Figure 11.19: Restriction endonuclease polymorphic sites in the β-globin gene cluster on chromosome 11. the arrow points to the approximate location where the restriction site may present (+) or absent (-)

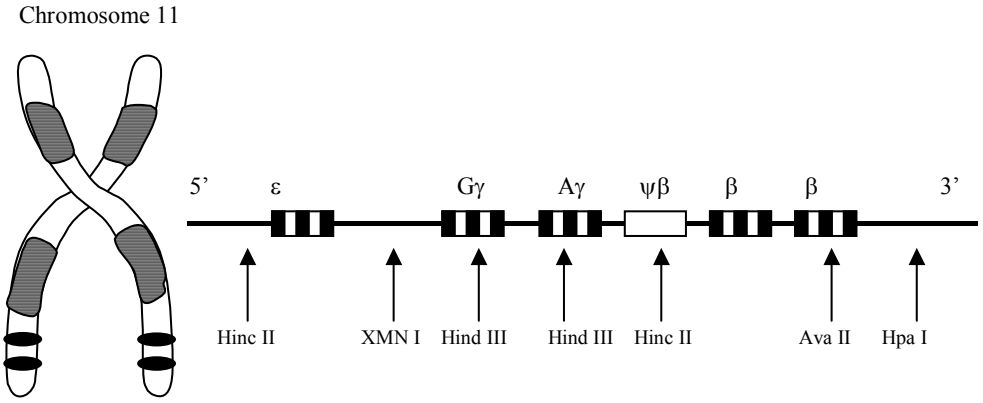


Figure 11.20: Frequency of Hinc II polymorphic site 5' to ε-gene in sickle cell disease patients with a mild or severe disease compared to normal Hb AA controls

