CHAPTER 10

GENE-GENE INTERACTIONS

10.1. Introduction

The presence of two or more genetic abnormalities in the same locality, particularly, those such as Saudi Arabia, where consanguinity and other forms of inter-marriages within the families and tribes are very common, show interesting interactions. Two or more abnormal genes may be co-inherited in the same individual and frequently result in modification of the clinical presentation of the abnormal genes. Such gene-gene interactions have been the subject of extensive research as they have played a significant role in the elucidation of the genetic basis for certain disorders, e.g. the thalassaemias, and are of special clinical significance (Weatherall & Clegg, 1981; Weatherall et al, 1965; El-Hazmi, 1985).

The most frequently encountered of the disorders are those resulting from interaction between haemoglobinopathies and the thalassaemias and include sickle cell-thalassaemias, and Hb E-thalassaemias, and those between sickle cell and G-6-PD deficiency (Weatherall & Clegg, 1981).

Saudi Arabia presents a unique environment for the study of interactions between different genetic abnormalities, since there is a high frequency of two or more abnormal genes in the same locality and the rate of consanguinity is high, two or more genetic abnormalities frequently occur in the same patient.

10.2. Genetic interactions of haemoglobinopathies and thalassaemias

The co-inheritance of and interaction between genes for haemoglobinopathies and the thalassaemias was first reported in the Italians during 1944-45. Later, such interactions were recognised in several other populations and were shown to be a heterogenous group both in terms of the molecular pathogenesis and the clinical presentation. With the

introduction of electrophoretic techniques during the 1950's and the molecular biology techniques during 1980, the phenotypic and genotypic classification of these disorders have been investigated in detail.

10.2.1 Classification

There are a large number of syndromes resulting from interaction between structurally abnormal haemoglobins and the thalassaemias. The following is a system of classification which groups the disorders into separate classes

I. Haemoglobin structural disorders - β-Thalassaemia

(i) Sickle cell-β°-thalassaemia: No Hb A on electrophoresis, resembles sickle cell anaemia (Hb SS) electrophoretically, clinically and haematologically.

(ii) Sickle cell-β^+thalassaemia: has 5-15% Hb A and is moderately severe clinically.

(iii) Sickle cell-β^+-thalassaemia: Appears as Hb S heterozygote but with 20-30% Hb A and a mild course.

(vi) Sickle cell -β°-thalassaemia: No Hb A on electrophoresis resembles Hb SS, with 15-25% Hb F and normal or low Hb A_2. Mild clinical course.

(v) Sickle cell-β^+-thalassaemia: A rare condition. 25-30% Hb A and 46-54% Hb S on electrophoresis. Mild clinical course.

(vi) Sickle cell - Hb Lepore syndrome.A rare condition. Hb S and Hb Lepore have been shown to coexist.

(vii) Other Structural Hb variants β-thalassaemia.

II. Haemoglobin Structural Disorders - α-Thalassaemias: α

(i) Sickle cell - α-thalassaemia.2 (heterozygotes; -α/αα). Electrophoretically as Hb SS, reduced or normal Hb A_2. Slightly milder than SCA. Lower red cell indices.

(ii) Sickle cell - α-thalassaemia.2 [(homozygous; -α-/-α)]. Sickle cell α-thalalssaemia [heterozygous, (--/αα)]. Electrophoretically as Hb SS, reduced or normal Hb A_2. Lower red cell indices. Slightly milder than Hb SS. Wide range of clinical presentations.

(iii) Sickle cell - Hb H disease (--/-α). Rare condition. Electrophoretically has Hb H and Hb S: Lower Hb A_2. Reduced red cell indices.

(iv) Other structural variants - α-thalassaemia.

III. Haemoglobin structural disorders - Haemoglobin structural disorders

(i) Hb S - other abnormal haemoglobins

IV. α-thalassaemia - β-thalassaemia

10.2.1.1 Structural haemoglobin variants-β-thalassaemia

These conditions are due to double heterozygocity to Hb S and β-thalassaemia mutations, one on each chromosome. If the latter is a β° mutation, no β-chains are synthesised and the condition appears as sickle cell anaemia. On the other hand, if the mutation is a β^+ mutation, some β-chains are synthesised and Hb A is present on electrophoresis but is reduced. A wide range of Hb A level has been encountered depending on the nature of the mutation.

(i) Distribution

Areas where both sickle cell and β-thalassaemia genes occur, have coexisting sickle cell-β-thalassaemia. The sickle cell-β-thalassaemia have been reported in the Mediterraneans, Africans, Saudi Arabs, Indians, Black population of North Americas. However, the gene frequency varies considerably.

We investigated interaction between Hb S and β° and β^+-thalassaemias in the different regions of Saudi Arabia. Hb S/β° and Hb S/β^+-thalassaemias were identified in some of the regions.

(ii) Clinical Features

Hb S/β°-thalassaemia is clinically, haematologically and electrophoretically similar to sickle cell anaemia. While the Hb S/β^+-thalassaemia has a benign clinical course with a lower incidence of symptomatic presentation. The clinical findings in the Saudi Hb S/β°-Thalassaemia patients are presented in Table 10.1.

(a) Painful crises: Painful cirses are reported in both Hb S/β°-thal and Hb S/β^+-thal patients, though more frequently in the former. The pains are generally mild in Hb S/β^+-thal and severe in the Hb S/β°-thalassaemia. In addition, the frequency of severe painful crises is higher in the Hb S/β°-thalassaemia. In the Saudi Hb S/β^+-thal patients the clinical presentation is milder and the Hb S/β°-thal suffer from frequent episodes of painful crises.

(b) Dactylitis: Dactylitis has been reported in both Hb S/β° -thal and Hb S/β^+-

Clinical presentation	Frequency (%)		
	Hb S/β°-Thal.		
	Children	Female	Male
Crises:			
Vasoocclusive	50	100	58.3
Haemolytic	41.8	12.5	25
Aplastic	0	0	0
Sequestration	0	12.5	0
Hepatomegaly	66.6	62.5	56.2
Gallstone	8.3	25	5
Splenomegaly	8.3	-	21.8
Hypersplenism	-	12.5	78.3
Splenic infarction	16.6	25.0	-
Auto-splenectomy	16.6	0	8.3
Splenectomy	50	50	25
Renal problems	16.6	25	33.3
Cardiomegaly	8.3	-	-
Haemic murmur	16.6	25	8.3
Chest infection	8.3	0	33.3
Pnuemonia	8.3	12.5	0
Pulmonary infarction	8.3	0	25
Avascular necrosis	0	12.5	8.3
of head of femur			8.3
Abnormal bone scan	8.3	37.5	
Dactylitis	16.6	0	33.3
Osteomyelitis	0	12.5	0
Joint swelling	16.6	62.5	0
Hemiplagia	0	0	25
Intracranial	0	0	0
haemorrhage			0
Convulsions	0	0	0
Coma	0	12.5	0
Leg ulcers	0	0	8.3
Blood transfusion	75	62.5	41.7

Hospitalization	83.3	87.5	75

Saudi patients Dactylitis was not seen in Hb S/β^+-thal. patients. In the Hb S/β°-thal. the frequency was low (6.25% in total Hb S/β°-thal. cases).

(c) Splenomegaly: In both Hb S/β°-thal. and Hb S/β^+-thal. splenomegaly occurs in almost one third of the patients, though the size of the spleen is higher in the former. Splenectomy occurs at a significantly higher frequency in Hb S/β°-thal compared to Hb S/β^+-thal. In the Saudi Hb S/β° thal. splenomegaly was identified in only 6.25% of the patients .There is a tendency for splenomegaly to decline with age in Hb S/β° thal. but not in Hb S/β^+-thal.

Acute splenic sequestration occurs mainly in the Hb S/β° thal. patients though a few cases of Hb S/β^+-thal. have been reported to suffer from this condition. Splenic infarction has been reported in both conditions occurring either spontaneously or during flight in pressurized aircraft. In the Saudi Hb S/β° and Hb S/β^+-Thalassaemia patients acute splenic sequestration were identified though at a low frequency.

(d) Hepatomegaly: Hepatomegaly occurs more in the Hb S/β° thal. patients compared to Hb S/β^+-Thalassaemia .The frequency of hepatomegaly was high in the Saudi Hb S/β°-Thalassaemia patients (56.25%).

(e) Gallstones: Gallstones have been reported in both conditions at a higher frequency in the Hb S/β° Thalassaemia. This was also true for the Saudi Hb S/β°-thal. patients where 21.88% of the patients had gallstones. Majority were adults.

(f) Leg ulcers: In the African patients leg ulcers have been reported in both Hb S/β° -and Hb S/β^+-thal. patients though in Saudis the frequency of leg ulcers in Hb S/β°-thal. is very low and no case of leg ulcers was seen in Hb S/β^+-Thal. patients.

(g) Acute pulmonary episodes: Episodes involving the pulmonary system i.e. pneumonia and pulmonary embolism occur in both Hb S/β° and Hb S/β^+-thal. cases though the frequency and severity of such episodes is higher in the former. In the Saudi Hb S/β^+-thal. patients such episodes were not encountered.

(h) Cardiovascular disorders: Disorders of the cardiovascular system occur in both Hb S/β° and Hb S/β^+-thal. cases at a lower frequency in the latter. The abnormalities include cardiomegaly, mid-systolic murmurs and left-ventricular hypertrophy. In the Saudi Hb S/β^+-thal. cases, these abnormalities were rarely seen.

(i) Sexual development and frequency: In the African populations detailed investigation have been conducted on the sexual developments in Hb S/β°-thal. and Hb S/β^+-thal. patients. In the former menarche occurs at a significantly latter age compared to Hb S/β^+-thal. cases. The frequency of live birth was 1.8 and 3.7 and that of pregnancies was 1.3 and 2.4 per patient in the Hb Sβ°-thal. and Hb S/β^+-thal. patients, respectively. This aspect was not investigated during the studies.

(j) Physical development and habits: In the Hb S/β° thal. patients the height

(k) Bone defects: Bone marrow expansion occurs more commonly in the Hb S/β° thal. patients, while thickened cortex, possibly due to cortical infarction occurs more commonly in Hb S/β^+-thal. patients. Bone defects including osteomyelitis, joint swelling, abnormal bone scans dactylitis were present in the Saudi Hb S/β°-thal., but rarely in Hb Sβ^+-thal. patients

(l) Retinal complications: Eye complications including retinal vein tortuosity, retinal haemorrhage and detachment have been reported in Hb S/β° thal. while proliferative retinopathy is more frequent in Hb Sβ^+- thal. This aspect was not investigated in the Saudi patients.

10.2.2. Comparison of the Hb S/β° thal. and homozygous sickle cell disease in Saudis

During our investigation on the Saudi population two studies were conducted to compare the Hb SS and Hb S/β°-thal. patients. Studies were conducted on the population from Eastern province and from the Western province. Tables 10.2 to 10.3 present the clinical and haematological parameter values in the Hb S/β°-thal. patients compared to Hb SS patients from the Western province and Tables 10.4 to 10.6 present the data comparing the Hb S/β°-thal. and Hb SS from the eastern province. Significant differences were obvious between the patients from the different province. In general, in the Hb S/β°-thal.

