% prepared in 35% methanol was used as protein fixative solution. The gel was dried at 50 – 60 °C for about 20 minutes.
To confirm any abnormal band seen in position of Hb S or any other haemoglobins on the IEF gel, the sample was sent to University Hospital (UHW) Haematology Research Laboratory, to undergo DNA level investigations to confirm the band as Hb S or to identify the unknown haemoglobins.

11.7.3. DNA-PCR analysis

11.7.3.1. Determination of Hb S and other β globin gene mutations

The β^S mutation was detected by a previously published method involving PCR amplification of the first two exons of the β globin gene followed by *Dde*I digestion of the PCR product (125). Further mutation analysis of the β globin gene coding region was carried out by PCR amplification of the first two exons

of the β globin gene using the forward primer

5'GGCCAATCTACTCCCAGGAG3' and the reverse primer

5'ACATCAAGGGTCCCATAGAC3', and PCR amplification of the third exon of the β globin gene using the forward primer

5'CAATGTATCATGCCTCTTTGCACC3' and the reverse primer

5'CACTGACCTCCCACATTCCC3', followed by direct sequence analysis using the same primers and the ABI PRISM™ BigDye fluorescent dideoxy chain terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The fluorescent products were separated by capillary electrophoresis and analysed using an ABI 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Adding of 1μl of extracted DNA sample to a mixture of 19 μl distilled water, 2.5 μl 10xPCR buffer (83mM $(NH4)_2SO_4$, 335mM Tris-HCl, pH 8.8), 1 μl dNTPs, 0.18 μl Taq Gold and 1.5 μl primer mix of equal volume of them at concentration of 10 μM. Then incubated in thermal cycler at 95 °C for 9min, then for 40 cycles. Size marker (*OX 174, Hae III*) was use to evaluate the size and concentration of the DNA products. Also blank sample was used to make sure there was no contamination in the any of the mixtures.

The PCR product was tested to evaluate the DNA amplification. This performed by running of agarose gel electrophoresis. In this method, PCR sample products were loaded into the gel that placed in the electrophoresis tank contained 400ml of TBE buffer (0.89M Tris, 0.89M Boric acid, 0.02M Na_2 EDT, final pH 8.3). Ethidium Bromide (2.5 mg/ml) also was been added to the buffer at the concentration as the gel. After the bands had separated enough, the gel was placed onto an UV transilluminator and viewed and photographed.

All the DNA-PCR products of the samples were tested and showed a band in positions size 603 bp.

Digestion using *Dde* I enzyme for detection Hb S was run for all the samples, size marker (*OX174, Hae III*) and controls (homozygous Hb SS, heterozygous Hb AS, normal Hb AA). In this test 7 µl of the mixture 10xPCR buffer (83mM $(NH4)_2SO_4$, 335mM Tris-HCl), made of 5.6 µl distilled water, 1.4 µl NEB3 buffer (100 mN NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT) and 0.52 µl *Dde* I [10.000U/ml (NEB175)] was added to 7 µ l DNA-PCR product. Then incubated at 37 °C for overnight. After the incubation, agarose gel electrophoresis was run for the entire digested DNA samples with the six marker and controls at 140 volts for about 45 minutes. Finally the separated bands were placed onto an UV transilluminator and photographed.

11.7.3.2. DNA sequence analysis

Sequence of DNA was made in four separate steps

Step A: Purification of the PCR products for sequencing

The DNA-PCR product was purified for sequencing. In this step the PCR product was added to 225 µl of the binding buffer solution. Then this mixture was transferred to a spin column tube and spun. The solution in the collected tube was decanted. Washing solution (0.5 ml) was added to the spin column, spun and the collected elute was removed. Again 0.25 ml of washing solution was added to the column and spun and the collected solution was decanted. The spin column was placed onto broken tops tubes. Into each column 30 µl

sterile water was added, then spun. The collected elute in the tube contained the purified PCR product.

The concentration of the purified DNA-PCR product was determined by electrophoresing 5 µl of each product in agarose gel and 10 µl of the size marker (*OX174, Hae III*) at known concentration. Electrophoresis was run at 140 volts for 45 minutes.

Step B: Prism Dye Terminator Cycle Sequencing

The ideal concentration of PCR product is between 15 – 45ng, which was added per 10 µl sequencing reaction. All sequences were performed in forward and backward to minimise the chances of losing heterozygosity due to a very small signal from one of the nucleotides of single nucleotide deletions or insertions.

Diluted (1/6) the forward primer 5'GGCCAATCTACTCCCAGGAG 3' and the reverse primer 5'ACATCAAGGGTCCCATAGAC 3', both were used for detection mutation in first and second exons.

From each primer 1.2 µl was added to 2 µl PCR product and mixed with 2 µl of terminator ready reagent mixture (ABI Prism BigDye Terminator Ready Reaction Kit PE Applied Biosystems). Then distilled water was added to them making the total volume in each tube 10 µl. By using thermal cycler, this preparation was incubated for 25 cycles (96 °C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes).

Step C: Preparing the sequence reaction product for gel electrophoresis

All the entire contents of each reaction tube from step B was added a mixture of 8 µl distilled water and 2 µl 3M-sodium acetate. Then a 50 µl of 95% ethanol was added to the above mixture. Then this mixture was mixed thoroughly and placed on ice for 20 to 25 minutes. Then was spun for 25 minutes at high speed. The supernatant was removed and 250 µl of 70% ethanol was added. Then it was spun at high speed for 5 minutes. Again the supernatant was aspirated and the pellet was stored in dark at - 20 °C until electrophoresis was possible.

Step D: Referral of samples to University of Wales College of Medicine Molecular Biology Services for Automated Sequence Analysis.

11.7.3.3. Detection of mutation in exon 3

It was carried out using the same method as in first and second exons analysis, except the following:

The primers used were 5'CAATGTATCATGCCTCTTTGCACC3' for forward 5'CACTGACCTCCCACATTCCC3' for backward.

1 µl DNA extract was added to 15.5 µl distilled water, 2.5 µl 10xPCR buffer (83mM $(NH4)_2SO_4$, 335mM Tris-HCl), 1µl dNTPs, 5 µl primers mixture of equal volume from each one and 0.17 µl Taq Gold.

Then the mixture was incubated in thermal cycler at 96 °C for 9 minutes then for 37 cycles (90 °C for 1 min, 66 °C for 2min) then for 66 °C for10 min.

11.7.3.4. Detection of alpha thalassaemia mutations -$\alpha^{3.7}$ and -$\alpha^{4.2}$

The alpha thalassaemia alleles consist of either deletion mutations in and around the globin gene cluster or point mutations within one of the two-globin genes. The two common alpha thalassaemia deletions are -$\alpha^{3.7}$ and -$\alpha^{4.2}$ that can be identified by the polymerase chain reaction method (126,127,128), but the application of these PCR methods for detection alpha thalassaemia mutations has some difficulties. Southern blot analysis remains the recommended method for detection alpha thalassaemia mutations because of its ability to diagnose all alleles in one set. The primers in Table 19 were used for detection of alpha thalassaemia mutations (128).

Table 19 Primers used for detection of alpha thalassaemia mutations

$-\alpha^{3.7}$ multiplex PCR:	Forward: AAGTCCACCCCTTCCTTCCTCACC Reverse 1: ATGAGAGAAATGTTCTGGCACCTGCACTTG Reverse 2: TCCATCCCCTCCTCCCGCCCCTGCCTTTTC
$-\alpha^{4.2}$ multiplex PCR:	Forward: TCCTGATCTTTGAATGAAGTCCGAGTAGGC Reverse1:TGGGGGTGGGTGTGAGGAGACAGGAAAGAGAGA Reverse 2: ATCACTGATAAGTCATTTCCTGGGGGTCTG

Polymerase chain reaction was preformed using 5% DMSO, 200 µmol/l dNTPs and 1.25 units of AmpliTaq Gold polymerase, 2.5 µl 10x PCR buffer [83mM $(NH4)_2SO_4$, 335mM Tris-HCl, pH 8.8], primer concentration between 0.1 and 0.3 µM and 100 ng DNA.

The PCR conditions used are 95 °C for 9 minutes, then 40 cycles of 94°C for 30 seconds, 57 °C for seconds, and 72 °C for 2 minutes, then extension temperature of 72 °C 10 minutes. Gel electrophoresis (1.2% agarose gel) was applied and specific PCR product was determined.

11.7.3.5. Determination of the *Xmn* I polymorphic site

The –158 (C→T) G-gamma globin gene polymorphic site was detected by PCR amplification using 5'GAACTTAAGAGATAATGGCCTAA3' and 5'ATGACCCATGGCGTCTGGACTAG3' as forward and reverse primers respectively (129) followed by digestion of the PCR product with the restriction endonuclease *Xmn* I (New England Biolabs Inc., Beverly, MA, USA). Digested fragments were separated on agarose gel electrophoresis and analysed after ethidium bromide staining. The –158 C→T *Xmn* I polymorphism creates a restriction site for this enzyme and digested fragments of sizes 420 and 220 bp were observed.

11.7.3.6. Determination of β globin cluster haplotypes

Haplotypes were identified by the analysis of the polymorphic restictriction sites in the beta globin gene cluster: *Hind* II/ε, *Hind* III/$^{G}\gamma$, *Hind* III/$^{A}\gamma$, *Hinc* II/3'φβ γ , *Hinf* I/5'β in addition to *Xmn* I. Fragments containing each of these polymorphic sites were amplified by PCR DNA amplification using forward and reverse primers (Eurogentec Ltd, UK) (Table 20) specific for each polymorphic site and subsequently digested with the appropriate restriction endonuclease (New England Biolabs Inc., Beverly, MA, USA) for each polymorphic site (130,131).

Table 20 Primers used to identify the beta globin cluster

Polymorphic site	Primers
Hind II/ε	TCTCTGTTTGATGACAAATTC AGTCATTGGTCAAGGCTGACC
Hind III/$^{G}\gamma$	AGTGCTGCAAGAAGAACAACTACC CTCTGCATCATGGGCAGTGAGCTC
Hind III/$^{A}\gamma$	ATGCTGCTAATGCTTCATTAC TCATGTGTGATCTCTCAGCAG
Hinc II/3'φβ	TCTGCATTTGACTCTGTTAGC GGACCCTAACTGATATAACTA
Hinf I/5'β	CTACGCTGACCTCATAAATG CTAATCTGCAAGAGTGTCT

11.8. Prevalence of haemoglobin S

The overall prevalence of sickle cell trait was calculated as the percentage of sickle cell trait samples detected amongst the total tested. Prevalence for different localities was determined indirectly, from the information on the places of residence of the parents. If the parents were from different governorates that child was considered to come from either one or the other and lower and upper limits for prevalences for these areas were therefore given.

11.9. Survey to evaluate the sickle cell patient's health care in Yemen

Due to the lack of information in the literature regarding health care issues, a questionnaire was used to evaluate the available health care for sickle cell patients in Yemen. The questionnaire was constructed to collect information about age of the patient, time of diagnosis of sickle cell disorders, methods of sickle cell disorder diagnosis, health care after the diagnosis, sickle cell management, blood transfusion services, cost of health care and causes of death of sickle cell patients.

11.9.1. Sampling

Eighty-six known sickle cell anaemia patients registered in two hospitals (Al-Thawra, Kuwait University) were identified and their parents were interviewed. The questionnaire was completed by the researcher.

11.9.2. Interview Details

Interview took place during 5 months from September 2001 to January 2002. After informal consent was obtained from parents or their relatives, subjects were interviewed for 15 –20 minutes in the Haematology Department in Al-Thawra Hospital and in the Internal Medicine Department in Kuwait University Hospital. Data was also obtained from laboratory and hospital records.

11.9.3. Questionnaire Form

The table 21 below shows the questionnaire from.

