CHAPTER 11
POSSIBLE FACTORS MODULATING THE HAEMATOLOGICAL AND CLINICAL PRESENTATION OF SICKLE CELL DISEASE IN SAUDI ARABIA

11.1 Introduction

Sickle cell disease in the Saudi population presents as a clinically and haematologically heterogenous group with a wide range of clinical severity. At one end of the spectrum are patients who have a mild disease associated with a mild degree of haemolytic anaemia, none or few crises, nearly normal growth and development, and no other major complications [Perrine et al, 1972, 1981; Babiker and Taha, 1982; Gelpi, 1970; El-Hazmi et al, 1987, 1988]. While on the other hand, there are patients who suffer from a severe form of the disease which requires frequent blood transfusions due to a severe haemolytic anaemia and frequent episodes of vasoocclusive crises [El-Hazmi & Warsy, 1986; El-Hazmi et al, 1990; Acquaye et al, 1985]. In addition, the growth and development in these patients may be retarded, and they may suffer from severe complications associated with the sickle cell disease.

The wide spectrum of clinical and haematological presentation of sickle cell disease has attracted considerable attention and several studies have been directed to identify the possible factors involved in modulating the nature of sickle cell disease [Weatherall et al, 1969; Wood et al, 1980; Pembrey et al, 1978; Al-Awamy et al, 1986; El-Hazmi et al, 1990]. Among the factors that have so far been contemplated are included both genetic and acquired factors. These may be listed as follows:

Acquired factors:

(i) Iron deficiency

(ii) Penicillin prophylaxis and pneumococcal vaccination

(iii) Overall health status

(iv) Environmental conditions

(v) Socio-economic factors

(vi) Others - unknown

Genetic factors:

(i) Hb F level

(ii) Gγ/Aγ ratio

(iii) Associated thalassaemia

(iv) Associated G-6-PD deficiency

(v) Restriction endonuclease polymorphic sites on DNA

(vi) β-globin gene haplotypes

(vii) The locus control region (LCR)

(viii) Others

In this section, we summarize our studies conducted in the search of possible modulating factors of sickle cell disease.

11.2 Acquired factors affecting sickle cell disease presentation

As a general rule environmental factors influence the health status of the inhabitants. Dietary habits, socio-economic status, hygienic and sanitary conditions, climate and exercise are just a few of the factors that influence the health of even normal non-sickle cell disease individuals. It is therefore, expected that sickle cell disease patients living in healthy environments and belonging to a high socio-economic group may suffer from a few complications compared to those sickle cell disease patients who are devoid of proper health care and nutrition. In this respect we considered a few environmental factors which may modulate the sickle cell disease presentation. Though more detailed studies are required in this aspect, but a brief

overview of our findings are discussed.

11.2.1 Effect of iron level

Iron deficiency is one of the most frequently encountered nutritional deficiency in several populations of the world, particularly in children and adult females in the child-bearing age. Fe^{++} deficiency affects the haemoglobin synthesis and produces a hypochromic-microcytic anaemia. Since in sickle cell disease patients the level of total Hb S in the red cells is believed to play an important role in influencing the disease presentation , presence of Fe^{++} deficiency has been considered as a factor modulating severity of sickle cell disease. Generally, the sickle cell disease patients have sufficient iron stones in the body and do not suffer from iron-deficiency. This is due to the fact that the chronic anaemic state, has a positive influence on the absorption of the dietary iron and in addition, these patients often receive blood transfusion which produces an elevation in the body iron stores. However, a small percentage of the patients do develop iron deficiency, particularly those whose dietary habits influence the body iron stores.

We grouped our sickle cell disease patients, males, females and children into those with iron deficiency, normal iron level and iron overload and estimated the haematological and biochemical parameters and severity index in these patients. The results of haematological and biochemical parameters are presented in Tables 11.1 and 11.2. The results showed slight variations in the level of the haematological and biochemical and clinical severity with major abnormalities in the patients with iron overload. It is well documented in literature that longstanding overload results in deposition of iron in the organs and tissues and may produce major organ damage thus

Table 11.1: Haematological parameters in sickle cell disease patients with iron deficiency, normal iron status and iron overlaod