Table 10.2: Comparison of the clinical presentation in Hb SS and Hb S/β°-Thal. patients from the Western Province of Saudi Arabia

	Hb SS (45)		Hb S/β°-Thal. (32)	
	No.	Frequency (%)	No.	Frequency (%)
Crises:				
Vasoocclusive	38	84.44	21	65.6
Haemolytic	9	20	9	28.1
Aplastic	0	0	0	0
Sequestration	3	6.67	1	3.12
Hepatomegaly	34	75.56	18	56.25
Gallstone	10	22.22	7	21.88
Splenmegaly	8	17.78	2	6.25
Hypersplenism	2	4.44	1	3.125
Splenic infarction	3	6.66	5	15.625
Auto-splenectomy	8	17.78	5	15.625
Splenectomy	12	26.67	14	53.75
Renal problems	5	11.11	4	12.5
Cardiomegaly	8	17.78	2	6.25
Haemic murmur	26	57.78	8	25
Chest infection	3	6.66	1	3.125
Pnuemonia	13	28.9	5	15.625
Pulmonary infarction	1	2.22	2	6.25
Avascular necrosis of head of femur	10	22.2	2	6.25
Abnormal bone scan	13	28.89	8	25
Dactylitis	9	20	2	6.25
Osteomyelitis	7	15.6	1	3.125
Joint swelling	18	40	10	31.25
Hemiplagia	2	4.44	0	0
Intracranial haemorrhage	0	0	0	0
Convulsions	0	0	0	0
Coma	1	2.22	1	31.25
Leg ulcers			1	8.125
Blood transfusion	34	75.5	19	59.4
Hospitalization	44	97.78	26	81.25

( ) No. of patients investigated

Table 10.3: Comparison of the haematological parameter values in Hb S/β°-Thal and Hb SS patients from Western Province

Parameters	Sickle Cell/β°- patients		Sickle cell Anaemia patients	
	Males (n=14)	Females (n=8)	Males (n=37)	Females (n=24)
Hb (g/dl)	9.9 ± 1.4	9.6 ± 1.9	10.1 ± 1.3	8.4 ± 1.6
RBC ($X10^{12}$/l)	3.9 ± 0.7	3.8 ± 0.7	3.3 ± 0.8	2.7 ± 0.6
PCV (l/l)	0.28±0.03	0.27±0.04	0.29±0.05	0.24±0.06
MCV (fl)	72.9 ± 8.0	73.3 ± 7.3	82.7 ±24.4	88.6 ± 7.6
MCH (pg)	30.8 ±18.2	25.4 ± 3.1	31.6 ± 5.1	30.1 ± 4.5
MCHC (g/dl)	34.8 ± 2.8	35.3 ± 2.6	34.2 ± 2.4	32.8 ± 3.7
Hb A_2 (%)	5.0 ± 0.9	4.8 ± 1.3	3.1 ± 0.6	2.7 ± 0.8
Hb F (%)	8.0 ± 5.5	10.9 ± 3.4	9.5 ± 6.1	11.5 ± 7.3

Table 10.4: Comparison of the clinical data in Saudi Hb SS and Hb S/β°-thalassaemia patients in the Eastern Province of Saudi Arabia

Clinical Presentation	% of patients	
	Sickle Cell Anaemia	Hb S/β°-thalassaemia
No. investigated		
Anaemia	56	28.6
Pain in bones & joints	90	71.4
Abdominal pain	64	30.0
Leg ulceration	-	-
Hepatomegaly	36	30.0
Splenomegaly	45	15.0
General weakness	90	72.0
Jaundice	18	15.0
Oedematous feet	9	15.0
Loss of weight	20	30.0
Previous blood transfusion	73	30.0
Osteomyelitis	45	-

Table 10.5: Comparison of the Haematological parameters in Saudi Hb SS and Hb S/β°-thalassaemia patients from the Eastern province

Parameters	Sickle Cell/β° patients		Sickle cell Anaemia patients	
	Males (n=13)	Females (n=8)	Males (n=37)	Females (n=24)
Hb (g/dl)	10.76±2.01	9.36±3.16	11.17±3.16	10.08±2.07
RBC ($X10^{12}$/l)	4.13±0.97	3.98±0.79	4.00±1.15	3.53±0.78
PCV (l/l)	0.32±0.05	0.29±0.04	0.33±0.09	0.30±0.05
MCV (fl)	78.09±8.07	76.10±8.23	83.42±12.02	85.83±11.66
MCH (pg)	26.32±2.39	24.01±3.63	28.51±3.60	28.83± 3.20
MCHC (g/dl)	34.75±2.78	34.28±2.85	34.31±1.70	33.79±3.10
Hb A_2 (%)	4.83±0.40	4.87±0.40	2.77±0.37	2.84±0.46
Hb F (%)	7.26±3.37	10.53±3.14	10.55±4.41	12.40±7.03

Table 10.6: Comparison of the values of biochemical parameters in Hb S/β°-patients and Hb SS patients from Eastern Province

Parameters	Sickle Cell/β° patients		Sickle cell Anaemia patients	
	Males (n=13)	Females (n=8)	Males (n=37)	Females (n=24)
Liver function tests				
T. bil (μmol/l)	22.00± 9.00	20.10 ± 8.5	22.20±10.70	22.20 ± 10.70
D. Bil (μmol/l)	2.83 ± 0.98	2.97 ± 1.63	3.27 ± 1.50	2.64 ± 1.24
TAG (mmol/l)	1.20 ± 0.59	1.49 ± 1.43	1.14 ± 0.46	1.04 ± 0.46
Chol. (mmol/l)	3.53 ± 0.70	3.12 ± 1.26	3.0 ± 0.89	3.14 ± 0.87
ALP (U/l)	127.5±58.70	86.25±36.79	116.3±39.44	95.89±43.37
SGOT (U/l)	54.67±18.00	33.0±14.40	44.46±17.66	40.78±22.07
SGPT (U/l)	17.00±7.0	9.0 ± 6.68	13.0 ± 6.39	13.00± 5.29
T. Protein (g/l)	75.00± 4.58	75.5±5.97	76.5 ± 4.0	76.40± 4.50
Albumin (g/l)	45.33±3.06	44.00±3.46	45.0 ± 3.80	44.60± 2.50
Bone function tests				
Calcium (mmol/l)	2.33 ± 0.06	2.30 ± 0.08	2.31 ± 0.11	2.30 ± 0.05
Phosphate (mmol/l)	1.23 ± 0.24	1.16 ± 0.17	1.15 ± 0.20	1.20 ± 0.22
ALP (U/l)	127.5±58.70	86.25±36.79	116.3±39.44	95.89±43.37
Renal function test				
Creatinine (mmol/l)	58.67±13.30	46.25±8.61	58.50±17.26	49.89±11.69
Urea (mmol/ll)	3.70 ± 1.13	2.80 ± 0.42	3.54 ± 1.15	3.12 ± 0.81
Electrolytes				
Sodium (mmol/l)	137.0±1.00	139.0± 1.00	137.14±2.03	138.00± 1.00
Potassium (mmol/l)	4.47±0.49	4.12 ± 0.33	4.55± 0.52	4.21 ± 0.59
Chloride (mmol/l)	101.0± 3.00	104.25±1.89	102.15±2.30	115.44±32.86
Miscellaneous				
Glucose (mmol/l)	4.47 ± 0.90	4.47 ± 0.51	4.56 ± 0.76	4.91 ± 1.20
Uric acid (mmol/l)	354.00± 6.00	216.5±70.32	318.14±58.35	252.89±80.91

patients the MCV, MCH and reticulocytes were lower, while total haemoglobin, packed cell volume, red cell count, Hb A_2 level was higher.

10.2.3. Sickle Cell - δβ°-thalassaemia

This condition is rare and results from double heterozygocity to Hb S and δβ°-thalassaemia. The former results from deletion of different proportions of the δ-β cluster on the chromosome 11. Cases have been reported from Sicily, Greece and Italy.

The diagnosis is based on electrophoretic pattern showing Hb S, F, A_2. The Hb A_2 level is low, while the Hb F is high (15-20%). The Hb F shows an uneven intracellular distribution. Diagnosis is confirmed by family studies.

Haematologically the condition is mild with lower haemoglobin level and increased reticulocytes. Clinically, the course is benign, though painful crises occur.

10.2.4. Sickle Cell - Hb Lepore (Boston)

This is a rare condition and the electrophoretic pattern resembles Hb SS since at alkaline pH, Hb Lepore travels like Hb S. Diagnosis depends on family study, micro-cytosis, normal or low Hb A_2.

A mild haemolytic anaemia, microcytosis, hypochromia and anisocytosis are the major haematological findings. Clinically, a marked variability has been reported with symptoms ranging from a benign disease to a more severe one with bone pain, jaundice, splenomegaly and hematuria. No case with this interaction was identified in the Saudis.

10.2.5. Haemoglobin C-thalassaemia

The genetic interaction between Hb C and thalassaemia genes have been reported in Italians, American Negroes, North Africans, Turks and Sicilians. The disorders are remarkably heterogenous clinically and haematologically and between different racial

groups significant differences are encountered.

(a) **Hb C - β^+-thalassaemia**

This condition is associated with a mild degree of anaemia with no abnormal physical findings. The blood picture shows a mild degree of microcytosis and there are 20-50% target cells. We investigated cases in Saudi Arabia with this interaction. No case of coexisting Hb C-β^+-thalassaemia was identified in Saudis.

(b) Hb C -β° thalassaemia

A more severe disorder results from co-inheritance of Hb C and β° thalassaemia. The clinical findings include moderate to severe anaemia, icterus, bone changes and moderate to marked splenomegaly. These patients may suffer from crises and hypersplenism necessitating splenectomy. The haematological findings include haemoglobin level ranging from 7-10g/dl, hypochromic-microcytic cells, target cells, reticulocytes elevation (5-20%). No case of this interaction was identified during our studies in the Saudis.

(c) Hb C - Hb Lepore or δβ-thalassaemia

A few patients with coexisting Hb C and Hb Lepore have been reported in literature. The condition is generally mild with hypochromic-microcytic anaemia, reticulocytosis and presence of target cells. No other physical findings have been reported in these patients.

Coexistance of Hb C and δβ-thalassaemia is also rare and only a few case reports are found in the literature. The condition is generally mild and

splenomegaly and bone changes were not reported. We did not encounter any patient with either of these conditions.

10.2.6 Haemoglobin E-thalassaemias

The first report of coexisting Hb E and thalassaemias was from Thailand during the early 1950's. Later studies confirmed that this disorder occurs in Thailand and many parts of South-east Asia, the Indian subcontinent and the Far East. It is believed to be a major health problem in India, Pakistan and Bangladesh. Both Hb E β^+ and Hb E β° thalassaemias have been identified though the latter is more common in Southeast Asia and India.

Clinical Features

The homozygous Hb E disease is a mild disorder. Less severe than homozygous Hb C disease. However, the Hb E - thalassaemia is associated with a remarkable degree of variable clinical expression, ranging from a moderately severe state to a state as severe as β-thalassaemia major. The severe form, in the children, is associated with huge protuberant abdomen, generalised wasting, dry, wrinkled skin and sunken eyes. As the child grows marked skeletal deformities with typical faces became apparent. There is marked pallor and mild degree of icterus. Cardiomegaly is usually found with systolic murmur. Almost all the patients have hepatomegaly and majority have splenomegaly. Other complications include:

Growth retardation: in almost 60% of the patients.

Infections: are more frequent and are the commonest cause of death in all ages.

Hypersplenism: occurs in majority of the patients and requires splenectomy.

Aplastic crises: with worsening of anaemia has been reported

.Extramedullary bone

marrow tumours: are found in several patients due to massive erythroid hyperplasia.