Table 21 The questionnaire of the survey

1	Age	Year Month	Yes	No
2	Diagnosis of sickle cell disorders	At birth.		
		Before the appearance of sickle cell anaemia symptoms		
		After the appearance of the sickle cell symptoms		
		During sickle cell complications		
		At death		
3	How was the sickle cell disorder diagnosed?	From the clinical signs and symptoms		
		From the family health history		
		From the blood smear		
		From the sickling test		
		From the haemoglobin electrophoresis test		
		From other methods.		
		Are you aware of sickle cell patient registry?		
4	Health Care after the diagnosis sickle	Are you aware of sickle cell association?		
		Are you aware of sickle cell health centre?		

	cell disorder	Are you aware of sickle cell disorders card?		
		Are you aware of regular health evaluation?		
		What laboratory tests do you regularly or often tested?		
		Are you aware of your disorder?		
		Were you told what you should do during a crisis?		
		Were you or your relative told to avoid sickle cell disorder in the future?		
		Have you got leaflets about your disorder?		
		Where do you go in crisis?		
5	Sickle cell management	Does haematologist treat you?		
		Do you use haematinics?		
		Do you use antibiotic as prophylactic?		
		Have you got enough vaccines?		
		Have you been instructed to improve your hygiene?		
		Have you been instructed to improve your quality of food?		
		Do you use chelating agent to remove the extra iron from your body?		
		Do you use hydroxyurea?		
6	Have you been hospitalised in a year?	Once		
		Twice		
		Three times		
		More than three times		
7	How long do you spend in hospital each year?	7 days or less		
		One month or less		
		More than a month		
		Do you get fresh blood transfusion?		
		Do you get blood from relative donors?		
8	Blood transfusion services for sickle cell patient	Do you get blood from blood bank?		
		Is Kell group including in the blood cross matching?		
		Equal or less than 1000 ml		
		Less than 2000 ml		
9	How much do you get transfused blood per year?	More than 2000 ml		
		Do you pay the cost of blood transfusion?		
		Do you pay the cost of the prescriptions?		
10	Cost of health care for sickle cell patient	Do you pay the cost of hospitalisation?		
		Severe anaemia (lack of blood transfusion services).		
		Blood transfusion complications.		
11	Causes of death of sickle cell patient.	Sickle cell disorder complications (infection).		
		Negligence of the patient or his relative due to their poverty and/or illiteracy.		
		Misdiagnosis of sickle cell disorder		

12. RESULTS

12.1. Cord blood analysis

None of the intended mothers refused to take part in this study. Only one sample replaced by another cord blood sample after the pilot study showed it was adult blood sample (98% Hb A). Three hundred samples were left behind in Yemen due to delay in receiving the Guthrie cards form UK.

Table 22.a-b show distribution of collected samples by hospital and governorate.

Table 22.a Distribution of collected samples by governorate and hospital

Governorate	Total Samples	Samples Al-Sabain	Samples Al-Thawra	Samples Mother	Samples Kuwait
Sana'a and Sana'a City	745	484	229	9	23
Aden	18	7	10	0	1
Tiaz	171	79	73	4	15
Al-Hodeidah	27	20	6	0	1
Laheg	1	0	1	0	0
Ibb	179	64	104	5	6
Abyan	13	6	5	0	2
Dhamar	138	43	86	2	7
Shabwah	2	1	0	1	0
Haja	30	15	11	1	3
Al-Beida	53	27	25	0	1
Hadramout	16	5	9	0	2
Saadah	4	2	2	0	0
Al-Mahweet	45	19	23	0	3
Al-Mahrah	1	1	0	0	0
Mareb	16	7	7	0	2
Al-Jawf	4	3	0	0	1
Amran	22	7	11	0	4
Al-Daleh	15	5	7	2	1
Total	1,500	795	609	24	72

Table 22.b Distribution of the samples by hospital

Hospitals	Number of the Samples
Al-Sabain Maternity and Children Hospital	795
Al-Thawra General Hospital	609
Al-Kuwait University Hospital	72
Mother Hospital	24
Total	1,500

12.1. High performance liquid chromatography

Of the total 1500 cord blood samples analysed by HPLC, all chromatograms showed peaks eluting in the positions of Hb A and Hb F (Fig. 18). Thirty-eight samples showed abnormal haemoglobin peaks. Thirty-four of these had peaks in the position of Hb S (Fig. 18.b) one of which (sample Al-Kuwait 59) was unusually small and of uncertain significance.

The sample Al-Thawra 402 showed a peak between HbF and HbA and a minor peak in the Hb A_2 position. Sample Al-Sabain 1771 showed two peaks, one between Hb A and Hb A_2 and a minor peak eluting later than Hb S. Sample Al-Thawra 2 showed a single extra peak between Hb A and Hb F and sample Al-Sabain 1203 showed a single peak between Hb A and Hb A_2 (Fig.18 c--f).

Figure 1

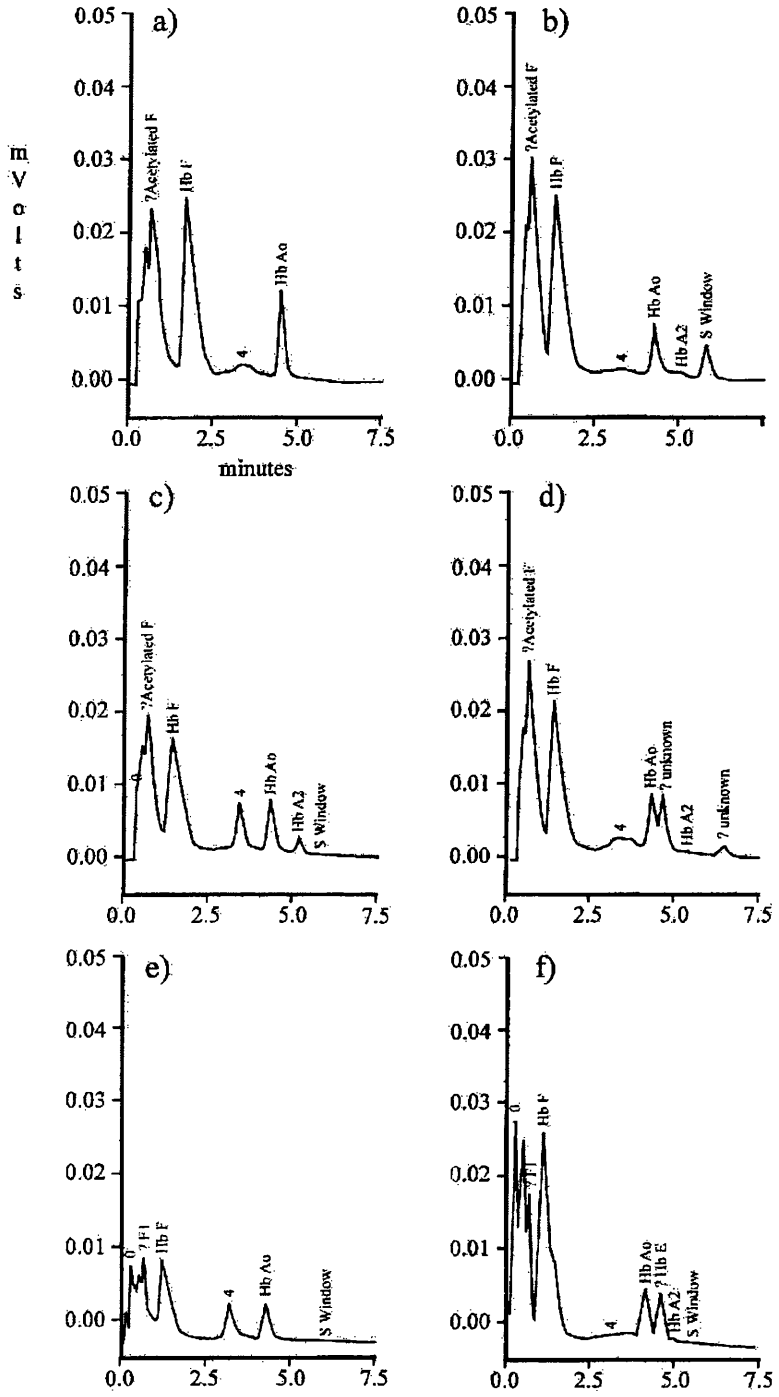


Figure 18 HPLC elution peaks

(a) sample showing no abnormalities, (b) sample from a baby found to have HbAFS, (c) sample Al-Thawra 402, (d) sample Al-Sabain 1771, (e) sample Al-Thawra 2, (f) sample Al-Sabain 1203

12.2. Iso-Electric focusing

All the samples showing abnormal haemoglobin peaks on HPLC were examined by IEF. Thirty-three of them were confirmed by IEF as probable Hb FAS. The sample Al-Kuwait 59 showed no variant haemoglobin band at all and was confirmed to lack the Hb S gene mutation by DNA studies. The sample Al-Thawra 402 showed a minor band at position Hb A –6 mm and sample Al-Sabain 1771 showed a minor band at position Hb A –16 mm. Sample Al-Thawra 2 showed two bands: a minor band at position Hb A –7 mm and a major band at position Hb A –13 mm and sample Al-Sabain 1203 showed a minor band at position HbA – 9 mm. These four samples were considered to contain unknown haemoglobin variants requiring further investigation for their identification. From the numbers of the peaks on HPLC and their relative intensities on IEF, it seems likely that Al-Thawra 402 and Al-Sabain 1771 contained slow alpha chain variants producing both fetal and adult haemoglobin variants detected most clearly by HPLC. Al-Thawra 2 could contain a gamma chain variant and Al-Sabain 1203 appeared likely to contain a beta globin chain variant.

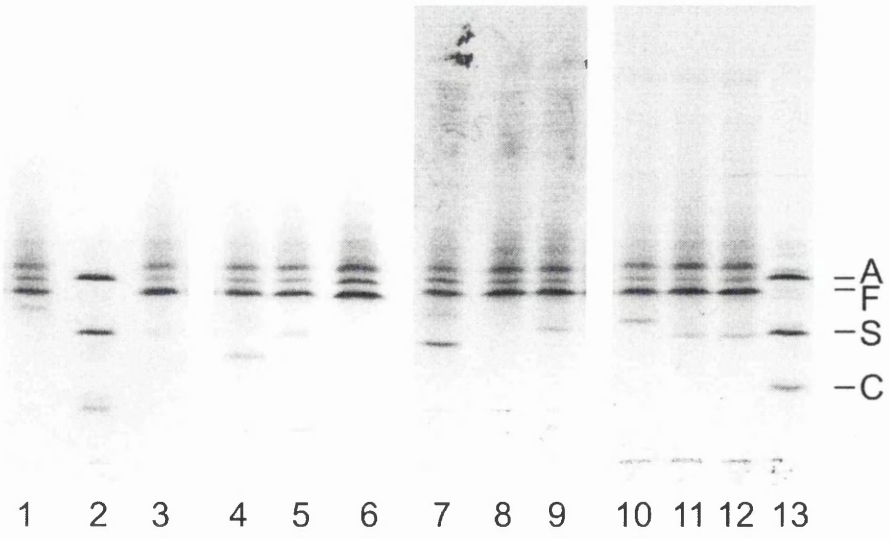


Figure 19 IEF plate of the samples with haemoglobin variants

(1) Al-Thawra 402. (2) Control ASC. (3) Sickle cell trait. (4) Al-Sabain 1771. (5) Sickle cell trait. (6) Normal. (7) Al-Thawra 2. (8) Normal. (9) Sickle cell trait. (10) Al-Sabain 1203. (11) Sickle cell trait. (12) Sickle cell trait. (13) Control ASC.

12.3. DNA analysis

All samples showed haemoglobin variants on HPLC were further examined at the DNA level.

12.3.1. Detection of Hb S mutation

All 33 samples showed a peak in the Hb S window on HPLC and a band in the HbS position on IEF were confirmed as heterozygous Hb S (Fig. 20) by the PCR-DNA studies. Sample Al-Kuwait 59 showed the usual fragment sizes only, as did the 4 samples with unidentified variant haemoglobins.

In lane 3 (Fig. 20), sample Al-Sabain 1046 showed a faint Hb S band with size of 351 bp. The HPLC result of this sample was Hb A (22.64%), Hb S (11.13%) and Hb A_2 (0.97). This result suggesting this faint Hb S band is due to maternal blood contamination. While in lane 7 (Fig. 20), the Hb AA sample showed a faint Hb S band with size of 351 bp which is due to partial digestion.