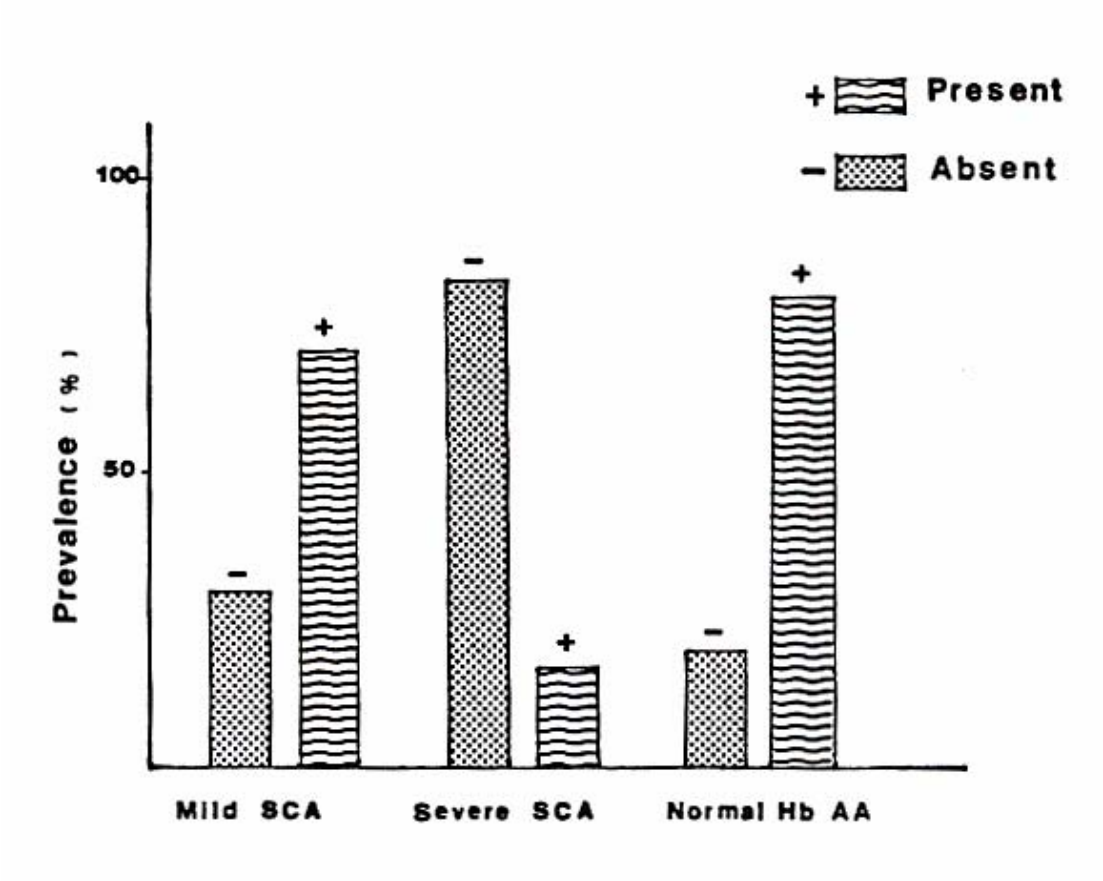


Figure 11.21: Frequency of Xmn I polymorphic site 5' to Gγ in sickle cell disease patients with a mild or severe disease compared to normal Hb AA controls

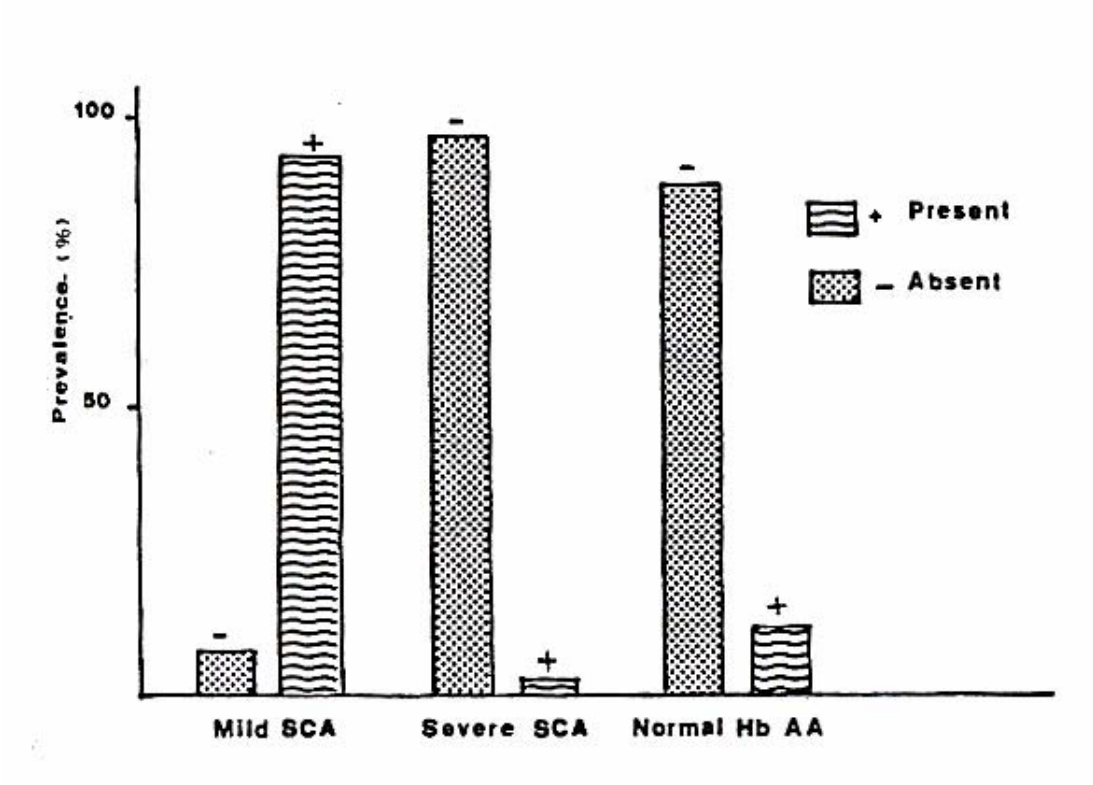


Figure 11.22: Hb F level in sickle cell disease patients with and without Xmn I polymorphic sote (7.0 kb fragment represents presence of Xmn I site: 8.0 kb fragment represents absence of Xmn I polymorphic site)