Iron-overload: occurs frequently due to increased gastrointestinal absorption and blood transfusion requirements.

Endocrine dysfunction: have been reported and include deficiency of the gonadotropins, cortisol, ACTH and growth hormone. Severe diabetes mellitus has been reported.

Haematological Features

The degree of anaemia is variable but is usually severe from early life and reticulocytosis is frequent. Red cell indices are reduced and resemble a β°-thalassaemia major picture. Red cell morphological defects are common target cell and inclusion bodies are identified. Though several cases heterozygous to Hb E were identified, but no case of Hb E-thalassaemia was encountered in the Saudi population.

10.2.7 Other abnormal haemoglobins - Thalassaemias

Sporadic cases of Hb D - thalassaemia and thalassaemia with rare structural haemoglobin variants have been reported. The clinical and haematological presentation is generally variable with a mild to moderate anaemia. No case of Hb D-thal. was identified in Saudis.

10.2.8 Structural haemoglobin variants - α-thalassaemia

The coexistance of α- and β-chain structural haemoglobin variants and different forms of α-thalassaemia is well documented. Diverse clinical disorders result from such

interactions and with the advances in molecular biology techniques the genotype-phenotype correlations are being clarified.

10.2.9 Sickle cell - α-thalassaemia

The interactions between Hb S and different forms of α-thalassaemia have been extensively investigated in the Americans, Jamaicans, Saudi Arabs, Turks and Indians. Such interaction produce a variable clinical and haematological picture and in the severe sickle cell disease states coexisting α-thalassaemia has been shown to have a modifying effect.

(i) Sickle cell anaemia - α-thalassaemia 1 or 2

The coinheritance of genes for sickle cell haemoglobin and α-thalassaemia 1 (--/αα) or 2 (-α/αα) are frequent in areas where both genes coexist at a high frequency and lead to alteration in the quantity of the structurally abnormal haemoglobin compared to that found in the absence of the thal.assaemic state. In addition, these interactions influence the molecular pathology and clinical manifestations of the Hb S in several populations (Weatherall et al, 1969; Weatherall & Clegg, 1981). The interactions of these genes is considered as one of the factors responsible for amelioration of the clinical manifestation of sickle cell disease (Weatherall et al, 1969). The association of α-thalassaemia and Hb S resulting in a 'new sickling disorder', characterized by a mild clinical manifestation, was first reported in a Saudi family from the Eastern province of Saudi Arabia (Weatherall et al, 1969). A report from an African study suggested that the beneficial effect of the association of α-thalassaemia is due to the lowering of the intracellular haemoglobin concentration. Subsequently, similar results were reported from

We conducted studies on the coexistence of Hb S and α-thalassaemia in the Saudi population in different regions of the country. The frequency of α-thalassaemia in Hb SS and Hb AS cases compared to Hb AA in the different areas of the country are presented in Table 10.7. The overall frequency of -α/αα,, -α/-α, ααα/αα in the Hb AA, AS and SS cases is presented in Figure 10.1.

The clinical manifestations and haematological findings were investigated in these patients. The results of clinical presentation in the patients from eastern and Western provinces are presented in Table 10.8 and 10.9, respectively, while the results of the haematological parameters in Hb SS patients with and without α-thalassaemia from the eastern and western provinces are presented in Tables 10.10. Comparison of the results from the two provinces shows significant differences between the two groups. Even the presence of α-thalassaemia does not ameliorate the Hb SS in the Western province patients to the same extent as that seen in the eastern province population. However, within patients of each province α-thalassaemia does ameliorate both the clinical presentation and the haematological parameters.

The results of the study in the eastern province and western province shows that

Table 10.7: Frequency of α-Thalassaemia in Hb AA, Hb AS and Hb SS cases in different regions of Saudi Arabia

Area	No. of cases	Frequency (%) *** α-gene arrangement		
		-α/αα	-α/-α	αα/αα
Al-Hafouf				
Hb AA	348	39.08 (136)	12.9 (45)	47.91 (167)
Hb AS	82	37.80 (31)	18.29 (15)*	43.90 (36)
Hb SS	24	33.3 (8)	37.5 (9)	29.2 (7)
Jaizan				
Hb AA	234	33.33 (78)	16.24 (38)	50.42 (118)
Hb AS	40	47.5 (19)	15.0 (6)	37.5 (15)
Hb SS	8	50.0 (4)	12.5 (1)	37.5 (3)
Al-Ula				
Hb AA	214	8.878 (19)	0.467 (1)	90.186 (193)
Hb AS	28	21.43 (6)	0	78.57 (22)
Hb SS	8	25.0 (20	0 (0)	75.0 (6)
Khaiber				
Hb AA	51	21.57 (11)	1.96 (1)	76.47 (39)
Hb AS	6	33.3 (2)	0 (0)	66.7 (4)
Hb SS	-			
Al-Baha				
Hb AA	31	25.8 (8)	6.45 (2)	67.74 (21)
Hb AS	6	50.0 (3)	16.7 (1)	33.3 (2)
Hb SS	2	0 (0)	0 (0)	100 (2)
Riyadh				
Hb AA	234	18.8 (44)	3.85 (9)	77.35 (181)
** Hb AS	151	36.42 (55)	6.62 (10)	56.95 (86)
* * Hb SS	186	37.63 (70)	11.83 (22)	50 (93)

* 1 case was leftward/rightward deletion
** Cases seen in Riyadh but belonging to different regions
( ) No. in bracket is the number of samples
*** Diagnosed using Bam H1

Figure 10.1: Frequency of α-Thalassaemia and triple α-gene arrangement
in the overall Hb AA, AS & SS population in Saudi Arabia

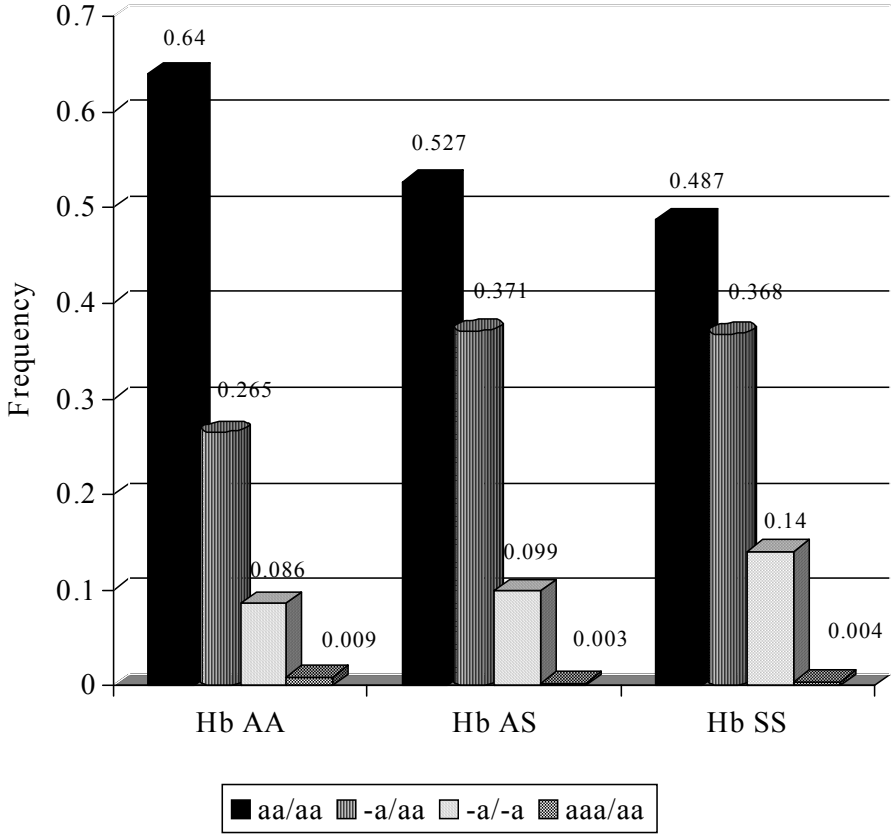


Table 10.8: Clinical presentation in Hb SS patients with and without α-Thalassaemia in Eastern Province of Saudi Arabia

Clinical Presentation	Hb SS patients (%)*	
	Without α-Thal.	With α-Thal.
Anaemia	56	30
Pain in bone & joint	90	70
Abdominal pain	64	29
Leg ulceration	-	-
Hepatomegaly	36	-
Splenomegaly	45	14
General weakness	90	86
Jaundice	18	14
Oedema feet	9	14
Previous Blood transfusion	73	30
Osteomyelitis	45	30

*Over 100 patients in each group followed for 3-4 years.

Table 10.9: Clinical manifestation in sickle cell anaemia patients with and without α-Thalassaemia in the Western Province of Saudi Arabia

Clinical manifestation	Hb SS Patients (%)*	
	With α-Thal.	Without α-Thal.
Pallor	38.0	95.4
General weakness	20.0	13.0
Pain in bones and joints	80.0	73.
Swelling of joints	13.0	13.0
Oedema feet	3.3	-
Jaundice	13.3	26.1
Splenomegaly	3.3	-
Crisis:		
Vaso-occlusive	53.3	43.5
Haemolytic	-	17.4
Blood transfusion	56.7	60.9

*Approximately 100 patients in each group followed for 3-4 years..

Table 10.10: Comparison of the Haematological parameters encountered in sickle cell anaemia patients with and without α-thalassaemia from eastern and western provinces of Saudi Arabia

Haematological Parameters	Sickle Cell Anaemia without α-thalassaemia		Sickle cell Anaemia with α-thalassaemia	
	WP	EP	WP	EP
RBC ($X10^{12}$/l)	2.4 ± 0.38	3.3 ± 1.15	3.58± 0.91	3.84 ± 0.61
Hb (g/dl)	7.84± 1.04	10.0 ±3.51	8.79± 1.23	10.84± 1.6
PCV (l/l)	0.204±0.032	0.27± 0.08	0.242±0.045	0.30 ± 0.30
MCV (fl)	89.20± 5.1	82.8 ± 2.0	73.45± 4.48	79.8 ± 6.90
MCH (pg)	32.6 ± 2.96	30.3 ± 1.5	25.18±4.20	28.3 ± 2.6
MCHC (g/dl)	37.96± 3.21	36.0 ± 3.2	36.03±2.78	34.8 ± 2.9
Hb A_2 (%)	2.71 ± 0.65	2.8 ± 0.13	1.24 ± 0.83	2.5 ± 0.26
Hb F (%)	12.02± 7.09	15.25± 2.56	10.43 ± 6.87	14.14± 6.24

the presence of α-thalassaemia in Hb SS patients decreases the value of MCV and MCH but increases the red cell count, total haemoglobin and haematocrit values. In Hb SS patients with homozygous α-thalassaemia 2, the MCV, MCH, Hb F, reticulocyte count and WBC count are significantly lower ($P<0.05$), while the RBC, total Hb levels, PCV and Hb A_2 levels are considerably higher ($P < 0.05$) than in the Hb SS patients without α-thalassaemia. The heterozygous α-thalassaemia cases have intermediate values for RBC and Hb, PCV and MCH while reticulocyte count and Hb F level are raised. In this group of patients from the western province comparison was made between Hb SS with and without α-thal. and the differences are shown in Figures 10.2 to 10.4.