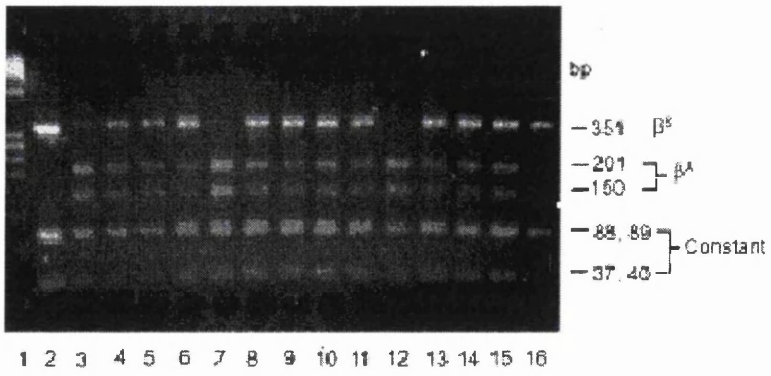


Figure 20 Gels of PCR-DNA of samples with haemoglobin variants
Marker in lane (1). HbSS in lane (2) and (16). HbAA in lane (7) and (12). Hb AS in lane (3), (4), (5), (6), (8), (9), (10), (11), (13) and (14).

12.3.2. Direct sequence analysis

Direct sequence analysis of all three β globin gene exons of the four samples containing unknown haemoglobin variants revealed an abnormality only in sample Al-Sabain 1203 which showed heterozygosity for an adenine to cytosine substitution in codon 22 (GAA→GCA). This predicts the replacement of alanine by glutamic acid, which occurs in Hb G Coushatta (132).

12.4. Maternal blood contamination

None of the samples in this study showed obvious signs of maternal blood contamination which is considered when adult Hb (A+ S+ any other adult Hb) level is more than Hb F level and a definite Hb A_2 peak on the HPLC or prominent A_2 band on IEF (133). However, examination of the *Dde*I digested PCR products of the first exon of the beta globin gene showed a discrepant beta-A:beta-S product ratio that might be a maternal blood contamination. On review of these discrepancies of beta-A:beta-S product ratio and then the other

HPLC results, a combination of both adult Hb > 20% and Hb A_2 >0.5% had been suggested as a criteria to assess what difference this would make to the conclusions of this study.

According to the suggested criteria in this study that arrived at empirically to indicate possible contamination with maternal blood, of 1500 samples 40 samples (2.7%) showed levels of Hb A_2 >0.5% and adult Hb>20%. Only two of them with Hb S gene which is not thought to have had a major effect on the conclusions drawn from this study.

12.5. Results of alpha thalassaemia mutations $-\alpha^{3.7}$ and $-\alpha^{4.2}$

PCR amplification of the common alpha thalassaemia mutations $-\alpha^{3.7}$ and $-\alpha^{4.2}$ did not show PCR products on the gel electrophoresis. This may be due to the nature of dried blood spots where deterioration of the blood samples from the time it is taken as oxidation of the haemoglobin occurs, resulting in methaemoglobin formation. This degradation is likely to be greater at higher temperatures and long storage. The blood samples on Guthrie cards used in this study were collected between July and December 2001. After one year, they were analysed for alpha thalassaemia genes. In addition, the PCR product (DNA) is large (1.8 kb) which makes it easier to degrade by long storage, in normal circumstances this should not prevent analysis using the DNA techniques. This states the prevalence of mutations $-\alpha^{3.7}$ and $-\alpha^{4..2}$ in Yemen and their interaction with Hb S remained unsolved in this study.

12.6. Prevalence of haemoglobin S

Tables 23 a-b show the places of origin of the parents of the children tested and found to have haemoglobin variants. The names of the parents recorded from the mother maternity file while their places of origin were reported by asking the mothers soon after delivery in the labour room. Figure 21 shows the distribution of sickle cell trait and total collected number of samples in each governorate of Yemen. No Hb S homozygotes were detected in this study (Fig. 22).

Table 23.c shows the calculated Hb S gene frequencies. The overall Hb S gene frequency was 0.011. The accurate estimation of the Hb S gene prevalence was calculated using 95% confidence interval. Thus the calculated standard error is 0.0027 and the 95% confidence limits would be 0.0164 to 0.0056. The Hb S gene frequency was highest (probably>0.01) in the following governorates: Haja: 0.0033 to 0.0500, Taiz: 0.0380 to 0.0439, Al-Hodeidah: 0.0185 to 0.0370, Amran: 0.0227 and Abyan: 0.0000 to 0.0385. The governorates of Sana'a and Sana'a City, Ibb, Dhamar, Al-Baida and Al-Mahweet appear to have lower (probably<0.01) Hb S gene frequencies (0.0067 to 0.0081, 0.0028, 0.0000 to 0.0036, 0.0000 to 0.0094 and 0.0000 to 0.0111 respectively).

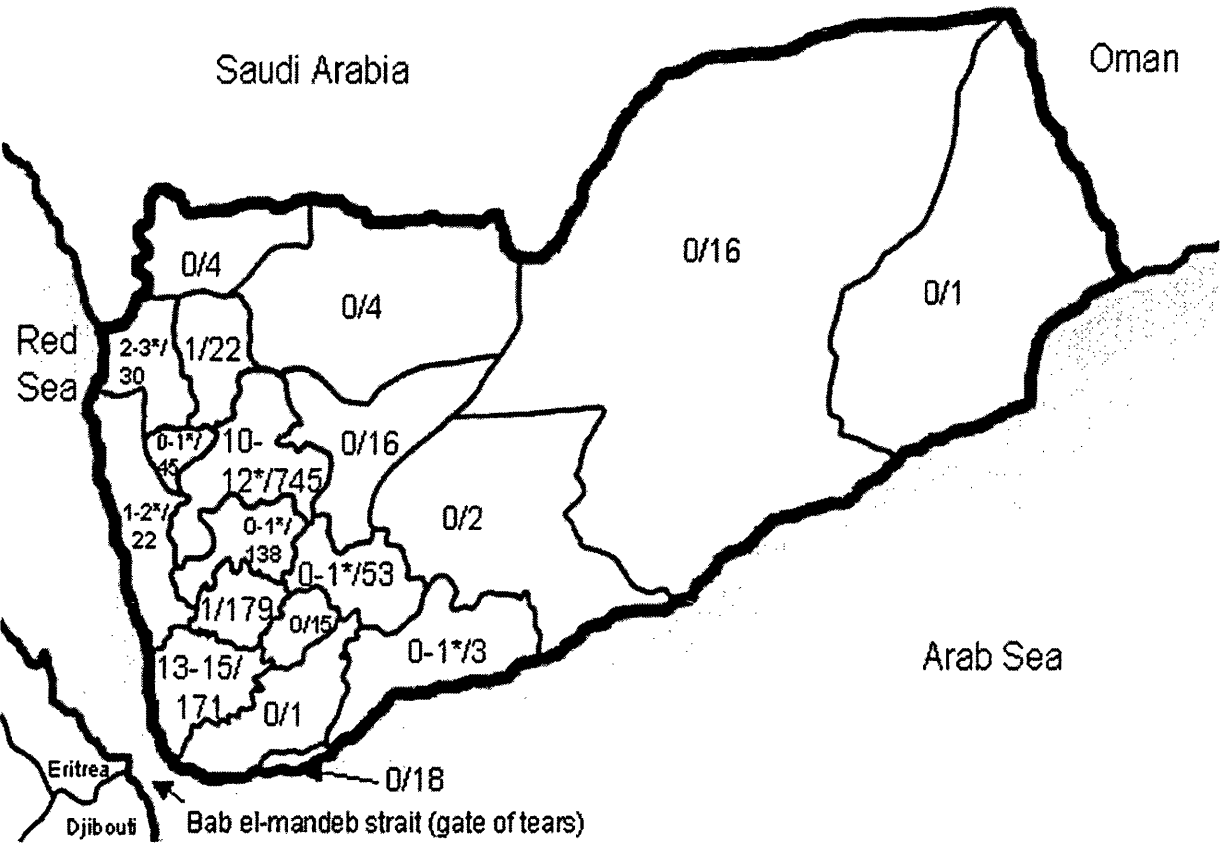


Figure 21 Distribution of sickle cell trait and the total number of sample in each governorate

Table 23.a The hospitals from which the blood samples found to contain variant haemoglobins were collected and the place of origin of each parent

Hb FSA	Hospital	Sample No.	Mother's Origin	Father's Origin
1	Mother	9	Taiz	Sana'a
2	Al-Sabain	1046	Taiz	Taiz
3	Al-Sabain	1144	Sana'a	Sana'a
4	Al-Sabain	1194	Taiz	Taiz
5	Al-Sabain	1217	Sana'a	Sana'a
6	Al-Sabain	1238	Haja	Al-Hodeidah
7	Al-Sabain	1300	Taiz	Taiz
8	Al-Sabain	1319	Taiz	Taiz
9	Al-Sabain	1321	Taiz	Taiz
10	Al-Sabain	1376	Sana'a	Sana'a
11	Al-Sabain	1591	Al-Hodeidah	Al-Hodeidah
12	Al-Sabain	1616	Taiz	Taiz
13	Al-Sabain	1753	Sana'a	Sana'a
14	Al-Sabain	1765	Taiz	Taiz
15	Al-Sabain	1794	Taiz	Abyan
16	Al-Sabain	1719	Sana'a	Sana'a
17	Al-Sabain	1732	Ibb	Ibb
18	Al-Thawra	34	Sana'a	Sana'a
19	Al-Thawra	87	Taiz	Taiz
20	Al-Thawra	553	Taiz	Taiz
21	Al-Thawra	163	Dhamar	Al-Beida
22	Al-Kawait	24	Sana'a	Sana'a
23	Al-Kawait	37	Sana'a	Sana'a
24	Al-Kawait	41	Taiz	Taiz
25	Al-Kawait	69	Sana'a	Al-Mahweet
26	Al-Kawait	76	Haja	Haja
27	Al-Thawra	834	Taiz	Taiz
28	Al-Thawra	250	Taiz	Taiz
29	Al-Thawra	261	Taiz	Taiz
30	Al-Thawra	264	Haja	Haja
31	Al-Thawra	269	Sana'a	Sana'a
32	Al-Thawra	423	Amran	Amran
33	Al-Thawra	493	Sana'a	Sana'a
HbFA?				
34	Al-Sabain	1203	Ibb	Ibb
35	Al-Sabain	1771	Al-Beida	Al-Beida
36	Al-Thawra	2	Al-Mahweet	Al-Mahweet
37	Al-Thawra	402	Dhamar	Dhamar

Table 23.b Summary of the abnormal haemoglobins results

Hospital	Sample	HPLC	IEF	DNA	Mother's Origin	Father's Origin
Sabain	1203	A+Hb?	A+Hb?	AG-Coushatta	Ibb	Ibb
Sabain	1771	A+Hb?	A+Hb?	A+non β globin	Al-Beida	Al-Beida
Thawra	2	A+Hb?	A+Hb?	A+non β globin	Mahweet	Mahweet
Thawra	402	A+Hb?	A+Hb?	A+non B globin	Dhamar	Dhamar

Table 23.c Prevalence of sickle cell trait in different governorates in Yemen

Governorate	Investigated sample number	Prevalence Hb AS #		Haemoglobin S gene frequency
		Number	%	
Sana'a&Sana'a City	745	10 -12*	1.34 - 1.61	0.0067 - 0.0081
Aden	18	0	0.0	0.0000
Taiz	171	13 - 15*	7.6 - 8.77	0.0380 - 0.0439
Al-Hodeidah	27	1 - 2*	3.7 - 7.4	0.0185 - 0.0370
Laheg	1	0	0.0	0.0000
Ibb	179	1	0.56	0.0028
Abyan	13	0 - 1*	0.0 - 7.69	0.0000 - 0.0385
Dhamar	138	0 - 1*	0.0 - 0.73	0.0000 - 0.0036
Shabwah	2	0	0.0	0.0000
Haja	30	2 - 3*	6.67 – 10	0.0333 - 0.0500
Al-Beida	53	0 - 1*	0.0 - 1.9	0.0000 - 0.0094
Hadramout	16	0	0.0	0.0000
Saadah	4	0	0.0	0.0000
Al-Mahweet	45	0 - 1*	0.0 - 2.22	0.0000 - 0.0111
Al-Mahrah	1	0	0.0	0.0000
Mareb	16	0	0.0	0.0000
Al-Jawf	4	0	0.0	0.0000
Amran	22	1	4.55	0.0227
Al-Daeh	15	0	0.0	0.0000
Total	**1,500**	**33**	**2.2**	**0.0110**