Parameters	Group	Iron status		
		Fe^{++} deficiency	Fe^{++} overload	Fe^{++} normal
Hb (g/dl)	M	ND	10.48 ± 2.34	10.72 ± 1.82
	F	8.5 ± 0.2	8.76 ± 1.00	9.00 ± 0.42
	C	9.7 ± 0.2	8.80 ± 1.56	8.83 ± 1.99
RBC ($x10^{12}$/l)	M	ND	4.16 ± 0.93	4.32 ± 0.92
	F	3.0 ± 0.32	3.55 ± 0.75	3.85 ± 0.64
	C	3.1 ± 0.32	3.34 ± 0.96	3.04 ± 0.92
PCV (l/l)	M	ND	0.334 ± 0.01	0.324 ± 0.06
	F	0.27 ± 0.04	0.27 ± 0.04	0.27 ± 0.01
	C	0.24 ± 0.02	0.264 ± 0.05	0.244 ± 0.05
MCV (fl)	M	ND	76.67 ± 8.98	76.36 ± 11.2
	F	53.0 ± 7.8	79.27 ± 12.41	71.00 ± 7.07
	C	71.5 ± 7.8	75.80 ± 9.90	79.90 ± 9.83
MCH (pg)	M	ND	25.50 ± 3.69	25.43 ± 4.47
	F	17.0 ± 2.8	25.45 ± 5.10	24.00 ± 2.83
	C	22.0 ± 2.8	27.40 ± 6.22	30.00 ± 4.15
MCHC (g/dl)	M	ND	33.00 ± 1.38	33.14 ± 1.41
	F	32.0 ± 0.7	33.27 ± 1.55	33.50 ± 0.71
	C	40.5 ± 0.7	33.50 ± 1.72	36.00 ± 1.80
WBC ($x10^{9}$/l)	M	ND	9.54 ± 4.45	8.37 ± 4.03
	F	6.0 ± 4.2	8.93 ± 6.23	7.35 ± 3.32
	C	6.5 ± 4.2	13.49 ± 6.20	13.69 ± 2.78

Table 11.2: Biochemical parameters in sickle cell disease patients with iron deficiency, normal iron status and iron overload

Parameters	Group	Iron status		
		Fe^{++} deficiency	Fe^{++} overload	Fe^{++} normal
T. Bilirubin (μmol/l)	M	ND	45.89 ± 38.39	62.93 ± 39.11
	F	ND	41.54 ± 19.45	36.50 ± 28.99
	C	16.00 ±16.97	35.10 ±16.96	56.15 ± 43.48
D. Bilirubin (μmol/l)	M	ND	8.79 ± 8.44	9.42 ± 7.27
	F	ND	8.00 ± 4.72	ND
	C	8.0 ± 1.0	5.87 ± 3.80	6.78 ± 4.92
T. Protein (g/l)	M	ND	77.18 ± 4.02	77.13 ± 4.17
	F	79.00 ± 2.1	79.90 ± 2.34	76.00 ± 5.65
	C	77.50 ± 2.12	77.50 ± 5.15	77.22 ± 4.84
Albumin (g/l)	M	ND	45.95 ± 3.41	46.87 ± 4.45
	F	49.0 ± 4.0	44.63 ± 4.86	43.50 ± 2.12
	C	49.0 ± 4.0	44.60 ± 3.86	46.11 ± 2.15
Ferritin (ng/ml)	M	ND	674.41 ± 267.84	115.03 ± 71.79
	F	4.80 ± 1.0	554.33 ± 310.07	71.40 ± 46.10
	C	3.7 ± 1.0	1224.33 ± 2392.16	70.45 ± 28.08
Glucose (mmol/l)	M	ND	4.93 ± 0.61	4.95 ± 0.46
	F	4.30 ± 0.2	5.14 ± 1.36	5.00 ± 0.28
	C	5.15 ± 0.2	5.90 ± 0.90	4.23 ± 0.83
Creatinine (mmol/l)	M	ND	60.05 ± 19.36	53.69 ± 15.10
	F	ND	33.90 ± 23.81	ND
	C	30.00 ± 1.41	30.10 ± 22.11	22.22 ± 19.09
Urea (mmol/l)	M	ND	3.88 ± 1.03	3.25 ± 0.83
	F	3.70 ± 1.0	3.37 ± 1.10	2.65 ± 0.35
	C	2.65 ± 2.19	3.82 ± 1.16	3.46 ± 1.39

leading to some of the complications associated with sickle cell disease, including liver dysfunction, endocrine abnormalities and cardiac disorders. Thus it is essential to maintain iron level in the normal range in sickle cell disease patients by giving chelation therapy in order to avoid further complications.

11.2.2 Penicillin prophylaxis and pneumococcal vaccination

Sickle cell disease patients have an increased susceptibility to bacterial infections due to various immune defects encountered in these patients. Bacterial infections are one of the major cause of increased morbidity and mortality in sickle cell disease and often lead to precipitation of the crises. The prophylactic use of pneumococcal vaccination with or without penicillin has been suggested by several investigators. Our studies on two groups of sickle cell disease patients, one who were vaccinated and received penicillin prophylaxis and the other who received neither, showed that the vaccinated group had significantly lower frequency of hospitalization, crises, blood transfusion requirements compared to the non-vaccinated group. Similarly other features frequently associated with sickle cell disease were more common in the non-vaccinated compared to the vaccinated group. Though haematologically there were no major differences in the two groups except for WBC counts, which were higher in the non-vaccinated group.

Our results also showed that vaccination and penicillin prophylaxis increase the crises-free intervals in sickle cell disease patients and hence play a significant role in decreasing the associated morbidity. Thus, we advocate the combined use of pneumo-coccal vaccination and penicillin prophylaxis for sickle cell disease patients particularly in areas where the disease is severe and bacterial and parasitic infections are more

prevalent. This environmental factor may also show a disease presentation to be mild since the patients have a higher level of acquire immunity and resistance against infections - a major cause of morbidity and mortality.