The level of Hb F and A_2 in Hb SS coexisting with α-thalassaemia has been the focus of several studies. Higgs et al (1982) reported a decrease in the level of Hb F and an increase in Hb A_2 level in Hb SS patients with homozygous α-thalassaemia 2, while Embury et al (1982) reported that the level of Hb F was significantly elevated, though there was no increase in the Hb A_2 level. The results of this study are in agreement with those of Higgs et al (1982). In addition, the results of this study showed a variable and inconsistent nature of sickle cell disease in Saudis. Some Hb SS patients with and without α-thalassaemia had Hb F levels as low as 2.5% and others had values as high as 25-30%. The haematological values and the clinical manifestations in the high F and low F groups were similar in some cases, while some patients with high Hb F levels showed a greater severity of the clinical manifestations and others with low Hb F levels and a mild presentation. This confirmed our previous report that a high Hb F level is not the only factor with a role in ameliorating the sickle cell disease in Saudis (El-Hazmi, 1980). Other

Figure 10.2: Haematological parameters in SCA with and without α-Thalassaemia

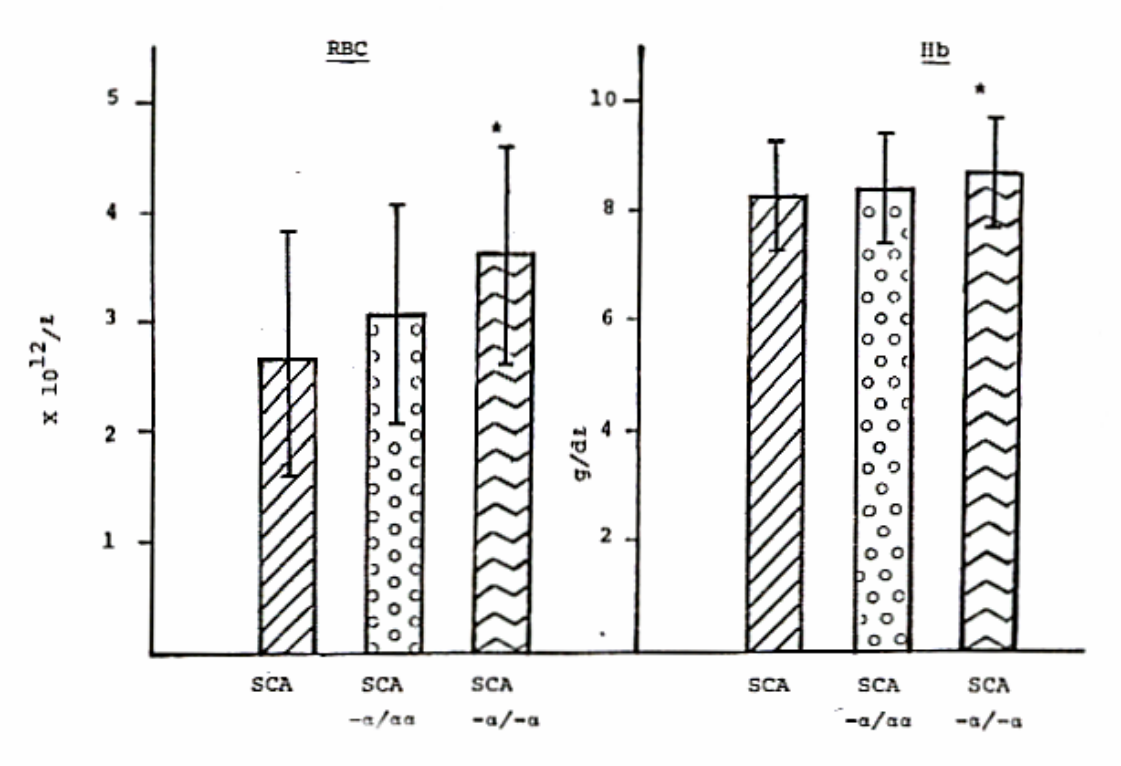


Figure 10.3: Haematological parameters in SCA with and without α-Thalassaemia

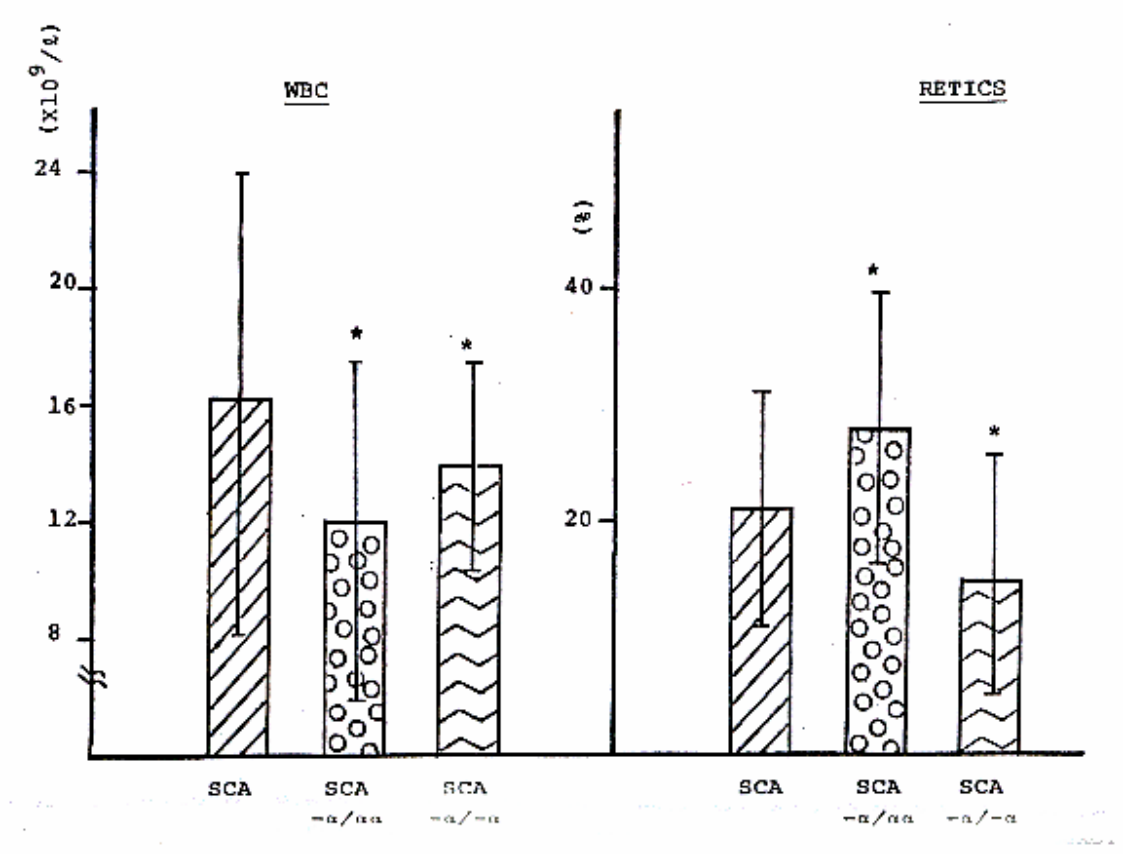
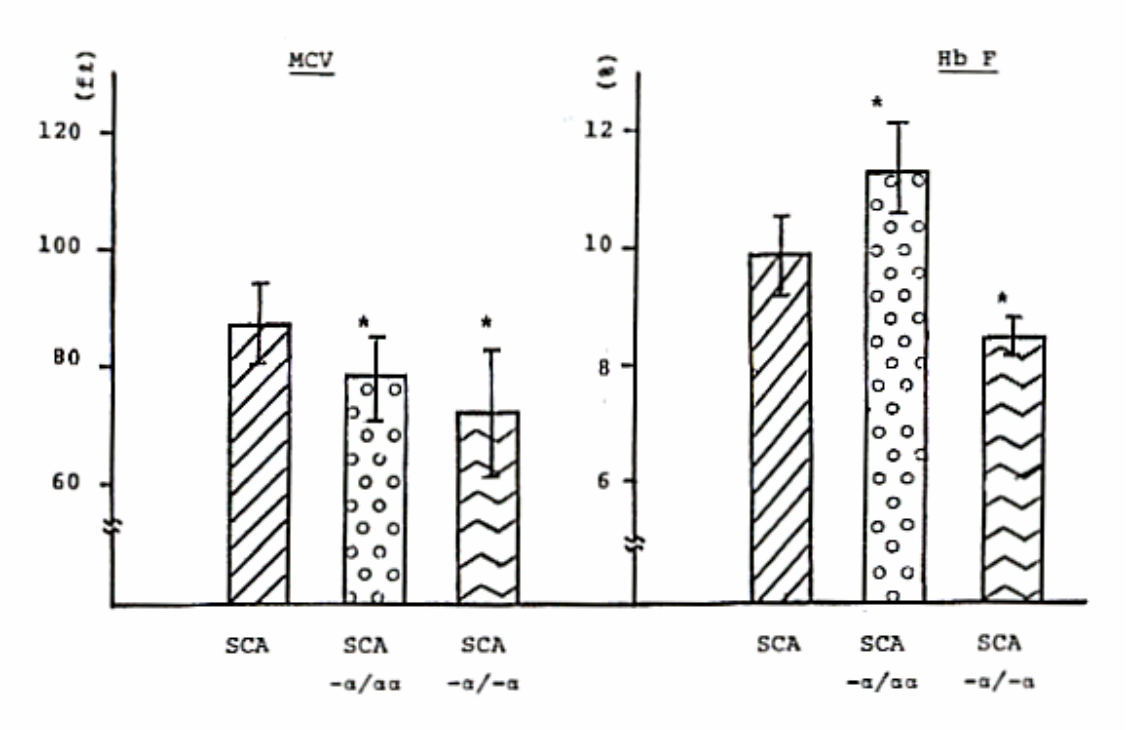


Figure 10.4: Haematological parameters in SCA with and without α-Thalassaemia



factors, both genetic and environmental, may be involved in modifying the clinical presentation of the sickle cell disease in this population.

The presence of α-thalassaemia, due to either 1 or 2 α-gene deletions, influences the biochemical parameters. The bilirubin level in Hb SS patients with α-thalassaemia was lower than in Hb SS patients without β-thalassaemia; however, the difference was not statistically significant. In addition, other liver function test abnormalities such as elevated ALP, SGOT and SGPT levels were more pronounced in the Hb SS patients with 2 gene deletions. Thus, the Hb SS patients with 2 α-gene deletions may be at a disadvantage compared to α-thalassaemia patients with 1 α-gene deletion, as the former condition on its own has a more severe clinical presentation than the latter condition. Creatinine and urea levels were lower in all Hb SS patients compared to the normal (Hb AA) group. This may be due to renal dysfunction in Hb SS patients which results in an inability to concentrate the urine.

The analysis of the clinical data revealed that Hb SS patients with α-thalassaemia have fewer complications such as hepatomegaly, splenomegaly, abdominal pain and osteomyelitis, and have received fewer blood transfusions compared to the Hb SS patients without α-thalassaemia. Leg ulceration and hand and foot syndrome are seen at a lower frequency in Saudi patients with or without α-thalassaemia. Furthermore, our studies show that the observed number of Hb SS cases detected in this population are considerably more than the expected value calculated by application of the Hardy-Weinberg equilibrium, suggesting the survival of Hb SS patients to adult life due to a mild disease.

The haematological findings in patients with Hb SS and α-thalassaemias show anaemia with reduced red cell indices and often slightly elevated Hb A_2. Family studies show Hb S heterozygocity in both parents. In addition, the α/β globin chain ratio is reduced. The Hb S level is lower in the red cells compared to the level in Hb SS patients and as a result the extent of red cell sickling and haemolysis is reduced, thus affecting the overall clinical severity of the Hb SS.