* The infant has parents who come from different governorates and the HbS gene is from one or the other.
# No Hb S homozygotes were found

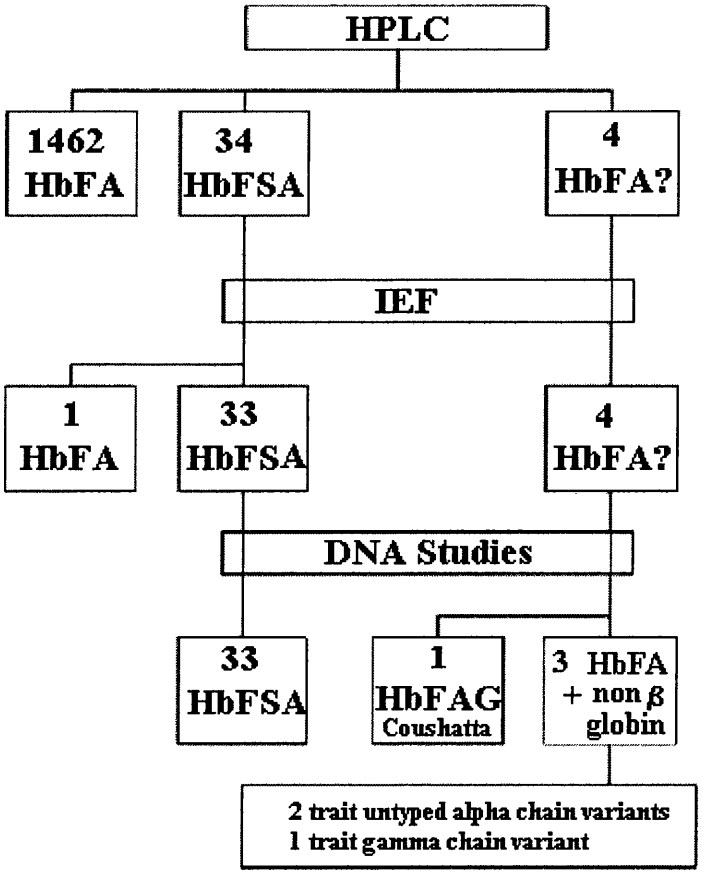


Figure 22 Summary of HPLC, IEF, DNA analysis results

12.7. Prevalence of the *Xmn* I polymorphism

Table 24.a shows only one of the 66 chromosomes (1.5%) from the samples indicating sickle cell trait (Hb FAS) showed the presence of the G-gamma globin gene promoter *Xmn* I site (Fig.23), i.e. 3.0% maximum of the Hb S chromosomes, compared with 27 (16.1%) of the 168 chromosomes from HbFA samples carefully selected from the same governorates as the Hb FAS samples. Table 24.b shows the prevalence of *Xmn* I among HbS chromosomes from different governorates in Yemen and which was only 1.5 % in Sana'a and Sana'a City. Table 24.c shows the prevalence of *Xmn* I among HbFA chromosomes which was highest in Abyan and Dhamar (50%), followed by Al-Hodiedah (30%), Sana'a and Sana'a City (18%) and were 16.7 % in Lahge

and Al-Mahweet, 12.9 in Taiz and not detected in the rest governorates. Table 24.d compares the prevalence of *Xmn* I among chromosomes with and without Hb S in both groups, and shows the higher prevalence of *Xmn* I among the non sickle cell carries.

The Hb S mutation in Yemen is therefore not usually on the Arab-Indian haplotype that is associated with a milder clinical course. The western coastal part of Yemen that consisted from Taiz, Al-Hodiedah and Haja governorates showed 11 *Xmn* I positive chromosomes from 84 total chromosomes among the non-Hb S samples and this results gives the prevalence of *Xmn* I of 13.1% in the western coast of Yemen while among the Hb S group is zero (Table 24.b).

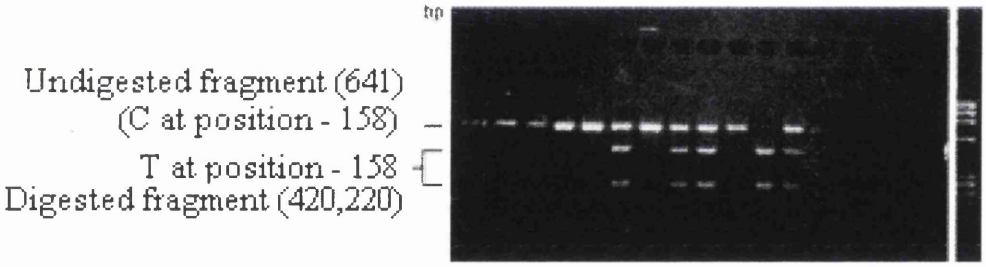


Figure 23 Gel electrophoresis of the polymorphism *Xmn* I

Table 24.a The *Xmn* I results among sickle cell trait and non-sickle cell samples

Hb FSA	Sample No.	Mother's Origin	Father's Origin	*Xmn* I
1	Mot[1]9	Taiz	Sana'a	- / -
2	Sab[2]1046	Taiz	Taiz	- / -
3	Sab 1144	Sana'a	Sana'a	- / -
4	Sab 1194	Taiz	Taiz	- / -
5	Sab 1217	Sana'a	Sana'a	- / -
6	Sab 1238	Haja	Al-Hodeidah	- / -
7	Sab 1300	Taiz	Taiz	- / -
8	Sab 1319	Taiz	Taiz	- / -
9	Sab 1321	Taiz	Taiz	- / -
10	Sab 1376	Sana'a	Sana'a	+ / -
11	Sab 1591	Al-Hodeidah	Al-Hodeidah	- / -
12	Sab 1616	Taiz	Taiz	- / -
13	Sab 1753	Sana'a	Sana'a	- / -
14	Sab 1765	Taiz	Taiz	- / -
15	Sab 1794	Taiz	Abyan	- / -
16	Sab 1719	Sana'a	Sana'a	- / -
17	Sab 1732	Ibb	Ibb	- / -
18	Tha[3]34	Sana'a	Sana'a	- / -
19	Tha 87	Taiz	Taiz	- / -
20	Tha 553	Taiz	Taiz	- / -
21	Tha 163	Dhamar	Al-Beida	- / -
22	Kuw 24	Sana'a	Sana'a	- / -
23	Kuw 37	Sana'a	Sana'a	- / -
24	Kuw 41	Taiz	Taiz	- / -
25	Kuw 69	Sana'a	Al-Mahweet	- / -
26	Kuw 76	Haja	Haja	- / -
27	Tha 834	Taiz	Taiz	- / -
28	Tha 250	Taiz	Taiz	- / -
29	Tha 261	Taiz	Taiz	- / -
30	Tha 264	Haja	Haja	- / -
31	Tha 269	Sana'a	Sana'a	- / -
32	Tha 423	Amran	Amran	- / -
33	Tha 493	Sana'a	Sana'a	- / -
Hb FA?				
34	Sab 1203	Ibb	Ibb	- / -
35	Sab 1771	Al-Beida	Al-Beida	- / -
36	Tha 2	Al-Mahweet	Al-Mahweet	- / -
37	Tha 402	Dhamar	Dhamar	+ / +
Hb FA				
38	Tha 59	Dhamar	Dhamar	+ / -
39	Sab 1736	Sana'a	Sana'a	- / -
40	Sab 1737	Sana'a	Sana'a	+ / -
41	Sab 1738	Sana'a	Sana'a	- / -
42	Sab 1739	Sana'a	Sana'a	- / -
43	Sab 1001	Sana'a	Sana'a	+ / -

44	Sab1002	Al-Hodeidah	Al-Hodeidah	- / -
45	Sab 1003	Al-Hodeidah	Al-Hodeidah	- / -
46	Sab 1004	Haja	Haja	- / -
47	Sab 1005	Dhamar	Dhamar	- / -
48	Sab 1006	Sana'a	Sana'a	- / -
49	Sab 1007	Taiz	Taiz	- / -
50	Mot 1	Ibb	Ibb	+ / -
51	Kuw^{4}5	Amran	Amran	- / -
52	Tha 3	Al-Mahweet	Al-Mahweet	- / -
53	Tha 23	Taiz	Taiz	- / -
54	Tha 102	Al-Beida	Al-Beida	- / -
55	Sab 1201	Haja	Haja	- / -
56	Sab 1009	Al-Beida	Al-Beida	- / -
57	Sab 1048	Al-Mahweet	Al-Mahweet	+ / -
58	Sab 1190	Ibb	Ibb	- / -
59	Sab 1465	Sana'a	Sana'a	+ / -
60	Sab 1466	Sana'a	Sana'a	- / -
61	Sab 1468	Sana'a	Sana'a	+ / -
62	Sab 1469	Sana'a	Sana'a	+ / -
63	Sab1470	Sana'a	Sana'a	- / -
64	Sab 1471	Sana'a	Sana'a	- / -
65	Sab 1472	Sana'a	Sana'a	- / -
66	Sab 1473	Sana'a	Sana'a	+ / -
67	Sab 1479	Sana'a	Sana'a	- / -
68	Sab 1480	Sana'a	Sana'a	- / -
69	Sab 1481	Sana'a	Sana'a	+ / -
70	Sab 1482	Sana'a	Sana'a	- / -
71	Sab 1483	Sana'a	Sana'a	- / -
72	Sab 1485	Sana'a	Sana'a	+ / -
73	Sab 1486	Sana'a	Sana'a	+ / -
74	Sab 1487	Sana'a	Sana'a	- / -
75	Sab 1488	Sana'a	Sana'a	- / -
76	Sab 1490	Sana'a	Sana'a	- / -
77	Sab 1493	Sana'a	Sana'a	- / -
78	Sab 1348	Taiz	Taiz	+ / -
79	Sab 1351	Taiz	Taiz	- / -
80	Sab 1356	Taiz	Taiz	- / -
81	Sab 1398	Taiz	Taiz	- / -
82	Sab 1401	Taiz	Taiz	- / -
83	Sab 1411	Taiz	Taiz	- / -
84	Sab 1420	Taiz	Taiz	+ / -
85	Sab 1444	Taiz	Taiz	- / -
86	Sab 1449	Taiz	Taiz	- / -
87	Sab 1461	Taiz	Taiz	- / -
88	Sab 1476	Taiz	Taiz	+ / -
89	Sab 1489	Taiz	Taiz	- / -
90	Sab 1492	Taiz	Taiz	- / -
91	Sab 1494	Taiz	Taiz	- / -

92	Sab 1498	Taiz	Taiz	+ / -
93	Sab 1507	Taiz	Taiz	+ / -
94	Sab 1515	Taiz	Taiz	- / -
95	Sab 1519	Taiz	Taiz	+ / -
96	Sab 1522	Taiz	Taiz	- / -
97	Sab 1527	Taiz	Taiz	- / -
98	Sab 1539	Taiz	Taiz	- / -
99	Sab 1547	Taiz	Taiz	- / -
100	Sab 1550	Taiz	Taiz	- / -
101	Sab 1593	Taiz	Taiz	- / -
102	Sab 1602	Taiz	Taiz	- / -
103	Sab 1606	Taiz	Taiz	- / -
104	Sab 1639	Taiz	Taiz	+ / -
105	Sab 1740	Taiz	Taiz	- / -
106	Sab 1756	Taiz	Taiz	+ / -
107	Sab 1303	Al-Hodeidah	Al-Hodeidah	+ / -
108	Sab 1312	Al-Hodeidah	Al-Hodeidah	- / -
109	Sab 1353	Al-Hodeidah	Al-Hodeidah	+ / +
110	Sab 1436	Abyan	Abyan	+ / -
111	Sab 1514	Abyan	Abyan	+ / -
112	Sab 1400	Haja	Haja	- / -
113	Sab 1434	Haja	Haja	- / -
114	Sab 1755	Haja	Haja	- / -
115	Sab 1777	Haja	Haja	- / -
116	Sab 1467	Amran	Amran	- / -
117	Sab 1529	Amran	Amran	- / -

1-Mother, 2- Al-Sabain, 3- Al-Thawra, 4-K-Al-Kuwait.