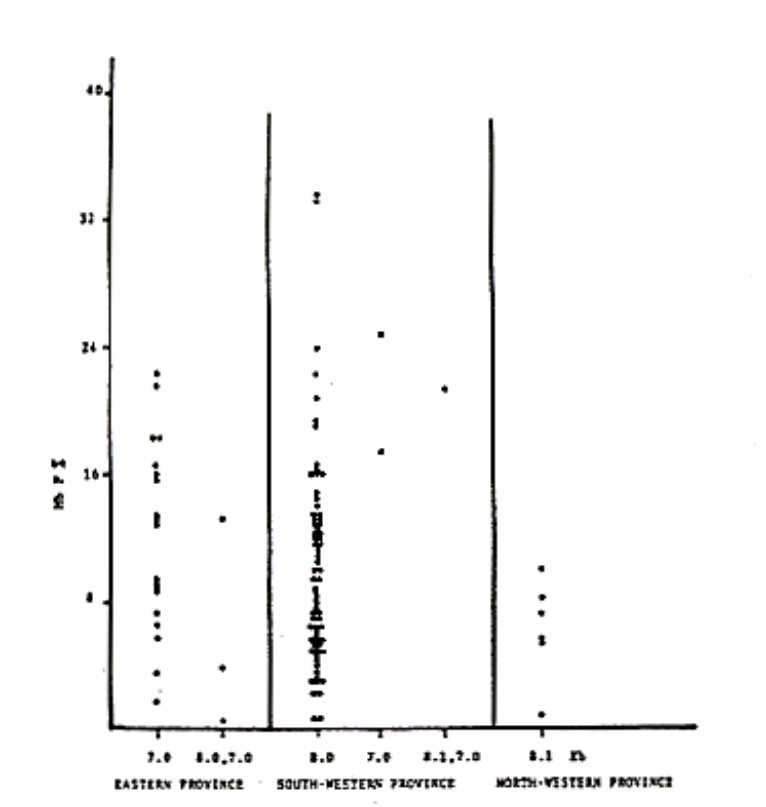
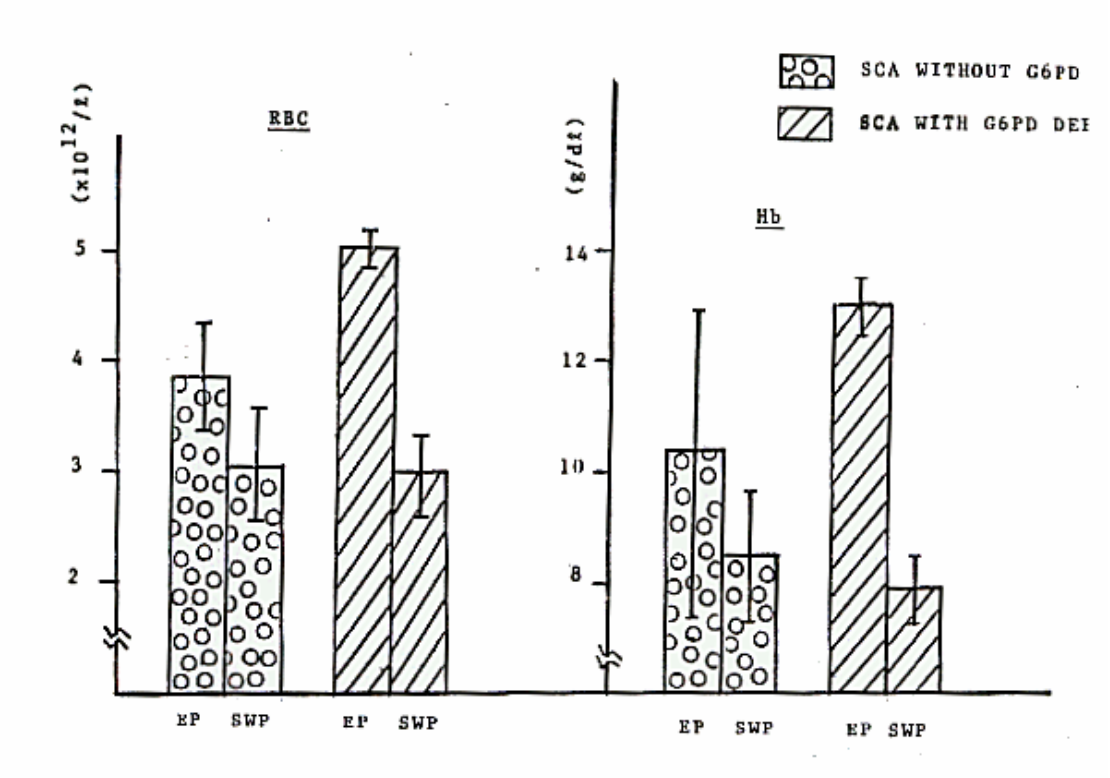