Earlier reports regarding the influence of α-thalassaemia on clinical severity of Hb SS contained conflicting data. Some studies showed an ameliorating effect while others failed to do so. These discrepancies were due mainly to difficulties in the diagnosis of the α-thalassaemia geno-types. Using biophysical studies it was recently shown that presence of α-thalassaemia lowers Hb S level in red cells and this would ameliorate the clinical severity of Hb SS. Using gene mapping studies the modifying influence of α-thalassaemia on the severity of Hb SS was confirmed. The rate of haemolysis, reticulocyte count, mean cell volume and mean cell haemoglobin in Hb SS with α-thalassaemia are lower compared to the Hb SS without α-thalassaemia. Differences are encountered depending upon whether one or two α-genes are deleted.

Studies on Saudi Hb SS patients with Hb H disease have shown the mild nature of this combination. The patients suffered from a mild hypochromic-microcytic anaemia and clinically had no major problems. Thus confirming the ameliorating effect of associated α-thalassaemia. A study showed that Hb SS patients with α-thalassaemia had a longer life expectancy than non-thalassaemia Hb SS patients. This suggests that the degree of anaemia may be a more significant determinant of survival.

Recently, molecular biology studies have shown that there are multifactorial consideration of the effect of α-thalassaemia on the clinical severity of Hb SS. The coexisting DNA polymorphic sites i.e. the Xmn I polymorphic site, the β-globin gene haplotypes, the G γ/Aγ ratio and possibly other unknown genetic factors also contribute to the clinical severity of Hb SS. Studies in Saudi Arabia have confirmed that α-thalassaemia in Hb SS patients with Benin haplotype and absence of Xmn I polymorphic site, have a lower ameliorating effect than α-thalassaemia in Hb SS patients with Saudi-Indian haplotype and Xmn I polymorphic site (discussed later).

10.2.10 Sickle cell trait-α-thalassaemia

As discussed in the section on Hb S heteroygotes in the Hb AS, the presence of α-thalassaemia influences the Hb S level and values ranging from 25-45% have been reported in different populations. We conducted several studies on the Hb S heterozygotes with associated α-thalassaemia in different regions of the country. Table 10.7 presents the frequency of α-thalassaemia in the Hb S heterozygote cases in different regions of Saudi Arabia.

Several haematological studies were conducted on Hb S heterozygotes with associated α-thalassaemia. A common finding was a lower level of Hb S in these individuals. The frequency distribution histogram of Hb S was obtained and Hb S level showed a trimodal distribution depending on whether there were 0, 1 or 2 α-gene deletions. The lowest Hb S level was found in individuals with two α-gene deletions. The haematological parameters were estimated in these individuals and no major abnormalities could be identified except for decrease in the red cell indices. The mean cell volume,

mean cell haemoglobin were lower in the individuals with α-thalassaemia. An increase in the level of Hb S resulted in an increase in the value of MCV and MCH, as discussed in the earlier section.

The proportion of Hb S in heterozygotes is considered an indicator for determining the presence or absence of α-thalassaemia. It has been shown that lower values of Hb S are indicative of co-existing α-thalassaemia and furthermore, the quantity of Hb S in heterozygotes is inversely related to the number of α-thalassaemia genes present. The heterogenous distribution of the Hb S level has been explained by a genetic model, according to which the number of active α-gene loci can modify the net synthesis of Hb S. This model indicates that the presence of α-thalassaemia is a cause for the decreased amount of available α-chains. This, in turn, leads to a decreased net synthesis of Hb S because of the lower affinity of β^S chains, compared to β^A chains, for α-chains. This genetic model has been confirmed by biosynthetic studies and DNA analysis. Applying this model, the genotypes in Hb AS heterozygotes with low, medium and high amount of Hb S are -α-/α ,β/β^S, -α/αα ,β/β^S and αα/αα ,β/β^S, respectively. The results of the present study show that in the Saudi Hb S heterozygotes the value of Hb S has an overall mean of 31% and a range of 17-45% of the total haemoglobin. The lower mean and range are indicative of co-existing α-thalassaemia. Similar results have been reported from Canada, Georgia, California and India for Hb S heterozygotes.

The Hb S values in Saudis show a trimodal distribution on the frequency distribution histograms. The peaks A, B and C have mean Hb S value of 23, 31.5 and 40% and a range of 18-28, 28-35 and 35-45%, respectively. The peak with values of Hb S 35%

The α-thalassaemia genes in Hb S heterozygotes modify the haematological parameters, although only slightly. The effect is mainly on the level of MCV, MCH and the α/β/ chain ratio. Most of the individuals with Hb S above 38% have normal haematological values, red cell indices and a balanced globin ratio. Individuals with associated α-thalassaemia 2 gene (heterozygotes) have normal total haemoglobin but show slight microcytosis and hypochromia, while those with homozygous α-thalassaemia 2 gene have a slightly reduced haemoglobin level and a more pronounced microcytosis and hypochromia. The MCV and MCH decreases as the Hb S concentration in the Hb S heterozygotes decreases. However, some individuals with reduced level of Hb S have normal MCV and MCH. Their α/β ratio is reduced indicating the presence of α-thalassaemia.

In addition, the results of this study show that in some regions a high percentage of the Hb S heterozygotes have co-existing α-thalassaemia. Though differences are encountered in the different regions. The high rate of co-existence of these two genetic abnormalities cannot be explained on the basis of any genetic linkage as the α-globin genes are located on chromosome 16 and β-globin genes on chromosome 11. The presence of Hb S is believed to play a protective role against harmful environmental factors such as malaria. The population investigated in this study lives in areas in which malarial parasites are endemic. In addition, the presence of α-thalassaemia has been shown to play a role in ameliorating the clinical manifestation of the sickle cell disease. The increased fitness of Hb S carriers and homozygotes compared to normal individuals against malaria on the one hand, and the increased fitness of Hb S homozygotes due to the presence of α-thalassaemia, on the other hand, may result in an increased frequency of these abnormal genes. Furthermore, consanguinity and other forms of inter-marriages may result in further concentration of these genetic abnormalities.

10.2.11. Haemoglobin C-α-thalassaemia

Coexisting Hb C and α-thalassaemia have been reported in the Negro populations. The homozygous Hb C disease with α-thalassaemia is generally benign with a mild degree of hypochromic-microcytic anaemia, and with elevated Hb F level. In Hb C heterozygotes the level of Hb C is less than 40% Hb C and about 4.5% Hb F.

10.2.12. Haemoglobin SC - α- thalassaemia

A few Negro cases with Hb SC disease and associated α-thalassaemia have been reported. The anaemia was slightly more severe than in Hb SC disease and the MCV and

MCH were significantly reduced. No such case was identified in Saudis.

10.2.13. Haemoglobin E - α-thalassaemia

In Southeast Asia where both Hb E and α-thalassaemia occur at a high frequency, there is a high frequency of coexisting Hb E and α-thalassaemias. In Hb E heterozygotes, which is a common condition in Thailand, the associated α-thalassaemia reduces Hb E level to 15-22% and 23-32% depending on 2 or 1 α-gene deletions, respectively. A few Thai families with homozygous Hb E - α-thalassaemia have been reported. the clinical picture is similar to that of thalassaemia major, with low haemoglobin level, elevated Hb F and presence of Hb Barts. No case of Hb E-α-thalassaemia was seen in Saudis.

10.2.14. Other abnormal haemoglobins - α-thalassaemia

Sporadic cases with other abnormal haemoglobins and associated α-thal.assae-mia have been reported. These include Hb J Bangkok-α-thalassaemia, Hb N Baltimore- α-thalassaemia, Hb-Riyadh-α-thalassaemia, Hb D-α-thalassaemia and Hb Constant Spring-α-thalassaemia. The clinical and haematological values shows variations, but generally the conditions are mild.

The interactions between structural and biosynthetic disorders of haemoglobin result in considerable modification of the haematological and clinical presentation. The accurate diagnosis of such conditions is essential particularly for genetic counselling and hence for control and prevention.

10.2.15. Triple alpha-gene in a patient with homozygous sickle cell anaemia

An unusual case who had inherited triple α-gene and Hb SS was diagnosed during our investigations and detailed studies revealed interesting results.

This pateint was a 17 years old male student from Fifa in the south-western province of Saudi Arabia and was suffering from severe form of Hb SS. Two age and sex matched Hb SS patient and one normal Hb AA control from the same region were included in this study to compare the results with those of the propositus. One of the Hb SS patient had normal α-gene arrangement while the other had one α-gene deletion. The normal control had normal α-gene arrangement. The propositus was diagnosed as a Hb SS patient based on the results of electrophoresis at acid and alkaline pH and confirmed by treatment of DNA with the restriction endonuclease Mst II (i.e. a fragment 1.35 Kb containing the β-globin gene indicates homozygous sickle cell disease).

DNA extracted from buffy coat was treated with restriction endonucleases Bgl II, Bam HI, Hind III, Hpa I and Xba I according to the methods recommended by the manufacturer. The DNA fragment was separated by electrophoresis in 0.9% agarose gel and transferred to nitrocellulose sheets. The α-globin gene containing fragment was recognized by treatment with a radio-labelled probe of 1.5 Kb fragment of α-globin gene cloned into Pst II site of PLTNI released upon digestion with Pst II. The fragments were visualized by autoradiography.

The fragments generated by treatment with the different restriction endonucleases are presented in Table 10.11 and Figures 10.5 to 10.8. The propositus was confirmed to have ($\alpha\alpha/\alpha\alpha\alpha^{anti\ 3.7}$) while among the other two Hb SS patients, one had one α-gene deletion ($-\alpha/\alpha\alpha$) and the other had normal α-genes ($\alpha\alpha/\alpha\alpha$). The clinical manifestations, obtained as the severity index, was significantly higher in the propositus compared to the other two Hb SS patients. The manifestations recorded over a period of one year are

The level of bilirubin was significantly higher in the propositus compared to the other Hb SS patients indicating a higher rate of haemolysis.

These results suggest that genetic factors affect the clinical presentation of sickle cell disease and contribute to the heterogeneity of clinical and haematological presentation of Hb SS. The heterogeneity is well documented and a number of genetic and environmental factors have been implicated as possible modifiers of the clinical presentation of sickle cell anaemia.