Table 24.b The prevalence of *Xmn*I among Hb AS samples

Governorate	Number of chromosomes	Positive *Xmn* I	Prevalence *Xmn* I (%)
Sana'a &Sana'a City	20-24	1	1.5
Aden	0	0	0
Taiz	26-30	0	0
Al-Hodiedah	2-4	0	0
Laheg	0	0	0
Ibb	2	0	0
Abyan	0-2	0	0
Dhamar	0-2	0	0
Shabwah	0	0	0
Haja	4-6	0	0
Al-Beida	0-2	0	0
Hadramout	0	0	0
Saadah	0	0	0
Al-Mahweet	0-2	0	0
Al-Mahrah	0	0	0
Mareb	0	0	0
Al-Jawf	0	0	0
Amran	2	0	0
Al-Daleh	0	0	0
Total	66	1	1.5

Table 24.c The prevalence of *Xmn* I among non-HbAS samples

Governorate	Numbers of chromosomes	No.of Positive *Xmn* I	Prevalence *Xmn* I (%)
Sana'a &Sana'a City	50	9	18
Aden	0	0	0
Taiz	62	8	12.9
Al-Hodiedah	10	3	30
Laheg	0	0	0
Ibb	6	1	16.7
Abyan	4	2	50
Dhamar	6	3	50
Shabwah	0	0	0
Haja	12	0	0
Al-Baida	6	0	0
Hadramout	0	0	0
Saadah	0	0	0
Al-Mahweet	6	1	16.7
Al-Mahrah	0	0	0
Mareb	0	0	0
Al-Jawf	0	0	0
Amran	6	0	0
Al-Daleh	0	0	0
Total	168	27	16.1

Table 24.d Comparison between the prevalence of *Xmn* I among HbAS and non-HbAS samples in different governorates in Yemen

Governorate	Hb AS (%)	Non-Hb AS (%)
Sana'a&Sana'a City	1.5	18
Aden	0	0
Taiz	0	12.9
Al-Hodiedah	0	30
Laheg	0	0
Ibb	0	16.7
Abyan	0	50
Dhamar	0	50
Shabwah	0	0
Haja	0	0
Al-Baida	0	0
Hadramout	0	0
Saadah	0	0
Al-Mahweet	0	16.7
Al-Mahrah	0	0
Mareb	0	0
Al-Jawf	0	0
Amran	0	0
Al-Daleh	0	0
Total	1.5	16.1

12.8. Results of the β globin gene polymorphisms

Twenty six of the thirty three of the sickle cell trait samples (79%) in this study showed absence of *Xmn* I, *Hind* III/$^{G}\gamma$ and *Hind* III/$^{A}\gamma$ polymorphic sites (Table 25.a) and this pattern is seen in Benin sickle cell haplotype. The polymorphic site *Hind*/$^{G}\gamma$ showed heterozygous in other 5 samples while *Hind* III/ $^{A}\gamma$ showed absence in 31 samples. The result of the polymorphic site *Hind* II/ε showed that 6 samples are absence and twenty six are heterozygous (-/+) where the results of the polymorphic sites *Hinf* I/5'β appeared only 2 samples are absence and 2 homozygous positive and the rest are heterozygous (Table 25.b). In addition to the 26 samples with Benin pattern haplotype, samples number 11

and 13 have another Benin haplotype pattern while samples 28 and 30 show pattern can be seen in either in Benin or Bantu haplotypes, and sample 32 showed polymorphic sites pattern seen in either Benin or Cameroon haplotypes. Finally, the pattern of the polymorphic sites seen in sample 31 is found in the Bantu sickle cell haplotype.

The high heterozygousity results of *Hind* II/ε, *Hinc* II/3'φβ and *Hinf* I/5'β do not exclude the Benin sickle cell haplotype because the absence polymorphic site might be located on the allele which carries the hemoglobin S mutation.

Table 25.a Results of the beta globin gene polymorphic sites

Hb FSA	Sample number	Mother's Origin	Father's Origin	*Hind* II/ε	*Xmn* I	*Hind* III/$^{G}\gamma$	*Hind* III/$^{A}\gamma$	*Hinc* II/3'φβ	*Hinf* I/5'β
1	Mot[1] 9	Taiz	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
2	Sab[2] 1046	Taiz	Taiz	-/+	- / -	-/-	-/-	-/-	-/+
3	Sab 1144	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
4	Sab 1194	Taiz	Taiz	-/+	- / -	-/-	-/-	-/-	+/+
5	Sab 1217	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
6	Sab 1238	Haja	Hodeidah	-/+	- / -	-/-	-/-	-/+	-/+
7	Sab 1300	Taiz	Taiz	-/+	- / -	-/+	-/-	-/+	-/+
8	Sab 1319	Taiz	Taiz	-/-	- / -	-/-	-/-	-/+	-/+
9	Sab 1321	Taiz	Taiz	-/+	- / -	-/-	-/-	-/-	-/+
10	Sab 1376	Sana'a	Sana'a	-/+	+ / -	-/+	-/-	-/+	-/+
11	Sab 1591	Hodeidah	Hodeidah	-/-	- / -	-/-	-/-	+/+	-/-
12	Sab 1616	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
13	Sab 1753	Sana'a	Sana'a	-/-	- / -	-/+	-/-	+/+	-/+
14	Sab 1765	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
15	Sab 1794	Taiz	Abyan	-/+	- / -	-/-	-/-	-/+	-/+
16	Sab 1719	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
17	Sab 1732	Ibb	Ibb	-/+	- / -	-/-	-/-	-/+	-/+
18	Tha[3]34	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/-
19	Tha 87	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
20	Tha 553	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
21	Tha 163	Dhamar	Al-Beida	-/+	- / -	-/-	-/-	-/+	-/+
22	Kuw 24	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
23	Kuw 37	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+
24	Kuw 41	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
25	Kuw 69	Sana'a	Mahweet	*	- / -	-/-	-/-	-/+	-/+
26	Kuw 76	Haja	Haja	-/+	- / -	-/-	-/-	-/+	-/+
27	Tha 834	Taiz	Taiz	-/+	- / -	-/-	-/-	-/-	+/+
28	Tha 250	Taiz	Taiz	-/-	- / -	-/+	-/-	*	-/+
29	Tha 261	Taiz	Taiz	-/+	- / -	-/-	-/-	-/+	-/+
30	Tha 264	Haja	Haja	-/+	- / -	*	-/-	-/+	-/+
31	Tha 269	Sana'a	Sana'a	-/-	- / -	*	*	-/-	-/+
32	Tha 423	Amran	Amran	-/-	- / -	-/+	*	+/+	-/+
33	Tha 493	Sana'a	Sana'a	-/+	- / -	-/-	-/-	-/+	-/+

*No result (no band on the gel electrophoresis)

Benin sickle cell haplotype	-	-	-	-	+	-

Table 25.b Summary of beta globin gene polymorphic sites results

Results	*Hind* II/ε	*Xmn* I	*Hind* III/$^{G}\gamma$	*Hind* III/$^{A}\gamma$	*Hinc* II/3'φβ	*Hinf* I/5'β
Homozygous negative	6 (18%)	32 (97%)	26 (79%)	31 (94%)	6 (18%)	2 (6%)
Heterozygous Neg/Pos	26 (39-79)	1 (1.5-3%)	5 (8-15%)	0	23 (35-70%)	29 (44-8%)
Homozygous positive	0	0	0	0	3 (9%)	2 (6%)
No result	1	0	2	2	1	0

12.9. Survey results of the sickle cell anaemia patients

Because the sickle cell anaemia patients were children, their parents or relatives answered the questions of this survey following verbal consent. In addition, due to incomplete medical records for most of the sickle cell patients, many of these questions were answered inadequately.

Questions about the sickle cell diagnosis and blood transfusion answered with the medical records support.

Table 26.a Age of the patients (n=86)

Age in years	Number of patients	%
Less than 5	33	38
6 – 12	49	57
13 – 15	4	5

Table 26.b Diagnosis of sickle cell disorders for the first time (n=86)

When Diagnosed	Number of patients
At birth	0
Before the appearance of the symptoms	4
After the appearance of the symptoms	69
During sickle cell complications	13
On death.	0

Eighty percent of the patients were diagnosed for first time with sickle cell disorder after the appearance of clinical signs and symptoms while 15% during the complication of the disorder and 5% before the appearance of the clinical symptoms. No patients were diagnosed at birth.

Table 26.c How was sickle cell disorder diagnosed? (n=86)

Method of sickle cell diagnosis	Number of patients
Clinical signs and symptoms, family health history, blood smear	26
Clinical signs and symptoms, family health history, blood smear, sickling test	30
Clinical signs and symptoms, family health history, blood smear, sickling test, haemoglobin electrophoresis test	30
Other methods (HPLC, IEF, DNA Studies)	0

In addition to the clinical, family history and physical signs in 30% of the patients the only other laboratory test done to confirm the diagnosis was blood smear. In 35% of the patients, the diagnosis was confirmed with a sickling test while the reminder had haemoglobin electrophoresis. None of the patient had HPLC, IEF or DNA analysis.

Table 26. d Health care after the diagnosis sickle cell disorder (n=86)

Heath care after diagnosis	Number of patients
Knowing sickle cell patient registry	0
Knowing sickle cell association	0
Knowing sickle cell health centre	0
Having sickle cell disorders card	0
Doing regular health evaluation	9
Doing regular or often laboratory tests	52
Knowing what is sickle cell disorder	42
Knowing what to do during crisis	58
Avoiding sickle cell disorder in the future	82
Having written materials about sickle cell	0

Table 26.e Sickle cell management (n=26)

Sickle cell management	Number of patients
In crisis, do you go to hospital or clinical centre	82
Treating by haematologist	0
Using haematinics	86
Using antibiotic as prophylactic	0
Having immunizations (BCG, Diphtheria, Tetanus, Portusis, Measles, Polio, Hepatitis B)	82
Instructing to improve your hygiene	59
Instructing to improve your quality of the food	61
Avoiding extreme weather	82
Avoiding dehydration	82
Using chelating agent	0
Using hydroxyurea	0

hygiene and nutrition. Ninety-five precent of the patients was advised to avoid extreme weather and dehydration.

Table 26.f Hospitalisation of sickle cell patients each year (n=86)

Hospitalisation each year	Number of patients
One	4
Twice	9
Three times	30
More than three times	43

Most of the patients (84%) were hospitalised three or more times per year where few (15%) of the patients were hospitalised twice or less.

Table 26.g Hospitalisation period of sickle cell patients each year (n=86)

Period of hospitalisation each year	Number of patients
7 days or less	2
One month or less	76
More than a month	8

Most of the patients (88%) hospitalised for a period between one week and a month.

Table 26.h Blood transfusion services for sickle cell patients (n=86)

Transfused blood	Number of patients
Fresh blood	0
Stored blood	86
Blood from relatives	44
Blood from blood bank	42
Cross matching with ABO Rh	86
Cross matching with ABO Rh +Kell antigen	0

About 36% of the patients had transfused blood between 1000 and 2000 mm with whole blood each year where 31% had more than 2000 ml and only 6% of the patients had less than 1000 ml of blood. Twenty-three patients (27%) did not know the quantity of the transfused blood.

Table 26.j Cost of health care for sickle cell patient (n=86)

Cost of health care	Number of patients
Cost of blood transfusion	86 (partially paid)
Cost of the prescriptions	86
Cost of hospitalisation	86 (partially paid)

All the patients paid all the cost of their prescriptions and partially for the hospitalisation and blood transfusion.

Causes of death

All the conducted patients were alive at the time of the interview. Three doctors worked with sickle cell patients in Al-Thawra Hospital were interviewed to answer this question, and they suggested that the major causes of death among the sickle cell patients were severe anaemia and infections. The blood transfusion services are existed only in main hospitals that were built in large cities where only 26% of the population live in. Patient's family

Haja, Al-Hodeidah and Taiz governorates the Hb S gene frequency probably ranges from 0.0185 to 0.0500, and is 0.035 to 0.044 for these combined regions. The southern governorates such as Abyan shows a Hb S gene frequency of zero to 0.0385, and no Hb S was detected amongst samples from the eastern and southern (desert) parts of the country such as Mareb, Al-Jawf, Shabwah, Hadramount, Al-Mahrah, Aden, Laheg and Al-Daleh.