11.2.3 Overall health status

Though we could not conduct a study whereby patients could be classified on the basis of the health status, but in general we observed that patients from the south-western province with severe disease who had better health as a result of proper dietary, vitamin and mineral intake were more resistant to crises and complications compared to patients from the same area but with poor health status. On the other hand it is also appreciated that a severe disease may influence the health status. Yet proper dietary management and increased mineral and vitamin supplementation may play a role in modifying the presentation of sickle cell disease. It must be emphasized that the recommended daily requirements (RDA) for these patients need to be obtained for all nutrients and may be different from normal individuals due to the high rate of turnover in their body and or chronic state of haemolytic anaemia.

11.2.4 Environmental conditions

The environmental conditions including the altitude may also play some role in modifying the clinical presentation of the sickle cell disease. Both very cold and very hot weather may precipitate crises. The latter results in dehydration while the former is a factor leading to sickling. In addition, patients living in healthy, clean environments tend to get fewer complications compared to those from areas of low sanitations. We noticed these among the patients though no statistical data collection or analysis was carried out on this aspect.

11.2.5 Socio-economic factors

In general, proper care and management of sickle cell disease patients is believed to play a significant role in decreasing the clinical severity and associated complications of sickle cell disease. In patients belonging to higher socio-economic groups the proper care and management is more available compared to the patients belonging to lower socio-economic groups.

The socio-economic status contributes to all the factors mentioned above i.e. improved nutrition, proper medical care and prophylactic measure, improved overall health status, clean and healthy environment and good and proper sanitary measures. Observations showed very clearly that patients belonging to higher socio-economic status had fewer complications compared to the low socio-economic group e.g. patients attending KKUH were from a high socio-economic group with a higher level of education among the patients, compared to the patients attending the children hospital, Ministry of Health, and the latter group suffered from significantly more severe disease. However, further studies and proper statistical data analysis are required to confirm this observation during our studies.

11.2.6 Other factors

There may be several other environmental or acquired factors that are at present unknown which play a role in influencing sickle cell disease presentation and may be revealed as more detailed investigations are carried out on the role of environmental factors in modulating sickle cell disease.

11.3 Genetic factors affecting sickle cell disease presentation

Several genetic factors have been implicated as possible modulators of sickle

cell gene expression. Various mechanisms have been proposed though the exact mechanism is not elucidated for any. The factors are briefly discussed in the following sections with data from our studies on Saudis.

11.3.1 Effect of Hb F level

Fetal haemoglobin level has been one of the factors which has been proposed as a modulator of Hb SS presentation. Some of the earlier reports from the Eastern province of Saudi Arabia which showed a mild nature of sickle cell disease suggested that the presence of high Hb F level may be responsible for amelioration of the sickle cell disease (Pembrey et al, 1978). Other investigations have also considered elevated Hb F level, since it interferes with the intramolecular attraction between the Hb S molecules and hence decreases sickling. Though it has been shown quite clearly that Hb F level more than 20% are required to produce such an ameliorating effect [Powars et al, 1984].

We studied the clinical and haematological presentation in sickle cell disease with different Hb F levels. The Hb SS patient from Eastern and western provinces were grouped into those with high Hb F (> 10%) and low Hb F level (< 10%). The haematological parameters were analysed separately in each group and student `t' test was performed to determine the significance of the difference in the two groups from the two regions. The results are presented in Table 11.3. High and low Hb F level group from the Eastern province had significantly higher haematological parameter values compared to the high and low Hb F group from the western province, respectively, when the data in the high Hb F group was compared to the low Hb F group in each region, no statistically significant differences were obtained. This showed that Hb F

Table 11.3: Comparison of haematological parameters in homozygous sickle cell disease children with high (>10%) or low (<10%) Hb F level from Eastern and Western Provinces

Province	SS Patients	No.	Age	Haematological Parameters							
				RBC $x10^{12}/l$	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb $A_{2\,(\%)}$	Hb F (%)
Western	High Hb F (>10%)	24	4.1 ± 3.2	2.88 ± 0.76	8.20 ± 1.1	0.22 ±0.043	85.1 ± 8.9	28.8 ± 5.1	37.7 ± 2.9	3.66 ± 1.1	14.62 ± 4.1
Eastern	High Hb F (>10%)	24	9.7 ± 2.9	4.03 ± 0.74	11.2 ± 1.7	0.30 ±0.003	76.1 ± 7.9	28.4 ± 4.7	36.6 ± 3.3	2.85 ±0.55	16.7 ± 4.1
P*		0.002	0.005	0.005	0.003	0.0001	0.041	0.865	0.453	0.053	0.309
Western	Low Hb F (< 10%)	29	1.7 ± 2.6	3.2 ± 0.82	8.34 ±1.11	0.23 ±0.034	79.1 ±11.7	26.9 ± 4.6	36.3 ± 2.7	3.94 ± 1.6	5.3 ± 2.9
Eastern	Low Hb F (< 10%)	17	9.0 ± 2.9	4.0 ± 0.79	10.0 ± 2.1	0.30 ±0.006	73.8 ±10.4	24.6 ± 3.5	33.3 ± 2.3	2.9 ±0.45	7.1 ± 2.0
P*		0.350	0.020	0.020	0.041	0.007	0.243	0.181	0.008	0.020	0.071