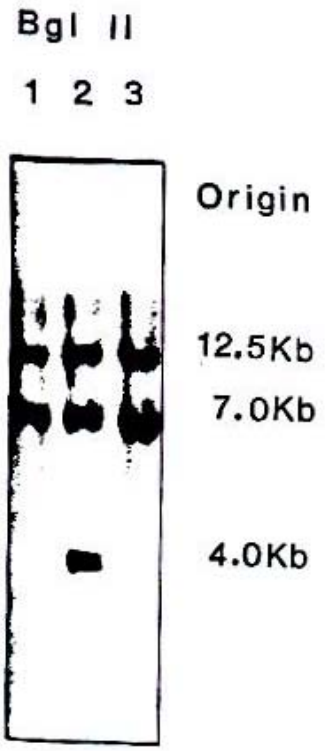
Several factors are believed to contribute to the sickle cell disease heterogeneity, including associated α- or β-thalassaemia, glucose-6-phosphate dehydrogenase deficiency, elevated Hb F level, iron deficiency and various β-globin gene haplotypes (Weatherall et al, 1969; Pembury et al, 1978; El-Hazmi and Warsy, 1984; 1987). However, to date no single genetic or environmental factors is identified which influences the nature of Hb SS. As the studies at the gene level have made significant progress due to the advancement made in the recombinant DNA technology, it has advancement made in the recombinant DNA technology, it has become possible to search for the possible genetic modulators of Hb SS. Several studies have shown the presence or absence of a

Table 10.11: Restriction endonuclease and band pattern in the propositus and Hb S homozygotes with normal α-globin gene (αα/αα) and one α-globin gene deletion

Restriction Enzyme	Bands in Propositus $\alpha\alpha\alpha^{anti\ 3.7}$	Bands in Hb S homzygotes with	
		Normal α-gene αα/αα	α-gene deletion -α/αα
Bgl II	12.5 7.0 4.0	12.5 7.0	12.5 7.0 15.8
Bam HI	17.5	14.5	14.5 10.5
Hind III	16.0 4.5 3.7	16.0 4.5 3.7	ND
Hpa I	14.5 4.3 3.7	14.5 4.3	ND
Xba I	19.0	15.6	ND

ND = Not done

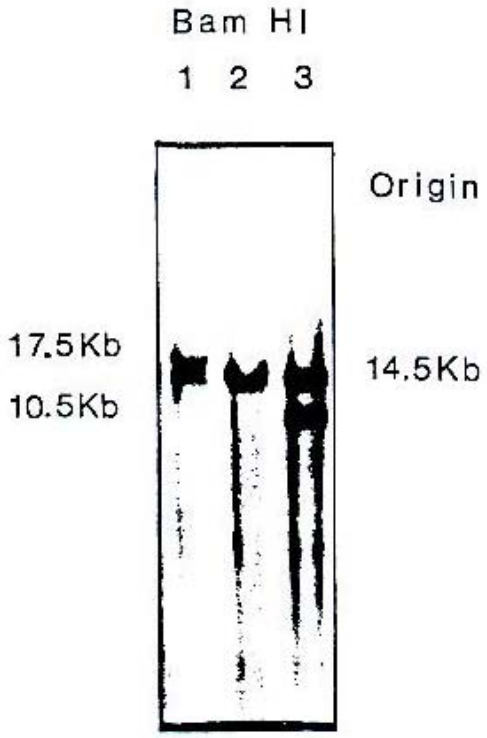
Figure 10.5: Atutoradiography of the DNA fragments obtained on digestion of the DNA from Hb SS patients with triple α-genes and normal gene arrangemwnt with Bgl II



Sample No. 1 & 3: Normal α-gene (αα/αα)

Sample No. 2 Triple α-gene/normal α-gene ($\alpha\alpha\alpha^{Anti\ 3.7}/\alpha\alpha$

Figure 10.6: Autoradiography of the DNA fragments obtained on digestion of the DNA from Hb SS patients with triple α-genes and normal gene arrangement with Bam HI



Sample 1: Triple α-gene/normal gene ($\alpha\alpha\alpha^{anti}$ 3.7/αα)

Sample 2: Normal α-gene (αα/αα)

Sample 3: One α-gene deletion (-α/αα)

Figure 10.7: Autoradiography of the DNA fragments obtained on digestion of the DNA from Hb SS patients with triple α-genes and normal gene arrangements with Hind III

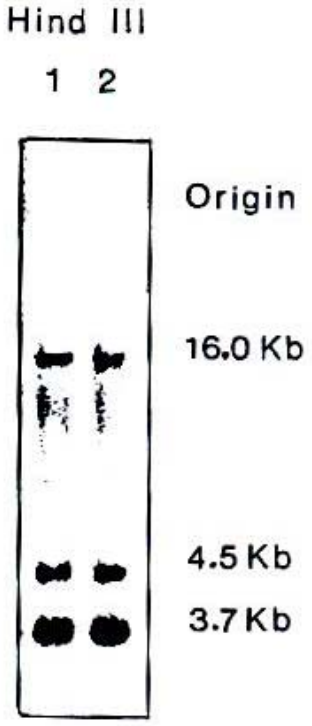


Figure 10.8: Autoradiography of the DNA fragments obtained on digestion of the DNA from Hb SS patients with triple α-genes and normal gene arrangements with Hpa I

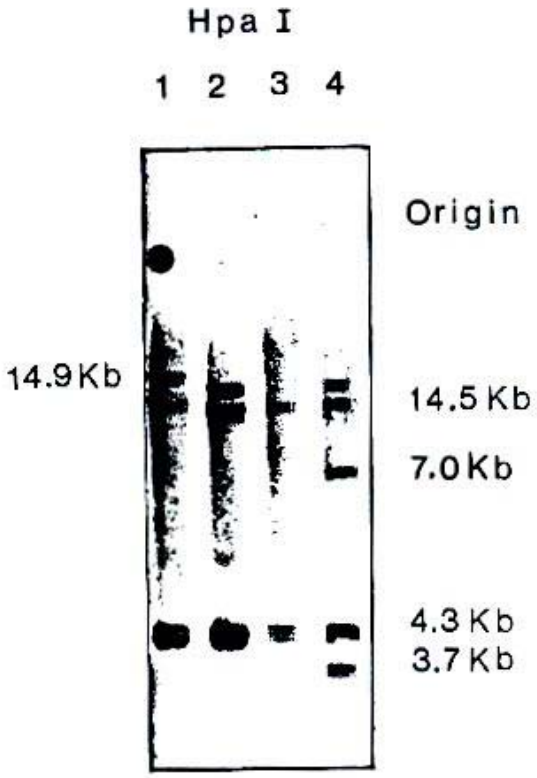


Table 10.12: Clinical manifestation and severity index in the propositus compared to the sickle cell anaemia patients with and without α-gene deletion

Signs/Symptoms	Frequency/Year		
	Propositus (Hb SS, αα/αα)	With no α-gene deletion	With one α-gene deletion
Severity of anaemia*	2	2	1
Chronic pain	1	0	1
Bilirubin level	1	1	1
Painful crises/year	2	1	1
Aseptic necrosis	1	0	0
Osteomyelitis	0	0	0
Leg ulcers	0	0	0
Gallstone	0	0	0
Hyposlenism	0	1	0
Priapism	0	0	0
Chest infection	1	0	0
Retinopathy	0	0	0
Cerebro-vascular accident (CVA)	0	0	0
Deep vein thrombosis (DVT)	0	0	0
Blood transfusion/year	1	0	0
Hospitalization/year	2	1	1

0 = Not present 1 = Present

* = Severity of anaemia > 11.0 g/dl = 0
9.0 - 11.0 g/dl = 1
7.0 - 8.9 g/dl = 2
< 7.0 g/dl = 3

Table 10.13: Haematological parameter value in the propositus compared to Hb S homozygotes with normal α-gene and one α-gene deletion (-α/αα)and normal control (Hb AA)

Parameters	Hb S Homozygotes			With two α-gene deletion (-α-/α)	Normal (Hb AA)
	Propositus with triple α-gene $\alpha\alpha\alpha^{anti\ 3.7}$	With no α-gene deletion (αα/αα)	With one α-gene deletion (-α/αα)		
Hb (g/dl)	8.4	8.92	9.57	11.6	12.5
RBC ($X10^{12}$/l)	2.41	2.77	3.07	4.29	5.0
PCV (l/l)	0.23	0.25	0.25	0.34	0.42
WBC ($X10^{9}$/l)	17.1	12.61	12.73	8.37	3.2
MCV (fl)	91	92.13	82.58	76	86
MCH (pg)	31	32	25	32	32
MCHC (g/dl)	34	34	32	32	32
Hb A_2 (%)	2.2	3.2	2.0	20	2.5
Hb F (%)	5.0	8.42	8.5	4.37	0.9
Retic (%)	11.41	11.41	9.93	2.37	0.9

Table 10.14: Biochemical parameter values in the propositus compared to the Hb SS patients with and without α-gene deletion and normal controls

Parameters	Hb S Homozygotes			Normal (Hb AA)
	Propositus Hb SS and αα/αα	With no α-gene deletion (αα/αα)	With one α-gene deletion (-α/αα)	
Total bilirubin (μmol/l)	85	84	34	9.0
Direct bilirubin (μmol/l)	30	25	1	2.2
Protein (g/l)	77	80	80	70
Albumin (g/l)	36	52	45	42
Alk. Phos (U/l)	223	192	238	100
ALT (U/l)	44	60	23	30
AST (U/l)	130	150	93	23
Uric Acid (μmol/l)	280	230	277	170
Calcium (mmol/l)	2.2	2.6	2.4	2.3
Phosphorous (mmol/l)	1.5	1.6	1.4	1.2
Cholesterol (mmol/l)	2.5	3.2	2.6	4.4

number of restriction endonuclease polymorphic sites in or around the β-globin gene which occur more frequently in the mild form of Hb SS. Though the exact mechanism involved in modifying the clinical presentation are not yet known, but one of the factors is believed to be an increased production of Hb F.

The results of this study showed also that in the Hb SS patients with α-gene deletion the clinical and haematological presentation are milder than in patients without α -gene deletion, while excess of α-chains, as that encountered in the propositus, who had five α-globin genes, resulted in a severe clinical presentation. Thus while α-thalassaemia improves the clinical and haematological presentation, presence of $\alpha\text{-}^{anti\ 3.7}$ makes the disease worse (Figures 10.9-10-11). This can be explained on the basis of excess of α-globin chains, which would result in production of higher amount of Hb S, producing more gelation, sickling and hence other complications. During our studies on Saudi patients, the presence of α-thalassaemia in sickle cell disease has been frequent, however, the triple α-gene arrangement in sickle cell disease patients is relatively rare. Of the several hundred sickle cell disease patients investigated during our studies since 1977, only one had this unusual α-gene arrangement which is believed to result from unequal crossing over during intra-chromosomal rearrangements (Figure 10.12)

10.2.16. Interaction between haemoglobin structural variants

Interaction between two different structural variants of haemoglobin are reported in literature and results in modified clinical presentation in these double heterozygotes. The most frequent double heterozygote states are those resulting from interaction between Hb S and Hb C, though others including between Hb S-Hb O-Arab and Hb S-HbE have

Figure 10.9: Severity Index in the propositus compared to the Hb SS patients with and without α-gene deletion