Three of the 38 samples showing haemoglobin variants contain non-β globin gene variants and further investigations are required to identify them. The study has demonstrated the first recorded case of Hb G Coushatta in the Yemeni population. One of the 66 chromosomes (1.5%) from the sickle cell trait samples showed the presence of the G-gamma globin gene promoter *Xmn* I site compared with 27 (16.1%) of the 168 chromosomes from Hb FA samples selected from the same governorates as the Hb FAS samples. In 79% of the sickle cell trait samples, absence of *Xmn* I, *Hind* III/$^{G}\gamma$ and *Hind* III/$^{A}\gamma$ polymorphic sites in same sample were found.

A survey of patients/families with sickle cell anaemia showed there is no national patient register for sickle cell diseases in Yemen, and no patient

nature of the blood tests that had done for them. About 49% of the patients knew that sickle cell is a sort of genetic blood disorder and to some extent that they have crescent shaped cells. Also 67% of the patients went to emergency departments in hospital during crisis.

13.2 Internal validity of the findings

Despite careful supervision and communication regarding the manner in which the cord blood should be collected maternal blood was present in a small number of samples. Neonatal blood collection would avoid this problem but would have been much more difficult to organise.

The total samples were collected from people from different regions of Yemen living in the capital, Sana'a City, therefore these samples were representative of the population of Yemen. The number of samples collected form the southern governorates was often too small for any firm conclusion to be drawn.

The absence of any homozygous Hb S samples in this study is probably due to a low number of samples from the west coastal governorates (Haja, Taiz and Al-Hodeidah) comprising only 15.2% of the total (228 of 1500).

prevalence of the common α^+ and α^0 thalassaemia mutations among the sickle cell trait samples in this study is not determined.

13.3 External validity of the findings

It is estimated that the annual number of malaria cases in Yemen is around 3 million (134) and the highest Hb S gene frequency was found in samples from the endemic malarial area that is the west coastal part of the country adjacent to the sea separating it from east Africa. In samples from the west coastal part of Yemen the Hb S gene frequency is 0.035 to 0.044. This is lower (p <0.01; χ^2 test) than the 0.0765 frequency recorded in southwest Saudi Arabia, the geographic extension of the west coastal strip of Yemen (66). A similar magnitude of difference was observed between samples from the highest frequency localities and those from their eastern neighbours including Sana'a city.

The prevalence of Hb S in the eastern governorates adjacent to Oman's border showed almost no cases of sickle cell trait and this agreed with the very low prevalence of sickle cell gene found in Dhofar region, West of Oman (70).

samples that were identified in this study.

The pattern of absence of *Xmn* I, *Hind* III/$^{G}\gamma$ and *Hind* III/$^{A}\gamma$ polymorphic sites is present in the Benin sickle cell haplotype and not in the Arab-Indian sickle cell haplotype. Therefore, the predominant sickle cell haplotype in Yemen is the African haplotype, more likely the Benin haplotype. This is supported by the findings of El-Hazmi (1999) study that showed the prevalence of the Benin sickle cell haplotype is 98.5% in the south western region of Saudi Arabia (69). This region is the geographic extension of the Yemeni western costal part, in which the prevalence of the sickle cell gene is the highest in this study.

The prevalences studies that were performed in Saudi Arabia (66), Oman (70), Bahrain (72) and United Arab Emirates (62) are completely or partially hospital based studies and samples were collected from both healthy and unhealthy individuals seeking medical help from health centres, outpatient clinics, hospital wards. Therefore, the results of these studies of such selected group of patients can be biased. In these studies, some of their samples were collected from adults, which may have underestimated prevalence of sickle cell

whose parents were from Taiz, Hajja and Al-Hodeidah respectively, which would predict a homozygous birth rate of approximately 1 in 520 to 1 in 692. This may be somewhat higher depending upon the proportion of consanguineous marriage and marriages within the same tribe, and easily missed in a sample size of 228. In those areas where double heterozygosity for β thalassaemia and Hb S occurs the rate of births affected by sickle cell disorders will be higher than that predicted from the frequency of Hb S alone.

Results of HPLC suggest the absence of β^0 thalassaemia, since all the samples showed presence of haemoglobin A. The absence of any of these haemoglobins C, E, and D indicates their prevalences are very low in Yemen.

Further investigations are needed to determine alpha chain and gamma chain variants involved in this study and reported as non-beta globin chain. Haemoglobin G Coushatta is not of clinical importance but other Hb variants and thalassemia may be, and it would therefore be important to study their prevalence in Yemen.

and *Hind* III/$^{A}\gamma$ polymorphic sites on the beta globin cluster in the sickle cell trait samples of this study.

The presence of the African sickle cell haplotype in Yemen, like the Benin haplotype, matches the clinical severity of the sickle cell clinical course among Yemeni patients as indicated by the survey results in this study.

The Benin sickle cell haplotype, which is found in south western Saudi Arabia (69,135), probably came from central West Africa through the slave trade routes and perhaps subsequently to the western region of Yemen. The Hb S gene in the other side of the Red Sea that separates Yemen from African countries- Ethiopia, Somalia and Djibouti showed prevalences of 0 - 1% which are much lower than that in western part of Yemen. Therefore, the existence of the Benin sickle cell haplotype in Yemen supports the argument of Nagel (1991) and Kulozik (1986) who suggested that population of West Africa that have African haplotypes have migrated to North Africa, the Mediterranean, then to Southwest of the Arabian Peninsula (136,137) where Yemen is located.

in Yemen are mainly children, perhaps due to poor survival of sickle cell patient to adult life. The high amount of the transfused blood (more than 1000 ml/year/patient) in 67.4% of the patients, the high frequency of hospital admission (three or more times in a year) in 84.9% of the patients and the long time of hospitalisations (8-30days) in 97.7% of the patients suggest that patients suffer frequently from health problems related to their sickle cell disorder due to either the severity of the sickle cell disorder or poor health care services or both.

Ninety-five precent of the sickle cell patients were diagnosed either after the appearance of clinical symptoms or complications. This suggests the absence of neonatal and antenatal screening programmes for sickle cell in Yemen. None of the patients were diagnosed by HPLC, IEF or DNA studies which demonstrate the limitations of clinical laboratory services in the hospitals of Sana'a City, Yemen, and suggests DNA analysis services have not been implemented yet in these laboratories.

diseases such as malaria infection, tuberculosis and schistosomiasis, in addition to the poor health administration in Yemen might be also responsible for the neglect of these disorders in Yemen.

The absence of use the hydroxyurea or chelating agent (desferal) in cases of iron overload, suggests doctors may have no experience of using these treatments in sickle cell patients.

No transfused blood had been cross-matched with Kell group, which suggests poor skill, limited knowledge or inadequate financial resources for the blood transfusion services. Only about half of the patients knew their sickle cell disorder and about half of the patients attended hospitals for regular laboratory tests or health evaluation, which indicates a poor level of knowledge in these patients or their relatives about sickle cell disorder.

A wider survey is required to determine the definite clinical characteristics of sickle cell disorder in Yemen, since the sickle cell patients that were included in this survey were only those who attended hospitals in the Sana'a City. They were seeking help because of their suffering from the sickle cell disorder, but

picture among Yemeni patients (107) but this was a hospital-based study which might have been missed the mild sickle cell or misdiagnosed patients who did not attended the hospital.

This survey was carried out among sickle cell patients attended hospitals in Sana'a City. The health situation may be worse for sickle cell patients who live away from these health institutes, particularly the western coastal part of Yemen, where we found the highest prevalence of sickle cell gene.

This study suggests a higher Hb S gene frequency amongst babies born in the capital city of Yemen than obtained previously on migrants from the Yemen (62,64). In addition, it goes further by demonstrating an uneven distribution of the Hb S gene amongst people from different governorates, and the predominant of non- Arab- Indian (Benin) sickle cell haplotype in Yemen. These findings are important because they raise the possibility that services can be targeted at those most in need thus saving valuable resources. The communities most at risk in Taiz, Al-Hodeidah and Haja are not those who reside near hospitals and would therefore benefit from the development of community health services. An obvious limitation of the study is the inability

more precisely. Nevertheless from this study findings sickle cell anaemia is expected to be a particular problem in the western part of Yemen to which health resources and health education for families affected could be directed.

15. CONCLUSION

The results of this study suggest an overall HbS gene frequency is 0.011 with a higher frequency in the western coastal part of Yemen than in the central mountainous and eastern desert areas. The incidence of affected homozygous births may reach 20/10,000 in western areas although it is lower than this overall. Analysis of sickle cell haplotype in Yemen indicated it most likely belongs to the Benin haplotype that has a severe sickle cell clinical course. Results of the sickle cell anaemia patients survey show that the clinical services provided to the sickle cell patients in Yemen are very poor.

Limited health resources can best be invested in developing a program of education, premarriage counselling, screening and health care, prioritising those communities residing in the western areas of Yemen with the highest Hb S gene frequency.

4) Herrick, JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. *Arch Intern Med* 1910; **6:** 517-21.

5) Emmel, VE. A study of the erythrocytes in a case of severe anemia with elongated and sickle-shaped red blood corpuscles. *Arch Intern Med* 1917; **20:** 586 - 98.

6) Hahn, EV. Gillespie, EB. Sickle cell anemia: report a case greatly improved by splenectomy;experimental study of sickle cell formation. *Arch Intern Med* 1927; **39:** 233 - 54.

7) Sherman, IJ. The sickling phenomenon with special reference to the differentiation of sickle cell anemia from the sickle cell trait. *Bull Johns Hopkins Hospita*l 1940; **67:** 309 - 24.

8) Ingram, VM. Specific chemical difference between the globins of normal human and sickle-cell anemia haemoglobin. *Nature* 1956; **178:** 792 - 94.

9) Bain, B. Haemoglobin and the genetics of haemoglobin synthesis. *Haemoglobinopathy diagnosis.* London: Blackwell Science; 2001, p.2.

10) Taketa, P. Attermeier, MH. Mauk, GA. Acetylated hemoglobin in feline blood. *J Bio Chem.* 1972; **247:**33 - 5.

11) Huisman, THJ. Carver, MFH. Fremov, GD. *A Syllabus of Human Hemoglobin Variants*. 2nd edn. Sickle Cell Anemia Foundation, MCG, Augusta, GA, USA; 1998.

12) Serjeant, GR. Sickle-Cell disease. *Lancet* 1997; **350:**725-30.

13) Steinberg, MH. Management of sickle cell disease. *New Engl J Med* 1999; **340:**1021- 30.

14) Aladin, A. Modell, B. Czeizel, A. Hamamy, H. *Community control of genetic and congenital disorders*. Alexanderia: WHO EMRO Technical Publication Series 24; 1997

of 14-filament fibers of hemoglobin S. *J Mol Biol* 1979; **130:** 451 – 72.

20) Bunn, HF. Forget, BG. *Hemoglobin: molecular, genetic, and clinical aspects.* Philadelphia: W.B. Saunders Company; 1986.

21) Pauling, L. Itano, HA. Singer, SJ. Wells, IC. Sickle cell anemia, a molecular disease. *Science* 1949; **110:**543 - 48.

22) Briehl, RW. Evert, S. Effects of pH, 2-3Diphospholycerate and salts on gelation of sickle cell deoxyhemoglobin. *J Mol Biol* 1973; **80:** 445 - 58.

23) Hofrichter, J. Ross, PD. Eaton, WA. Supersaturation in sickle cell hemoglobin solutions. *Proc Natl Acsd Sci* 1976; **73:** 3035-39.

24) Kaul, DK. Nagel, RL. Chen, D. Tsai, HM. Sickle erythrocyte-endothelial interactions in microcirculation: the role of von Willebrand factor and implications for vasoocclusion. *Blood* 1993; **81:**2429 - 38.

25) Modell, B. *Update estimates of the frequency of the haemoglobin disorders in each country. Guidelines for the Control of Haemoglobin Disorders.* UK: World Health Organization, Hereditary Diseases Programme, Annex 1;1994.

26) Lawrence, C. Fabry, M. Nagel, RL. The unique red cell heterogeneity of SC disease: Crystal formation, dense reticulocytes and unusual morphology. *Blood* 1991;**78**: 2104 – 12.

27) Monplaisir, N. Merault, G. Poyart, C. Rhoda, MD. Craescu, C. Vidaud, M. HbS-Antilles: a new variant with a lower solubility than HbS and producing sickle cell disease in heterozygotes. *Proc Natl Acad Sci* 1986; **83:** 9363- 67.