- P less than 0.05 is statistically significant.

level from 2-25 % did not affect the haematological parameter values significantly. When the Hb F level was correlated to the severity index in these patients no significant correlation could be seen (Figure 11.1) with a correlation coefficient (r) of 0.028 and $p = 0.803$. These results confirmed that Hb F level may not be a major factor in ameliorating sickle cell disease presentation, upto a certain level [Powars et al, 1984]. Though higher levels (possibly > 20%) may have an effect, as was seen during our studies during treatment of sickle cell disease patients with hydroxyurea and erythropoietin (Discussed latter), who had reached a significant elevation of Hb F level and a significant improvement in their disease presentation.

11.3.2 Associated thalassaemias an ameliorating factor of Hb SS

The role played by associated α- or β-thalassaemia in amelioration of sickle cell disease presentation has been the subject of considerable debate. We conducted extensive studies in sickle cell disease with and without α- or β-thalassaemia patients from Eastern and Western provinces. Our results showed an ameliorating effect of coexisting thalassemia genes in sickle cell disease patients. Where the disease severity was considerably reduced in sickle cell disease patients with α-thalassaemia both in the Eastern and Western province. However, the sickle cell disease in the Western province did not become as mild as the one in the Eastern province patients even in presence of α-thalassaemia. This suggested that though α-thalassaemia has an ameliorating influence on the severity of sickle cell disease, some other factors are also playing a role in producing a mild disease in the Eastern province patients.