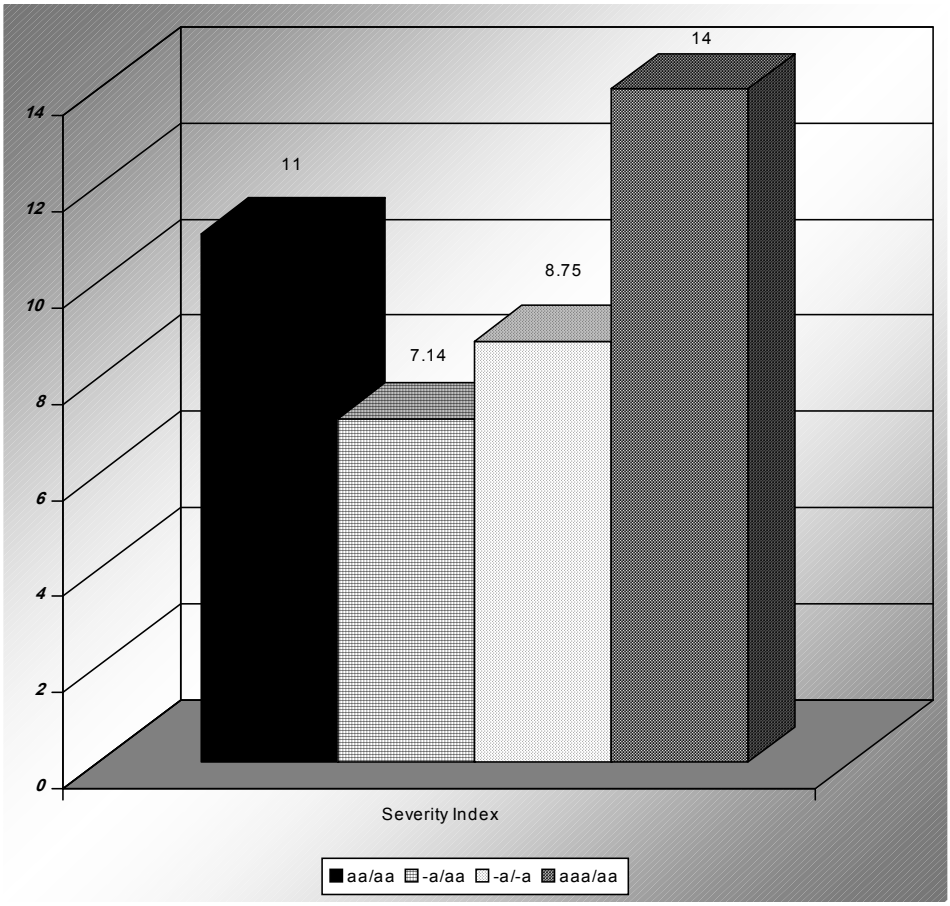


Figure 10.10: Haematological parameters in Hb SS with triple α-gene compared to Hb SS with and without α-gene deletion

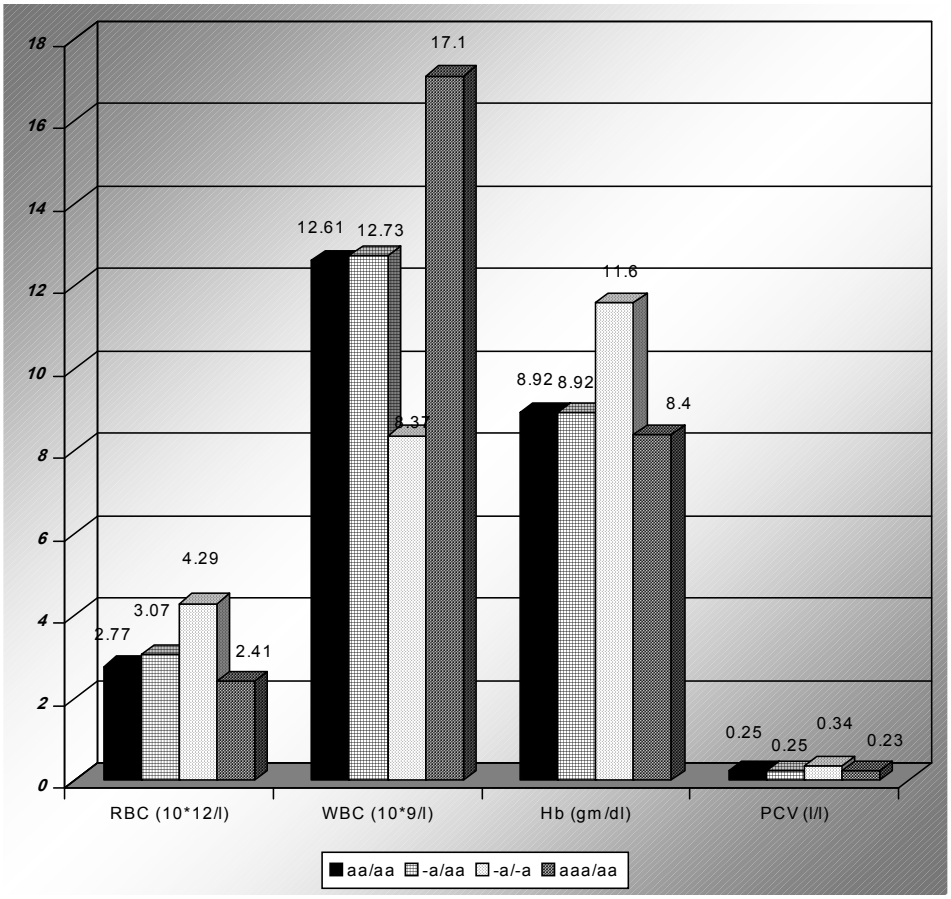


Figure 10.11: Haematological parameters in Hb SS with triple α-gene compared to Hb SS with and without α-gene deletion

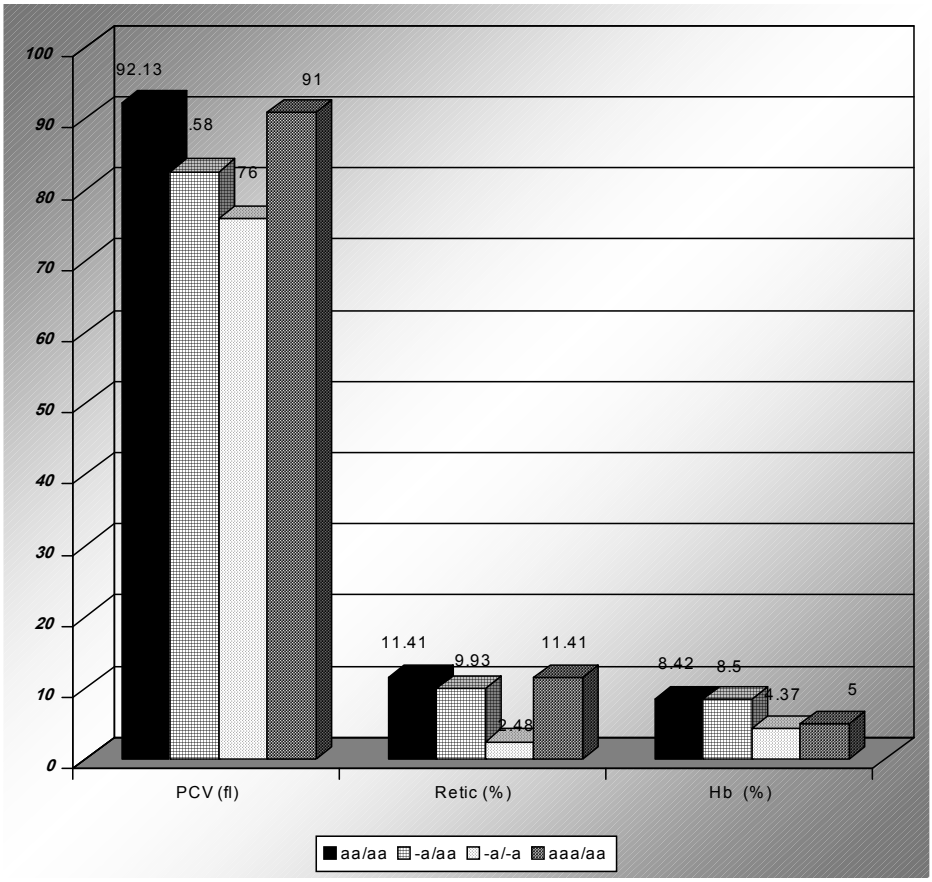
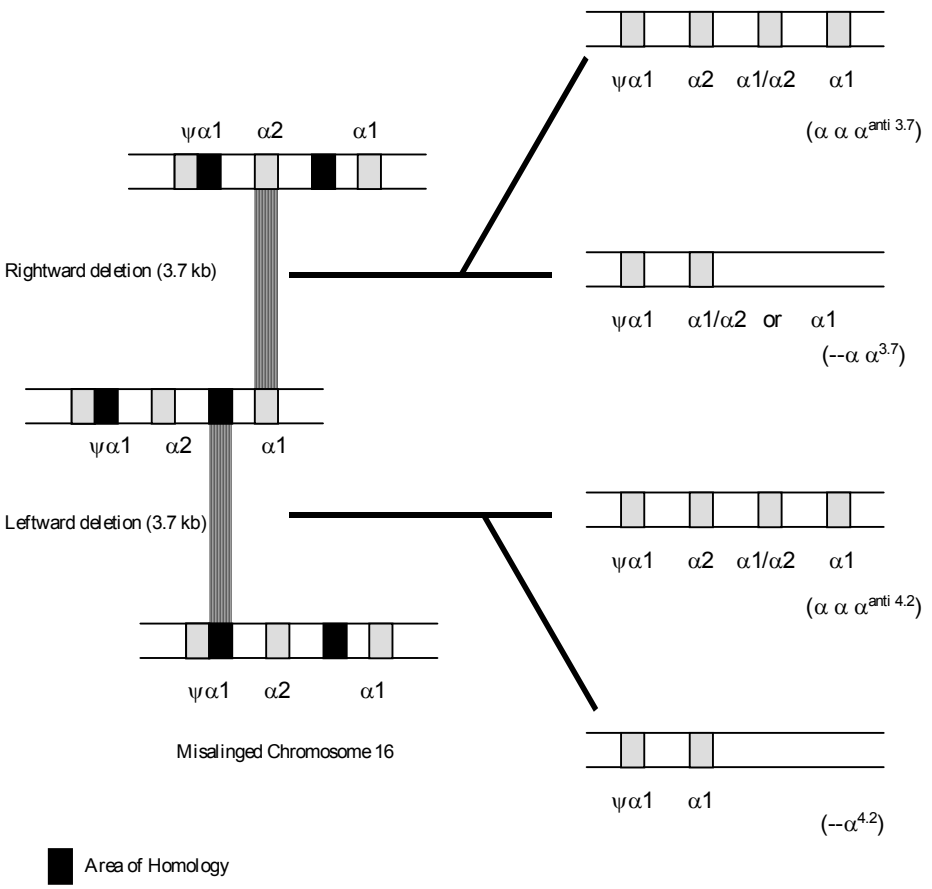


Figure 10.12: Unequal crossing between α-gene during intra-chromosomal translocations resulting in triple α-gene arrangement on one chromosome



been reported as isolated cases in populations where both abnormal exist. Among the Saudis we identified only HB SC interactions during the course of our investigations. Two Hb SC cases were identified in Riyadh, and two in Al-Baha, one of the grand parents in each case did not originally belong to Saudi Arabia. Compared to patients with Hb SS and Hb SC, these patients had a mild presentation and did not suffer from frequent episodes of crises and other complications associated with the homozygous sickle cell disease.

10.2.17 Genetic interactions between the thalassaemias

Several patients were diagnosed in different regions of Saudi Arabia who had coexisting α- and β-thalassaemia genes. The diagnosis in these patients was made using the molecular biology techniques. α/β globin chain ratio for the normal range as both α- and β-globin chains were decreased. The haematological and biochemical findings in these patients are presented in Table 10.15.

10.2.18. Genetic interaction between Hb S, α- and β-thalassaemia

Several cases were identified in some regions of Saudi Arabia who had Hb S, β-thalassaemia and α-thalassaemia due to either one or two α-globin gene deletion. The interaction of the three genes affected the clinical and haematological values in these patients. The Table 10.16 present the haematological and biochemical parameters in Hb S/β°-thalassaemia with one or two α-gene deletions.