28) Serjeant, GR. *Sickle Cell Disease.* 3rd edn. Oxford: Oxford University Press; 2001.

29) El-Hazmi, MA. Warsy, AS. Alpha Thalassaemia in Yemeni Children with Sickle Cell Disease. *J. Torp. Pediatr* 1999; **45:**370 - 74.

34) Langdown, JV. Williamson, D. Knight, CB. Rubenstein, D. Carrell, RW. A new doubly substituted sickling haemoglobin: HbS-Oman. *Br J Haematol* 1989; **71:** 445 - 46.

35) Jaiyesimi, F. Pandey, R. Bux, D. Sreekrishna, Y. Zaki, F. Krishnamoorthy, N. Sickle cell morbidity profile in Oman children. *Ann Trop Paediatr* 2002; **22:** 45-52.

36) Nagel, R. Daar, S. Romero, JR. Suzuka, SM. Gravell, D. Bouhassira, E. HbS-Oman Heterozygote: a new dominant sickle syndrome. *Blood* 1998;**92**: 4375 - 82.

37) McDonagh, KT. Nienhuis, AW. *The thalassemias. In: Nathan D.G, Oski F.A, eds. Hematology of Infancy and Childhood.* Philadelphia: Saunders; 1993.

38) Baglioni, C. The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. *Proc Nati Acad Sci* 1962; **48:**1880-86.

39) El-Hazmi, MA. Warsy, AS. Interaction between glucose-6-phosphate dehydrogenase deficiency and sickle cell gene in Saudi Arabia. *Trop Geogr Med* 1987; **39:**32 - 5.

40) Lewis, RA. Hathorn, M. Glucose-6-phosphate dehydrgenase correlated with S hemoglobin. *Ghana Med J* 1963; **3:**131.

41) Bouanga, JC. Mouele, R. Prehu, C. Wajcman, H. Feingold, J. Galacteros, F. Glucose-6-phosphate dehydrogenase deficiency and homozygous sickle cell disease in Congo. *Hum. Hered* 1998; **48:**192 – 97.

42) Piomelli, S. Reindorf, C. Arzanian, MT. Corash, LM. Clinical and biochemical interactions of glucose-6-phosphatase dehydrogenase deficient and sickle cell anaemia. *N Engl Med* 1972; **287:** 213 - 17.

43) Konotey-Ahulu, FID. Glucose-6-phosphate dehydrogenase deficiency and sickle cell anemia. *N Engl J Med* 1972; **287:**887 - 8.

48) Lehmann, H. Distribution of the sickle cell gene. A new light on the origin of the East Africans. *Eugenics Review* 1959; **46:**101-21.

49) Lehmann, H. Origin of the sickle cell S. *Afr. J. Sci* 1959;**50:**140 -1.

50) Kan, YW. Dozy, AM.. Polymorphism of DNA sequence adjacent to the human beta globin structural gene: relationship to sickle mutation. *Proc Natl Acad Sci* 1978;**75:**5631-35.

51) Kan, YW. Dozy, AM.Evolution of the hemoglobin S and C genes in world populations. *Science* 1980; **209:**388 -91.

52) Lanclos, KD. Oner, C. Dimovski, AJ. Gu YC. Huisman, THJ. Sequence variations in the 5'flanking and IVS-II regions of the G gamma and A gamma-globin genes of beta S chromosomes with five different haplotypes. *Blood* 1991; **77:**2488 - 96.

53) Antonarakis, SE. Kazazian, HH. Orkin, SH. DNA polymorphism and molecular pathology of the human globin gene clusters. *Hum Genet* 1985; **69:** 1-14

54) Padmos, A. Roberts, G. Lindahl, S. Kulozik, A. Thomas, P. Serjeant, B. Serjeant, G. Avsacular Necrosis of the Femoral Head in Saudi Arabian With Homozygous Sickle Cell Diseas- Risk Factors. *Ann Saudi Med* 1995; **15:**21-24.

55) Greenberg, JH. In: Skinner, EP. *Peoples and cultures of Africa. Garden City.* New York: Doubleday/National History Press; 1973.

56) Mears, JG. Lachman, HM. Cabannes, R. Amegnizin, KP. Labie, D. Nagel, RL. Sickle gene; its origin and diffusion from West Africa. *J Clin Invest* 1981; **68:** 606 - 10.

57) Luzzatto, L. Nwachuku-Jarrett, ES. Reddy, S. Increased sickling of parasitized erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet* 1970; **1:**319 – 22.

62) White, JM. The approximate gene frequency of sickle haemoglobins in the Arab peninsula. *Br J Haematol* 1983; **55:** 563 - 64.

63) 2004. UK Forum on Haemoglobin Disorders.[WWW] <URL: http://www.haemoglobin.org.uk/index.html> [Accessed 25 August 2004]

64) White, JM. Byrne, M. Richards, R. Buchanan, T. Katsoulis, E. Weerasingh, K. Red cell genetic abnormalities in Peninsular Arabs: sickle haemoglobin, G6PD deficiency, and alpha and beta-thalassaemia. *J Med Genet1986;* **23:** 245 – 51.

65) El-Hazmi, MA. Warsy, AS. Molecular studies on Yemen sickle-cell disease patients: Xmn I polymorphism. *East Mediterr Health J* 1999; **5:**1183-87.

66) El-Hazmi, MAF. Warsy, AS. The sickle cell gene as a multifaceted problem in Saudi Arabia. *Saudi Med. J*, 1997; **18:** 400-404.

67) El-Hazmi, MA. Beta-globin gene haplotypes in the Saudi Sickle cell anaemic patients. *Hum Hered,* 1990; **40:**177 - 86.

68) Talafih, K. Hunaiti, AA. Gharaibeh, N. Gharaibeh, M. Jaradat, S. The prevalence of hemoglobin S and glucose-6-phosphate dehyrogenase deficiency in Jordanian newborn. *J Obstet Gynaecol Res* 1996; **22:** 417 - 20.

69) El-Hazmi, MA. Warsy, AS. Bashir, N. Beshlawi, A. Hussain, IR. Temtamy, S. Qubaili, F. Haplotypes of the b-globin gene as a prognostic factors in sickle cell. *East Mediterr Health J* 1999; **15;**1154 - 58.

70) Rajab, AG. Patton, MA. Modell, B. Study of hemoglobinopathies in Oman though antional register. *Saudi Med J* 2000; **21:**1168 - 72.

71) Baysal, E.. Hemoglobinopathies in the United Arab Emirates. *Hemoglobin* 2001; **25:** 247- 53.

72) Al-Affayed1, SS. Haites, N. Features of sickle cell disease in Bahrain. *East Mediterr Health J* 1995; **1:**112 - 19.

76) Sunna, EI. Gharaibeh, NS. Knapp, DD. Bashir, NA. Prevalence of hemoglobin S and beta-thalassemia in northern Jordan. *J Obstet Gynaecol Res.* 1996; **22:** 17 - 20.

77) Al-Sheyyab, M. Rimawi, H. Izzat, M. Batieha, A. el Bashir, N. Almasri, N. Daoud, AS. Sickle cell anaemia in Jordan and its clinical patterns. *Ann Trop Paediatr* 1996; **16:** 249 - 53.

78) Eliakim, R. Rachmilewitz, EA. Hemoglobinopathies in Israel. *Hemoglobin* 1983; **7:** 479 - 85.

79) Powars, DR.. Natural history of sickle cell disease-the first ten years. *Semin.Hematol,* 1975; **12:** 267 - 85.

80) MRC working party on the evaluation of chorion villus sampling. Medical Research Council European Trial of chorion villus sampling *Lancet,* 1991;**337:** 1491 - 99.

81) Miller, ST. Sleeper, LA. Pegelow, CH. Enos, LE. Wang, WC. Weiner, SJ. Prediction of adverse outcomes in children with sickle cell disease. *N Engl J Med* 2001; **342:** 83- 9.

82) Berchel, C. Diara, JP. Loret, H. Foucan, L. Turduc, LE. Samuel, Y. Natural history of sickle cell anemia. *Rev Prat* 1992; **42**:1885- 91.

83) Davis, LR. Changing in blood picture in sickle cell anaemia from shortly after birth to adolescence. *J Clin Pathol* 1976; **29:** 898-901.

84) Thame, M. Grandison, Y. Mason, K. Thompson, M. Higgs, DR. Morries, J. Serjeant, BE. Serjeant, GR.The red cell distribution width in sickle cell diseases: is it clinical value? *Clin Lab Haematol* 1991; **13:** 229 - 37.

85) Balkaran, B. Char, G. Morris, JS. Serjeant, GR. Stroke in a cohort study of patients with homozygous sickle cell disease. *J. Pediatr* 1992; **120:**360 - 6.

86) Powars, DR. Sandhu, M. Niland-Weiss. J. Johnson, C. Bruce, S. Manning, PR. Pregnancy in sickle cell disease. *Obstet Gynecol* 1986; **67:**217 - 28.

91) Merghoub, T. Perichon, B. Maier-Redelsperger, M. Dibenedetto, SP. Samperi, P. Ducrocq, R. Dissection of the association status of two polymorphisms in the beta-globin gene cluster with variations in F-cell number in non-anemic individuals. *Am J Hematol* 1997; **56:**239 - 43.

92) Gonvcalves, I. Duroq, R. Lanvinha, J. Nogueira, PJ. Peres, MJ. Picanco, I. Combined effect of two different polymorphic sequences within the beta globin gene cluster on the level of Hb F. *Am J Hematol* 1998;**57:** 269-76.

93) Seltzer, WK. Abshire, TC. Lane, PA. Roloff, JS. Githens, JH. Molecular genetic studies in black families with sickle cell anemia and unusually high levels of fetal hemoglobin. *Hemoglobin* 1992; **16:**363 - 77.

94) Konotey-Ahulu, FID. Treatment and prevention of sickle cell crisis. *Lancet* 1971; **2:**1255 - 8.

95) Maharajan, R. Fleming, AF. Egler, LJ. Pattern of infections among patients with sickle cell anaemia requiring hospital admissions. *Nig J Paediatr* 1983; **10:** 13-7.

96) John, AB. Ramlal, A. Jackson, H. Maude, GH. Sharma, AW. Serjeant, GR Prevention of pneumococcal infection in children with homozygous sickle cell disease. *Br Med J* 1984; **288:**1567 -70.

97) Gaston, MH. Verter, JI. Woods, G. Pegelow, C. Kelleher, J. Presbury, G. Zarkowsky, H. Vichinsky, E. Iyer, R. Lobel, JS. Prophylaxis with oral penicillin in children with sickle cell anemia. *N Engl J Med* 1986: **314:**1593 - 99.

98) Adeyokunnu, AA. Hendrickse, RG.. Salmonella osteomyelitis in childhood. A report of 63 cases seen in Nigerian children of whom 57 had sickle cell anaemia. *Arch Dis Child* 1980; **55:**175 - 84.

99) Serjeant, GR. Topley, BM. Mason, K. Serjeant, JR. Pattison, SE. Mohamed, R. Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. *Lancet,* 1981;**2:**595 - 7.

haematological expression of haemoglobin S in Saudi Arabs. *Acta Haematol* 1992; **88:** 67 - 71.

104) El-Hazmi, MA. Warsy, AS. A comparative study of haematological parameters in children suffering from sickle cell anaemia (SCA) from different regions of Saudi Arabia. *J Trop Pediatr* 2001; **47:**136 - 41.

105) Nasserullah, Z. Al-Jame, A. Abu Srair, H. Al-Qatari, G. Al-Naim, S. Al-Aqib, A. Mokhtar, M. Neonatal screeningfor sickle cell disease, glucose-6-phosphatase dehydrogenase deficiency and alpha thalassaemia in Qatif and Al-Hasa. *Ann Saudi Med* 1998; **18:** 289 - 92.

106) Hawasawi, ZM. Nabi, G. Al-Magamci, M.F. Awad, KS. Sickle cell disease in childhood in Madina. *Ann Saudi Med* 1998; **18:**293 - 95.

107) Acquaye, JK. Omer, A. Ganeshaguru, K. Sejeny, SA. Hoffbrand, AV. Non-benign sickle cell anaemia in western Saudi Arabia. *Br J Haemtol* 1985; **60:** 99-108.

108) Thuilliez, V. Ditsambou, V. Mba, JR. Mba Meyo, S. Kitengue, J.Currents aspects of sickle cell disease in Gabon. *Arch Pediatr* 1996; **3:**668 - 74.