A summary of the effects of α- or β°-thalassaemia on the clinical severity of

Figure 11.1

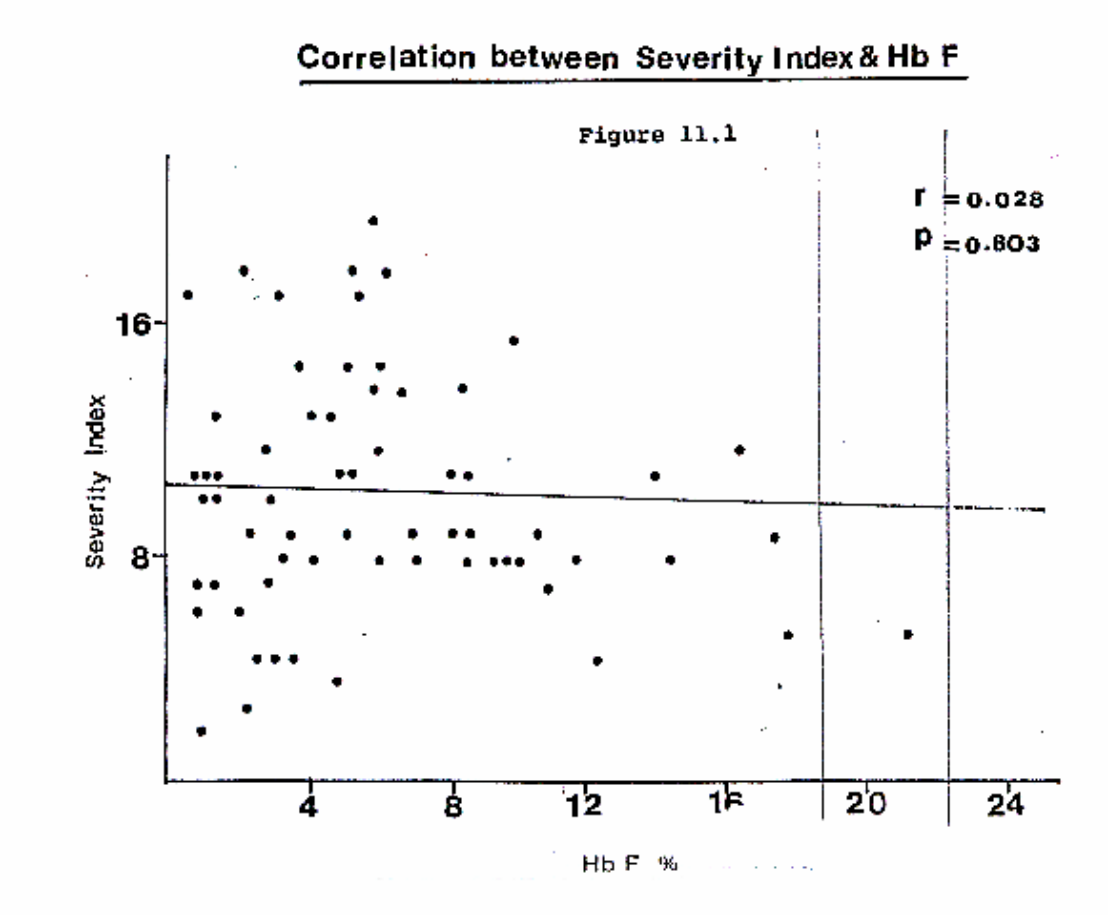
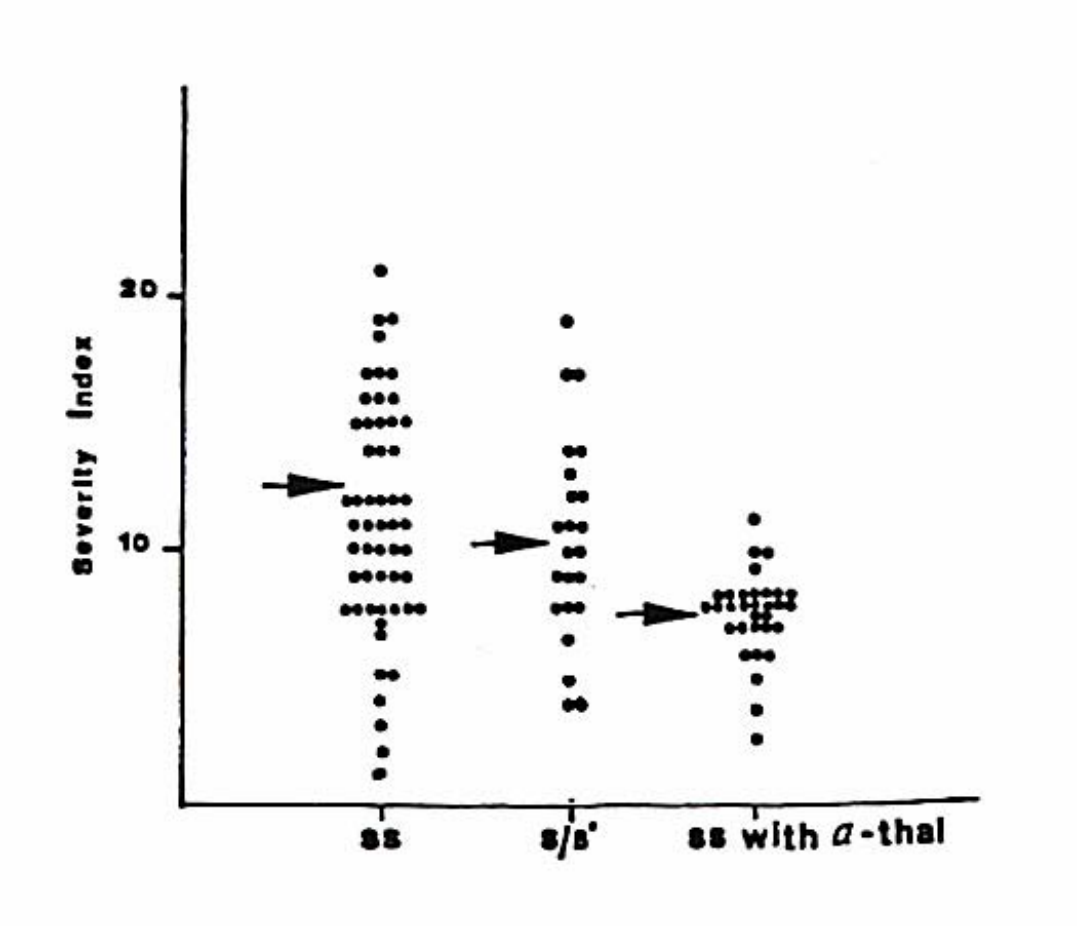

Correlation between Severity Index & Hb F
Figure 11.1
r = 0.028
p = 0.803
16
8
Severity Index
4
8
12
16
20
24
Hb F %

Figure 11.2: Severity index in a group of Hb SS patients with or without α- or β°-thalassaemia



sickle cell disease is presented in Figure 11.2 within α-thalassaemia group, when the one gene deletion group was separated from those with two α-gene deletions, different severity indices were obtained in the two groups as shown in Figure 11.3. A comparison of the haematological findings in the Hb SS with or without α- or β-thalassaemias are presented in Figures 11.4 to 11.6.

In summary associated α- or β°-thalassaemia have a modulating influence on the presentation of sickle cell disease, though the disease does not acquire a benign nature as the one identified in the Eastern province of Saudi Arabia. Thus suggesting the presence of some other associated factors involved in modulation of the Hb SS disease.