Figure 11.18: Comparison of the haematological results of patients from SWP and EP



produced, while in normal β^A cases a 7.6 Kb fragment was generated by Hpa I digestion. However, soon it was shown that the Hpa I site was not a sensitive marker of β^S since both β^S and β^A were shown to be linked to 7.6, 13.0, 5.6 and 7.0 Kb Hpa I fragments (Kan and Dozy, 1980). However, this was the stepping stone and led to the evaluation of several restriction endonuclease polymorphic sites and it was suggested that the incidence of polymorphism at the DNA level was 1 per 100 nucleotides outside the coding sequences of the globin genes [Jeffrey, 1979; Cooper et al, 1985). Several polymorphic sites were identified and these include the Hind III polymorphic site in the intervening sequence II of the Gγ/Aγ gene, Bam HI polymorphic site 3' to β-globin gene, Hinc II, polymorphic site within and 3' to ψβ-globin gene and 3' to ε-gene, Ava II polymorphic site in the β-globin gene and Xmn I polymorphic site 5' to Gγ .These polymorphic sites in the β-globin gene cluster on chromosome II are presented schematically in Figure 11.19.

We investigated the restriction sites in 80 sickle cell disease patients with a mild or severe disease presentation in an attempt to compare if the frequency of the polymorphic sites shows any variation in the different forms of disease presentation.

Hinc II polymorphic site 5' to ε-gene was present at a significantly higher frequency in patients with a mild sickle cell disease and it was also present at a high frequency in the normal group without sickle cell disease (Figure 11.20).

Xmn I polymorphic site 5' to Gγ '5 .to Gγ has been the subject of considerable interest as it was suggested that it was linked to a high γ-globin gene expression. In the Saudi patients the patients with a mild disease had a high frequency of this polymorphic

site, while in patients with severe disease and normal controls the frequency was significantly low (Figure 11.21). We determined the difference in Hb F level in patients from the different province with or without the Xmn I polymorphic site. The results are presented in Figure 11.22.

Hind III polymorphic sites in Gγ occurred at a higher frequency in the patients with mild sickle cell disease, while less than 20% of the patients with severe disease had this site as shown in Figure 11.23. On the other hand, Hind III polymorphic site in Aγ was absent in all the sickle cell disease patients with mild or severe disease and in the normal controls.

Hinc II polymorphic site in ψβ was encountered at a significantly higher frequency in sickle cell disease patients with mild disease, while it was absent from all patients with the severe form of the disease (Figure 11.24). The Hinc II polymorphic site 3' to ψβ did not show any significant difference between the patients with a mild or severe disease though it occurred at a considerably lower frequency in normal controls (Figure 11.25).

Finally, the Hpa I polymorphic site 3' to β-globin gene also showed differences in patients with a mild or severe disease. In the former it was encountered at a high frequency, at about the same frequency as in normal control, while the frequency was considerably low in patients with a severe sickle cell disease (Figure 11.26). Thus, these results show that the polymorphic sites in the β-globin gene cluster have a significant role to play in the modulation of the clinical presentation of the sickle cell disease and the clinical expression is controlled to some extent by linkage to these

Figure 11.23: Frequency of Hind III polymorphic site in Gγ in sickle cell disease patients with mild or severe sickle cell disease compared to normal controls