10.2.19. Genetic interactions of sickle cell haemoglobin and G-6-PD deficiency

In some studies interactions between sickle cell and G-6-PD deficiency genes have been reported and it has been shown that G-6-PD occurs at a higher frequency in sickle cell disease patients (El-Hazmi & Warsy, 1984). On the other hand, other studies have

Table 10.15: Haematological and biochemical parameters in patients with double heterozygotes to α- and β-thalassaemia

Parameters	β^+-thalassaemia	β^o-thalassaemia
	-α/αα	-α/αα
Haematological Parameters		
Hb (g/dl)	11.9 ± 3.0	8.5 ± 2.5
PCV (l/l)	0.35 ± 0.09	0.27 ± 0.06
WBC ($X10^9$/l)	8.9 ± 6.3	9.8 ± 7.0
MCV (fl)	79.0 ± 7.4	84.0 ± 5.0
MCH (pg)	26.8 ±3.3	24.0 ± 3.0
MCHC (g/dl)	34.2 ± 1.1	31.0 ± 2.3
Hb A_2 (%)	5.5 ± 1.6	2.6 ± 1.0
Hb F (%)	2.2 ± 1.6	7.9 ± 2.0
RBC ($X10^{12}$/l)	4.5 ± 1.5	3.5 ± 0.6
Biochemical Parameters		
T. Bil. (μmol/l)	13.4 ± 19.9	16.40 ± 4.0
D. Bil. (μmol/l)	2.5 ± 2.1	1.0 ± 0.1
T. protein (g/l)	60.0 ± 12.5	64.0 ± 23.3
Albumin (g/l)	39.2 ± 7.3	45.0 ± 1.4
ALP (U/l)	4.1 ± 1.3	292.0 ± 121.0
SGPT (U/l)	18.7 ± 20.4	223.0 ± 134.0
SGOT (U/l)	24.8 ± 2.8	-

Table 10.16: Haematological and biochemical parameters in Hb S/β°-thalassaemia patients with α-thalassaemia

Parameters	Hb S/β° -thalassaemia	
	-α-/α	-α/αα
No. investigated	9	11
Age	13.3 ± 4.0	18.8 ±4.0
Haematological Parameters		
Hb (g/dl)	9.3 ± 2.0	9.2 ± 1.5
PCV (l/l)	0.26 ± 0.05	0.27 ± 0.04
WBC ($X10^9$/l)	10.6 ± 3.9	10.7 ± 4.74
MCV (fl)	73.6 ± 4.6	76.8 ± 8.2
MCH (pg)	25.9 ± 2.6	26.5 ± 3.9
MCHC (g/dl)	36.0 ± 4.0	33.6 ± 2.2
Hb A_2 (%)	4.5 ± 1.0	4.6 ± 1.2
Hb F (%)	4.2 ± 2.1	7.8 ± 5.2
RBC ($X10^{12}$/l)	3.6 ± 0.77	3.51 ± 0.59
Biochemical Parameters		
T. Bil. (μmol/l)	25.2 ± 12.1	36.0 ± 33.0
D. Bil. (μmol/l)	4.4 ± 3.0	5.8 ± 4.5
T. Protein (g/l)	73.9 ± 11.0	72.6 ± 12.4
Albumin (g/l)	40.0 ± 3.6	40 ± 4.0
ALP (U/l)	142.0 ± 50.0	158.0 ± 115.0
SGPT (U/l)	38.4 ± 26.8	33.7 ± 17.8
SGOT (U/l)	76.6 ±44.1	62.1 ± 29

failed to show any correlation between the two genetic abnormalities. In

addition, there are contradictory reports regarding the possible beneficial influence of G-6-PD deficiency on the clinical manifestation of sickle cell disease and both ameliorating and worsening influence of coexisting G-6-PD deficiency in sickle cell disease patients have been reported. G-6-PD deficiency and sickle cell genes occur at a high frequency in several regions of Saudi Arabia (El-Hazmi, 1979, 1982, 1983; El-Hazmi and Warsy, 1984, 1986; Warsy & El-Hazmi, 1987; El-Hazmi et al 1986) and in some studies a significantly higher frequency of G-6-PD deficiency is reported in sickel cell disease patients compared to normal individuals (El-Hazmi and Warsy, 1984, 1987; Warsy, 1985). We determined the frequency of G-6-PD deficien-cy in Hb S homozygotes (Hb SS), Hb S heterozygotes (Hb AS) and normal individuals and in some regions encountered the highest frequency of G-6-PD deficiency gene in the Hb SS group, followed by the Hb AS group as shown in Table 10.17. However, no such association was observed in other regions e.g. Makkah, Al-Qateef and Al-Qunfuda. In several regions the difference in the frequency of G-6-PD deficiency in Hb SS group compared to the normal was statistically significant. Similarly in several regions except Najran, Makkah, Al-Qunfuda and Tehamat-Aseer the difference in the frequency of G-6-PD deficiency in Hb AS group was significantly higher than in Hb AA group. This led us to suggest that some interaction does seem to exist between the sickle cell and G-6-PD deficiency gene. The possible causes for such as interaction may be (a) increased survival of sickle cell disease patients with G-6-PD deficiency possibly as a result of amelioration of the sickle cell disease by presence of G-6-PD deficiency (b) beneficial effect of sickle cell gene to the G-6-PD deficiency gene and (c) beneficial effect of the

Table 10.17: Frequency of G-6-PD deficiency in Saudi male with different

haemoglobin phenotypes in different regions of Saudi Arabia

Region	Total No. of samples	Haemo-globin phenotype	No. of each phenotype	Frequency of G-6-PD deficiency	χ^2 analysis	P value
Al-Qateef, Al-Hafouf & neighboruing villages	851	AA AS SS	639 145 67	0.200 0.303 0.418	SS/AA AS/SS AS/AA	<0.05 <0.05 <0.005
Najran	231	AA AS SS	226 5 ND	0.257 0.400 ND	AS/AA	>0.05
Khaiber	280	AA AS SS	240 40 ND	0.225 0.425 ND	AS/AA	<0.05
Tehamat-Aseer	114	AA AS SS	81 27 6	0.210 0.150 0.667	SS/AA AS/SS AS/AA	<0.05 <0.05 >0.05
Riyadh	383	AA AS SS	370 13 ND	0.083 0.307 ND	AS/AA	<0.05
Al-Qunfuda	823	AA AS SS	655 147 21	0.1045 0.1219 ND	AA/AS	> 0.05
Al-Ula	409	AA AS SS	351 50 8	0.0796 0.278 0.167	AA/AS AS/SS AA/SS	< 0.05 < 0.05 < 0.05
Al-Qateef	960	AA AS SS	668 249 43	0.414 0.338 0.3125	AA/AS AS/SS AA/SS	< 0.05 > 0.05 < 0.05
Makkah	381	AA AS SS	359 20 2	0.056 0.050 ND	AA/AS	> 0.05
Bisha	469	AA AS SS	397 50 22	0.078 0.080 0.045	AA/AS AS/SS AA/SS	> 0.05 > 0.05 > 0.05

ND = Not determined

co-existing genes against the environmental factors such as hepatitis, or other viral and bacterial infections (El-Hazmi & Warsy, 1986).

Preliminary data also indicated a beneficial effect of associated G-6-PD deficiency on the clinical manifestation of sickle cell disease in the Eastern Province population (El-Hazmi & Warsy, 1984). In an attempt to confirm whether G-6-PD deficiency influences haematological parameter and clinical manifestations of sickle cell disease, we conducted this study on 81 children (mean age 5.5 years, range 5.5 ± 3.3) from the Western provinceof Saudi Arabia, where the sickle cell disease is more severe. Ten of these patients had G-6-PD deficiency due to the phenotype "G-6-PD Mediterranean". Two groups were formed, one with G-6-PD deficiency and the other without. The results of haematological parameters in G-6-PD normal and deficient sickle cell disease patients in comparison with the results reported earlier in the Eastern Province population (El-Hazmi & Warsy, 1984) are presented in the Table 10.18. The clinical signs and symptoms in the Hb SS patients with and without G-6-PD deficiency are presented in Table 10.19.

These results show that the presence of G-6-PD deficiency has a non-significant influence on values of the haematological parameters. Comparison of the clinical signs and symptoms in the two groups shows that general weakness, abdominal pain, pain in bone and joints, pallor and vaso-occlusive crisis are common complaints in majority of these sickle cell disease patients. Jaundice, haemolytic crisis, general weakness, vaso-occlusive crisis, loss of appetite, vomiting and weight loss, occur more frequently in the sickle cell disease patients with G-6-PD deficiency compared to the patients without G-6-PD deficiency. However, the latter are more prone to swellings and pain in bones and joints, abdominal pain and require more blood transfusions.

Table 10.18: The haematological parameters in Hb SS patients from Western province, with and without G-6-PD deficiency. The results are compared with the results of patients from Eastern Province

Parameters	Hb SS patients			
	Western Province		Eastern Province*	
	Normal G-6-PD (n = 71)	G-6-PD Deficient (n = 10)	Normal G-6-PD (n = 6)	G-6-PD Deficient (n = 5)
RBC ($X10^{12}$/l)	3.02± 0.94	2.97± 0.6	3.72± 0.84	5.0 ± 0.27
Hb (g/dl)	8.5 ± 1.26	7.85± 0.58	10.4± 2.11	13.1 ± 0.56
PCV (l/l)	0.22± 0.05	0.21±0.025	0.31±0.06	0.38 ± 0.02
MCV (fl)	80.3 ± 9.4	78.0 ±9.65	84.7± 3.7	78.0 ± 0.75
MCH (pg)	29.2 ± 5.9	26.8 ± 4.6	28.4 ± 3.0	27.3 ± 0.35
MCHC (g/dl)	37.5 ± 5.1	35.7 ± 2.7	34.0 ±0.96	34.2 ± 0.67
RBC ($X10^{12}$/l)	14.1 ± 5.9	14.3 ±6.05	7.7 ± 2.7	8.2 ± 1.45
Hb A_2 (%)	3.2 ± 0.9	3.47± 0.75	3.3. ± 0.6	3.0 ± 0.27
Hb F (%)	11.4 ± 6.8	9.23± 7.2	9.63± 5.4	2.6 ± 0.5

* From El-Hazmi and Warsy, 1989.

Table 10.19: Clinical signs and symptoms in Hb SS patients with and without G-6-PD deficiency

Clinical	Percentage of the Hb SS with normal G-6-PD (61)		Percentage of the Hb SS with G-6-PD deficiency (7)	
(a) Signs				
Pallor	88.5	(54)	100.0	(7)
Oedema feet	1.6	(1)	14.3	(1)
Jaundice	16.4	(10)	28.6	(2)
Splenomegaly	1.6	(1)	-	-
Crises:				
- Vaso-occlusive	47.5	(29)	57.1	(4)
- Haemolytic	8.2	(5)	-	-
(b) Symptoms				
General weakness	26.2	(16)	42.9	(3)
Pain in bones & joints	81.9	(50)	57.1	(4)
Swelling of joints	19.6	(12)	-	-
Weight loss	13.1	(12)	28.6	(2)
Loss of appetite	24.6	(15)	57.1	(4)
Vomiting	8.2	(5)	14.3	(1)
Abdominal pain	68.8	(42)	57.1	(4)

( ) The number in the bracket gives the number of patients

The amelioration of certain features of sickle cell disease due to associated G-6-PD deficiency could be attributed to preferential survival of the younger red cell population that have a higher G-6-PD activity compared to the older red cell population. This may also explain why the requirements for blood transfusion in these patients is lower. Compared to the results reported in the Eastern province patients several differences are encountered. The overall results show the disease to be mild in the eastern province patients with a higher prevalence of splenomegaly and hepatomegaly.

It is shown by these results that G-6-PD deficiency in sickle cell disease patients with severe form of the disease does not seem to ameliorate the disease though it appears to be beneficial in patients who already have a mild disease.