11.3.3 Effect of Gγ/Aγ ratio on sickle cell disease presentation

The γ-globin genes of Hb F exist as a pair of linked genes located on chromosome 11, 3' to the ∘ gene. The two genes of γ-globin chains differ in a single codon, which may code for an alanine at position 136 producing AγI or a glycine producing Gγ. In addition in AγI the isoleucine at position 75 may be replaced by a threonine producing a variant AγT [Schreoder et al, 1968; Efremov et al 1979]. The relative location of the γ-globin genes and the gene products are presented schematically in Figure 11.7. The relative expression of the γ-genes changes during ontogeny and in genetic disorders affecting the globin chain. Both the level of Hb F and the proportion of Gγ to Aγ shows significant variation in patients with sickle cell disease and β-thalassaemias [Huisman et al, 1977]. The postnatal period is characterized by a switching over from Gγ chain of Hb F to β-chains of Hb A and is associated with an

Figure 11.3: Severity Index in the SCA patients
with and without α-Thalassaemia

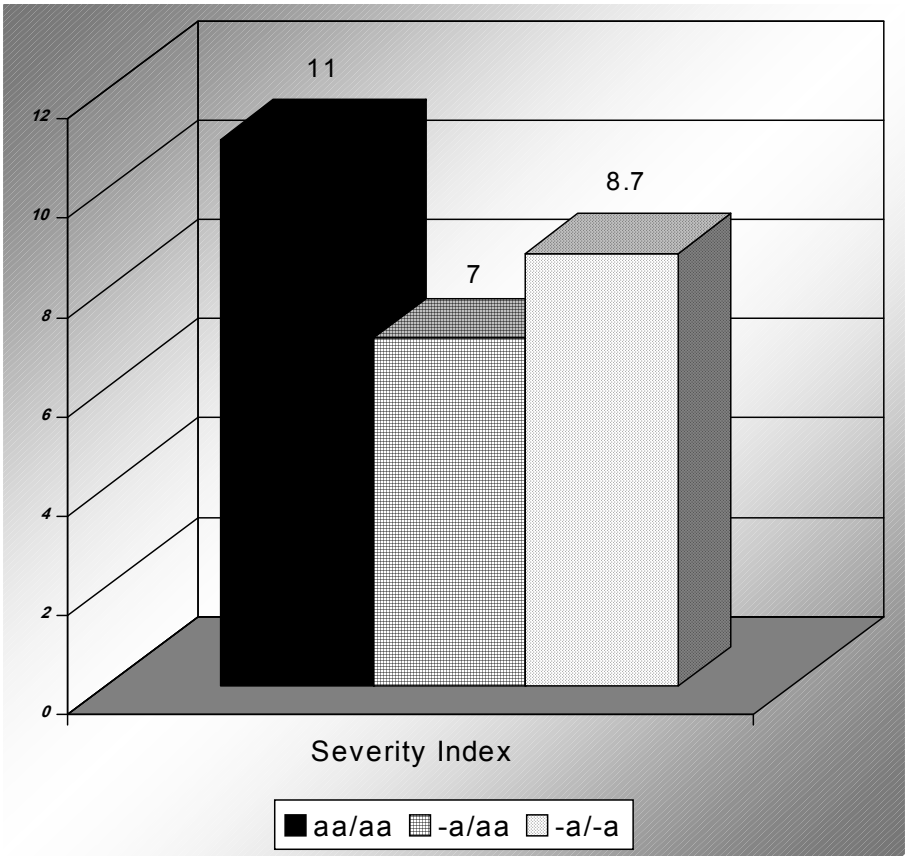


Figure 11.4: Total haemoglobin and RBC level in Hb SS patients
with and without α- or β°–thalassaemia

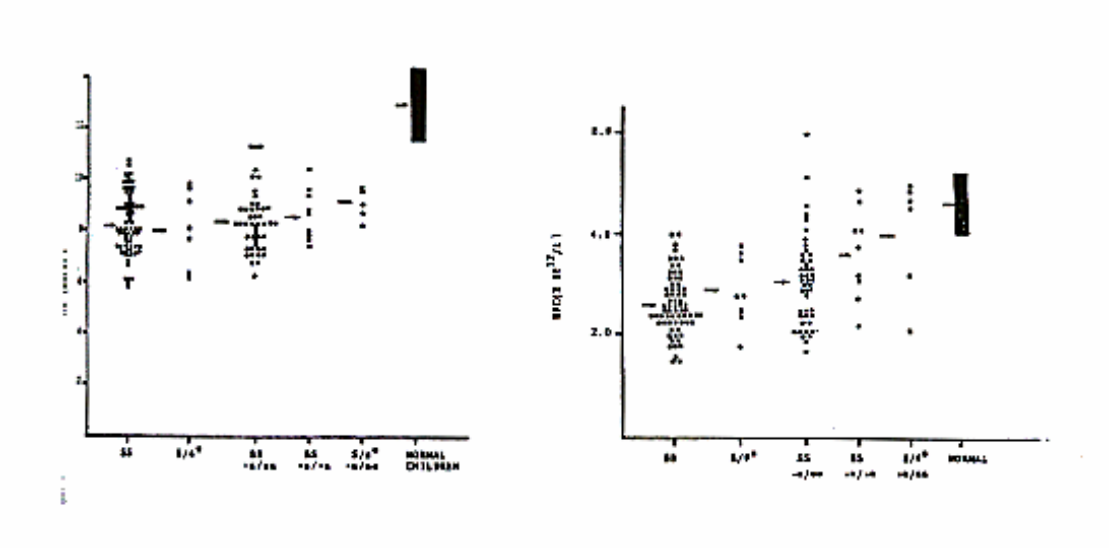


Figure 11.5: Packed cell colume and WBC count in Hb SS patients with and without α- or β°–thalassaemia