109) Weatherall, DJ. Clegg, JB. *The Thalassemia Syndromes.* 4th edn. London: Blackwell Science Publishing Co; 2001. p. 801.

110) Labie, D. Richin, C. Pangnier, J. Gentiline, M, Nagel, RL. Hemoglobin S and C in Upper Volta. *Hum Genet* 1984; **65:**300 – 2.

111) Kar, B. Satapathy, RK. Kulozik, AE. Kulozik, M. Sirr, S. Serjeant, BE. Serjeant, GR.. Sickle cell disease in Orissa State, India. *Lancet* 1986; **2:**1198 - 1201.

112) Leikin, SL. Gallagher, D. Kinney, TR. Sloane, D. Klug, P. Rida, W.. Mortality in children and adolescents with sickle cell disease. *Pediatrics* 1989;**84:** 500 – 8.

971 - 82.

117) Kutlar. F. Kutlar, R. Nuguid, E. Usefulness of HPLC methodology for the characterzation on cominations of the common β chain variants Hbs S, C, and O-Arab and the α chain variant Hb G-Philadelphia. *Hemoglobin* 1993;17:55 – 66.

118) Davies, SC. Cronin, E. Gill, M. Greengross, P. Hickman, M. Normand, C. *Screening for sickle cell disease and thalassamia: a systematic review with supplementary research.* Health Technology Assessment Report. 4(3). 2000.

119) Chepman, C. Champers, K. *Neonatal haemoglobionpathy screening methods. MDA Evaluation Report MDA/97/64.* London: Medical Devices Agency; 1997.

120) Arya, R. Rolan, PE. Wootton, R. Posner, J. Bellingham, A J. Tucaresol increases oxygen affinity and reduces haemolysis in subjects with sickle cell anaemia. *Br J Haematol* 1996; **93:**817-21.

121) Smith, CM. Hebbel, RP. Tukey, DP, Clawson, CC. White, JG. Vercellotti G. et al. Pluronic F-68 reduces the endothelial adherence and imporves the rheology of liganded sickle erythrocytes. *Blood* 1987; **69:**1631-6.

122) Central Statistical Organization. *Statistical Year--Book 2000.* Sanaa, Republic of Yemen: Ministry of Planning and Development; 2001.

123) Central Statistical Organization. *Mother and Child Health Report.* Sana'a, Republic of Yemen: Ministry of Planning and Development;1997.

124) Riou, J. Godart, C. Hurtrel, D. Mathis, M. Bimet, C. Bardakdjian-Michau, J. Prehu, C.; Wajcman, H.; Galacteros, F. Evaluation of cation-exchange high-performance liquid-chromatography for presumptive indentification of hemoglobin variants. *J Clin Chem* 1997; **43:** 34 - 9.

125) Old, JM, Ludlam, CA. Antenatal diagnosis. In: Hann IM, Gibson BES, eds. Paediatric Haemat*ology. London: Bailliere Tindall, Bailliere's Clinical Haematology* 1991; **4:**391-428.

monitoring of Thalassaemia, Nicosia, Cyprus: The Cyprus Thalassaemia Centre and The Cyprus Institute of Neurology and Genetics; 1999.

130) Sutton, M. Bouhassira, EE. Nagel, RL. Polymerase chain reaction amplification applied to the determination of β like globin gene cluster haplotype. *Am J Hematol* 1989; **132:**66 - 9.

131) Weatherall, DJ. Clegg, JB. *The Thalassemia Syndromes.* 4th edn. London: Blackwell Science Publishing Co; 2001.p.716.

132) Li, J. Wilson, D. Plonczynski, M. Harrel, A. Cook, CB. Scheer, WD. Zeng, YT. Coleman, MB. Steinberg, MH. Genetic studies suggest a multicentric origin for Hb G-Coushatta [beta 22(B4)GluàAla]. *Hemoglobin* 1999; **23:** 57-67.

133) Bain, B. Haemoglobin and the genetics of haemoglobin synthesis. *Haemoglobinopathy diagnosis.* London: Blackwell Science; 2001, P.207.

134) Lambeth, S. Health care in the Yemen Arab Republic. *Int J Nurs Stud* 1988;**25**:171- 7.

135) Padmos, MA. Roberts, GT. Sackey, K. Kulozik, A. Bail, S. Morris, JS. Serjeant, BE. Serjeant, G.R. Two different forms of homozygous sickle cell disease in Saudi Arabia. *Br J Haematol* 1991;**79:**93 - 8.

136) Nagel, RL. Pathobiology, epistatic effects, and genetic markers in sickle cell anemia. *Semin Hematol* 1991; **28:**180 - 201.

137) Kulozik, AE. Wainscoat, JS. Serjearnt, GR.Kar, BC. Al-Awamy, B. Essan, GJ. Falusi, AG. Haque, SK. Hilali, AM. Kate, S. Geographical survey of beta S globin gene haplotypes: evidence for an independent Asian origin of the sickle-cell mutation. *Am J Hum Genet* 1986; **39:**239 - 44.

138) [WWW]< URL: http://www.cia.gov/cia/publications/factbook > [Accessed 16 October 2004]

143) Jane, SM. Cunningham, JM. Understanding fetal globin gene expression: a step towards effective Hb F reactivation in haemoglobinopathies. *Br J Haematol* 1998;**102**:415-22.

144) Miyoshi, K. Kaneto, Y. Kawai, H. Ohchi, H. Niki, S. Hasegawa, K. X-linked dominant control of F-cells in normal adult life: characterization of the Swiss type as hereditary persistence of fetal hemoglobin regulated dominantly by genes on X chromosome. *Blood* 1988;**72**:1854-60.

145) Carig, JE. Rochette, J. Sampietro, M. Wilkie, AO. Barnetson, R. Hatton, CS. Demenais, F. Thein, SL. Genetic heterogeneity in hetrocellular hereditary presistence of fetal hemoglobin. *Blood* 1997; **90**:428-34.

146) Tadmouri, GO, Yuksel, L. Basak, AN. Hb S /beta (del) thalassemia associated with high hemoglobin A2 and F in a Turkish family. *Am J Hematol* 1998;**59**:83-6.

147) Serjeant, BE. Msaon, KP, Kenny, MW. Stuart, J. Higgs, DR. Weatherall, DJ. Hayes, RJ. Serjeant, GR. Effect of alpha thalassaemia on the rheology of homozygous sickle cell disease. *Br J Haematol* 1983;**55**:479-486.

148) Mukherjee, MB. Lu, CY. Ducrocq, R Gangakhedkar, RR. Colah, RB. Kadam, MD. Mohanty, D. Nagel, RL. Krishnamoorthy, R. Effect of alpha thalassaemia on sickle cell anemia linked to the Arab-Indian haplotype in India. *Am J Hematol* 1997;**55**:104-9.

Publ ished article

Al-Nood, H. Al-Ismail, S. King, L. May, A. Prevalence of the Sickle Cell Gene in Yemen: A Pilot Study, *HEMOGLOBIN* 2004, **28** (4): 277- 287.

Conference

9th International conference on Thalassaemia and the Haemoglobinopathies &11th International TIF conference for Parents and Thalassaemics, Città del Mare (Palermo), Italy – 15/19 October, 2003, P097

4. The disc is then removed and the vial spun at 15,000 rpm for 1 minute, to sediment and debris.
5. The HPLC run is then set up using a prime sample.
6. The gradient used and run criteria were follows:

Acquisition Set up

Sampling frequency	5.0 Hz
Runtime	8.5 minutes
Acquisition Delay	0.0

Gradient

Time	**%Concentration of buffer B***
0.10	18.0
5.80	68.0
5.81	100.0
6.60	100.0
6.61	18.0
8.50	00.0 STOP

*Supplied with beta thalassaemia testing kit (Chromsystems Instruments & Chemicals, GmbH, Munchen, Germany)

Analysis window set up

Name of Window	**Retention time (min)**	**Window width (min)**
?F1 (acetylated Hb F)	0.7	0.4
Hb F	0.8	0.6
HbA	4.6	0.8
HbA_2	5.3	0.8
Hb S	6.23	0.8
Hb C	8.0	0.8

Following the analysis run, the chromatograms are analysed quantitatively for the presence of peaks not normally present in neonatal dried blood spot samples.

Samples extraction

A 6 mm disc out of the dry blood spot is used to prepare the haemolysate of eac blood sample. A 50ul of haemoglobin elution solution is added on the dry blood disc small tube. Then the tube is mixed for 15 minutes. After the disc has been removed t tube is spun and the clear elution of the haemolysate can be used for analysis.

Method

1. Turn on water to cool plate. Clean plate.
2. Remove gel form packing.
3. Put a drop of water on the centre of the plate.
4. Place gel on the plate.
5. Blot gel gently with one Gel Blotter.
6. Set up anode and cathode wicks.
7. Place the sample template onto the gel so that it buts-up against the cathode wick.
8. Pipette 7.5 µl of sample lysate onto the appropriate well on the template.
9. Put on internal IEF chamber top. Connect the electrode connectors.
10. Put on the top cover.
11. Run at 1.5kV, 18 mA for 1 hour and 30 minutes.
12. Place gel in protein fixation for 10 minutes.
13. Wash in large quantities of distilled water for 15 minutes.
14. Dry the gel at 50 – 60 0C for about 20 minutes.
15. If staining is required. Use Isolab Gel Stain.
16. Wash in water for 5 minutes.
17. De-stain plate until background is clear in (5 parts water/ 5 parts methanol/ 1 part glacial acetic acid)

Into each tube 24 µl of the mastermix and 1µl DNA extract (sample).

Conditions of PCR:

95 °C 9 min
40 cycles:
94 °C 30 sec
62 °C 30 sec
72 °C 1 min
72 °C 10 min

Dde I Digestion for HbS

Per tube: 5.6 µl water
1.4 µl NEB3
0.52 µl Dde I, 10,000 U/ml

7 µl buffer + 7 µl PCR product

Incubate at 37 °C for 3-6 hours or preferably overnight.

Gel electrophoresis

3.0% NuSieve agarose gel electrophoresis:
(1.5 g in 50 ml 0.5xTBE buffer + 1 µl 2.5 mg/ml ethidium bromide)
TBE buffer (0.89M Tris, 0.89M Boric acid, 0.02M Na_2 EDT, final pH 8.3)

Electrophoresis tank preparation:
(400 ml buffer + 12 µl ethidium bromide)
15 µl of the PCR product + 2 µl of loading dye (200 µlIM Tris HCL - pH 8.0, 5 ml glycerol, 4.8 ml water, 1.0 mg Bromophenol blue)

Running time is 40 minutes at 150V.
Photographing of gel: The gel is placed onto an UV transilluminator and viewed and the gel can be photographed.

Interpretation:
The undigested fragment is 351 bp in size
5 Dde I restriction sites exist normally one of which is abolished by the HbS mutation.

µl dNTPs
µl primers
017 µl Taq Gold

Into each tube 23.5 µl of the mastermix and 1µl DNA extract (sample).

Conditions of PCR:

95 °C 9 min

37 cycles:
93 °C 1 min
66 °C 2 min

66 °C 10 min

The product size is 861 bp

Digestion with EcoR1

36 µl water
4 µl React 2 10x buffer
2 µl 12 Units/µl Eco R1

5 µl enzyme/ buffer + 5 µl PCR product,
incubate at 37 °C for at least 3 – 6 hours.

Gel electrophoresis as in Appendix C

Interpretation
HbAA gives bands of sizes 554 and 307 bp.

Hb AD gives bands of sizes 861, 554, and 307 bp.

Into each tube 23.5 µl of the mastermix and 1µl DNA extract (sample).

Conditions of PCR:

95 °C 10 min
40 cycles:
94 °C 30 sec
56 °C 30 sec
72 °C 1 min

72 °C 10 min

Digestion

Per tube:

5.45 µl water
1.4 µl NEB3
0.14 µl BSA
0.24 µl enzyme *Xmn* I 20U/µl

7 µl of this tube + 7 µl PCR product

Incubate at 37 °C for 6 hours or preferably overnight.

Gel electrophoresis as in Appendix C except the agarose gel is 2%

Interpretation

Undigested fragment size is 641 bp (C at position –158)
Digested fragments (420 + 220 bp) (T at position – 158)