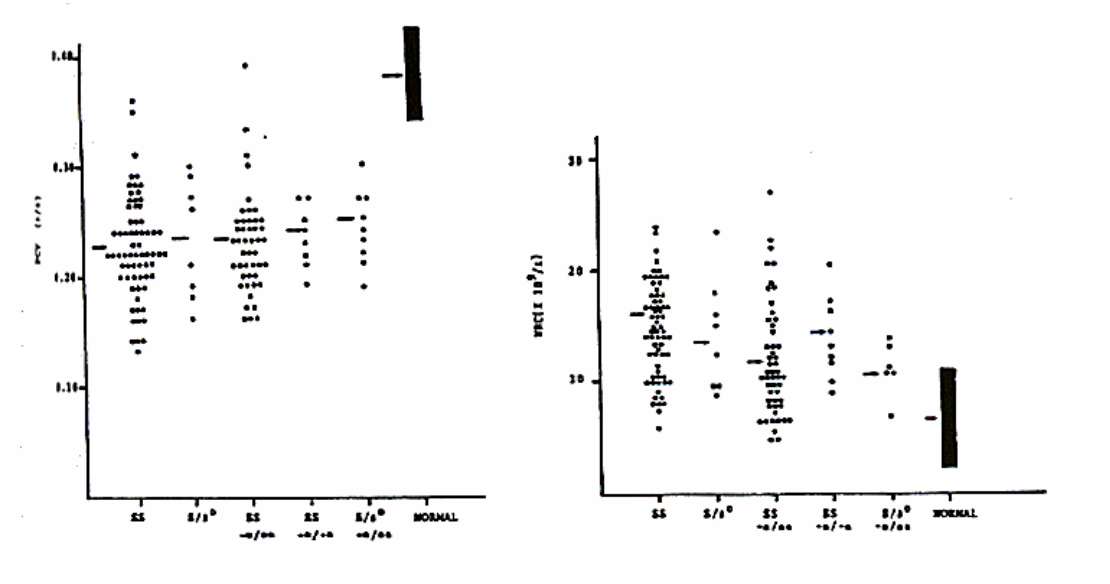


Figure 11.6: Fetal haemoglobin and RBC level in Hb SS patients
with and without α- or β°–thalassaemia

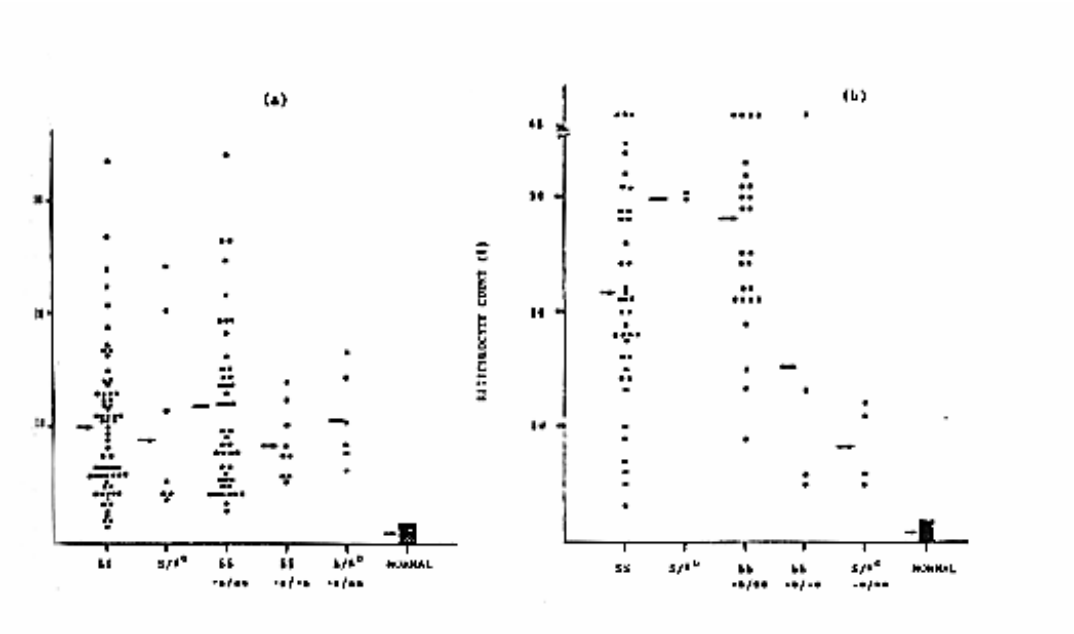
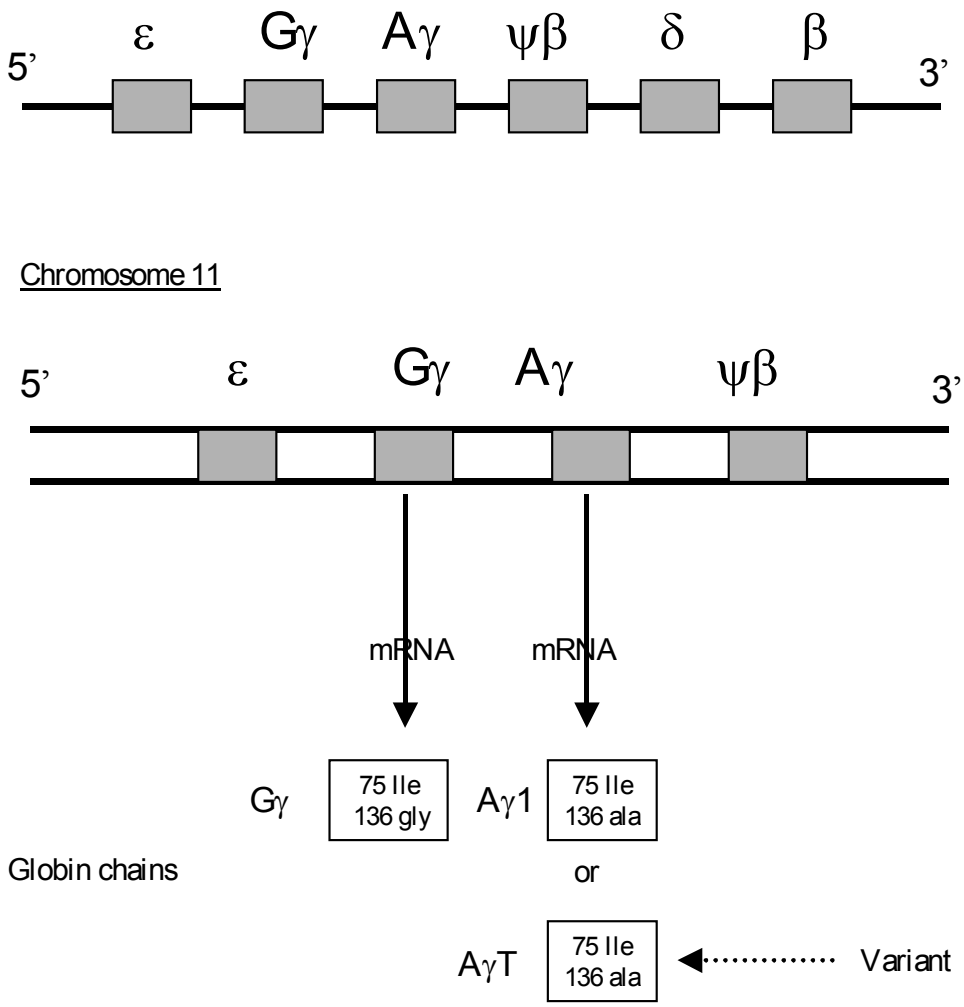


Figure 11.7: The Gγ/Aγ-globin genes and γ-globin chains



Normal Gγ/Aγ ratio
(a) at birth 70 : 30
(b) in adulty 40 : 60

additional developmental switching of 70% Gγ and 30% Aγ in most newborns to 40% produced in presence of a high Gγ/Aγ ratio and in newborns with a Gγ/Aγ ratio of 70:30, the Hb F level is significantly higher than in adults who have ratio of 40:60. Since Hb F level has been considered as one of the determinants of sickle cell disease severity [Powars et al, 1984], it is suggested that alterations in the Gγ/Aγ ratio, which will alter Hb F level may also alter the sickle cell disease severity. To investigate the ratio of Gγ/Aγ in Saudi patients and to correlate it to disease severity, we investigated

Gγ and Aγ by HPLC in eighty Saudi Hb SS patients. The patients were grouped with two groups i.e. those with a severity index <6 (range 3.93±1.2) and those with a severity index >6 (range 11.09±3.78). The levels of Gγ, AγI and AγT in the patients with mild or severe sickle cell disease are presented in Figure 11.8. The difference in the level in the two groups of patients was statistically significant where Gγ occurred at higher level in patients with mild disease unlike Aγ occurred at a higher level in patients with severe disease. Thus, the Gγ/Aγ ratio was higher in the former group compared to the latter (Figure 11.9). Interestingly, within the Aγ differences were found in the level of AγI and AγT in the two groups where AγT was significantly higher in patients with severe disease while the reverse was true for AγT (Figures 11.10 and 11.11). Table 11.4 presents the mean and range of the various γ-globin chains in the two groups. The Hb F level was estimated in the two groups and the result showed no significant difference between the two groups (Figure 11.12).

To correlate the Hb F level to the Gγ/Aγ ratio, we conducted regression analysis and determined the correlation coefficient. No correlation was obtained between the Hb F