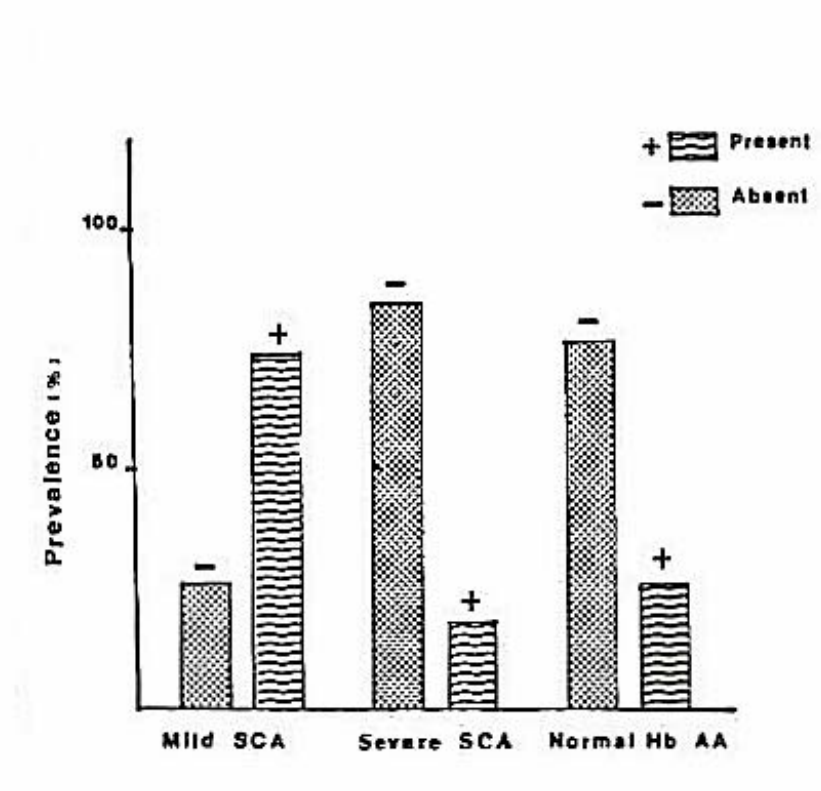


Figure 11.24: Frequency of Hinc II polymorphic site in ψβ in sickle cell disease patients with mild or severe sickle cell disease compared to normal controls

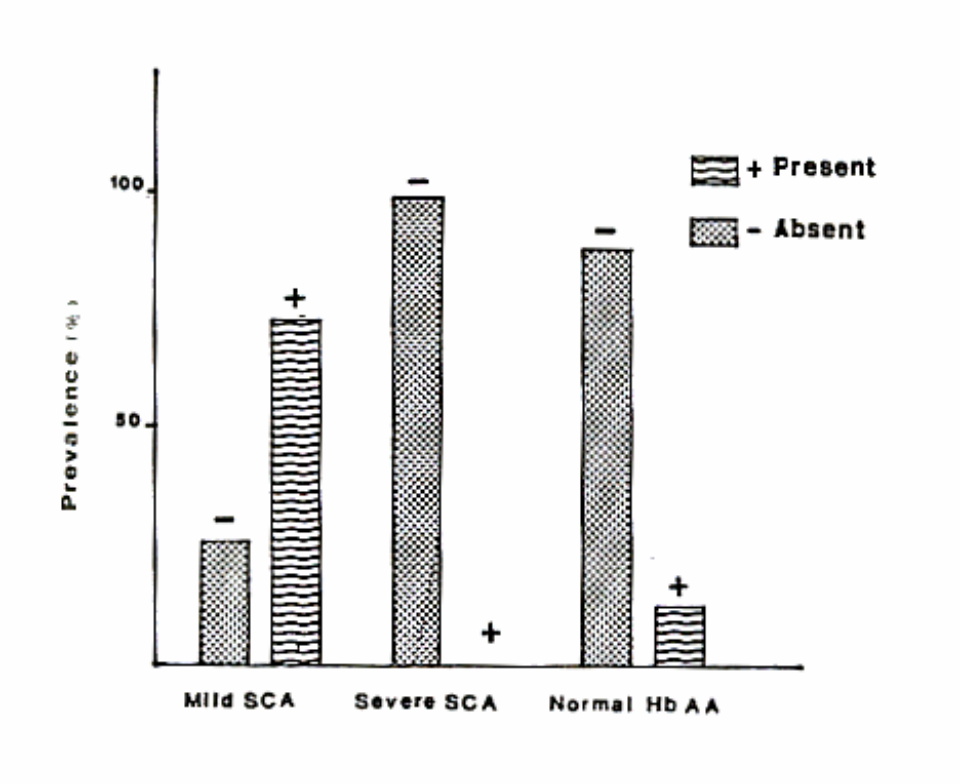


Figure 11.25: Frequency of Hinc II polymorphic site 3' to ψβ in sickle cell disease patients with mild or severe disease compared to normal controls

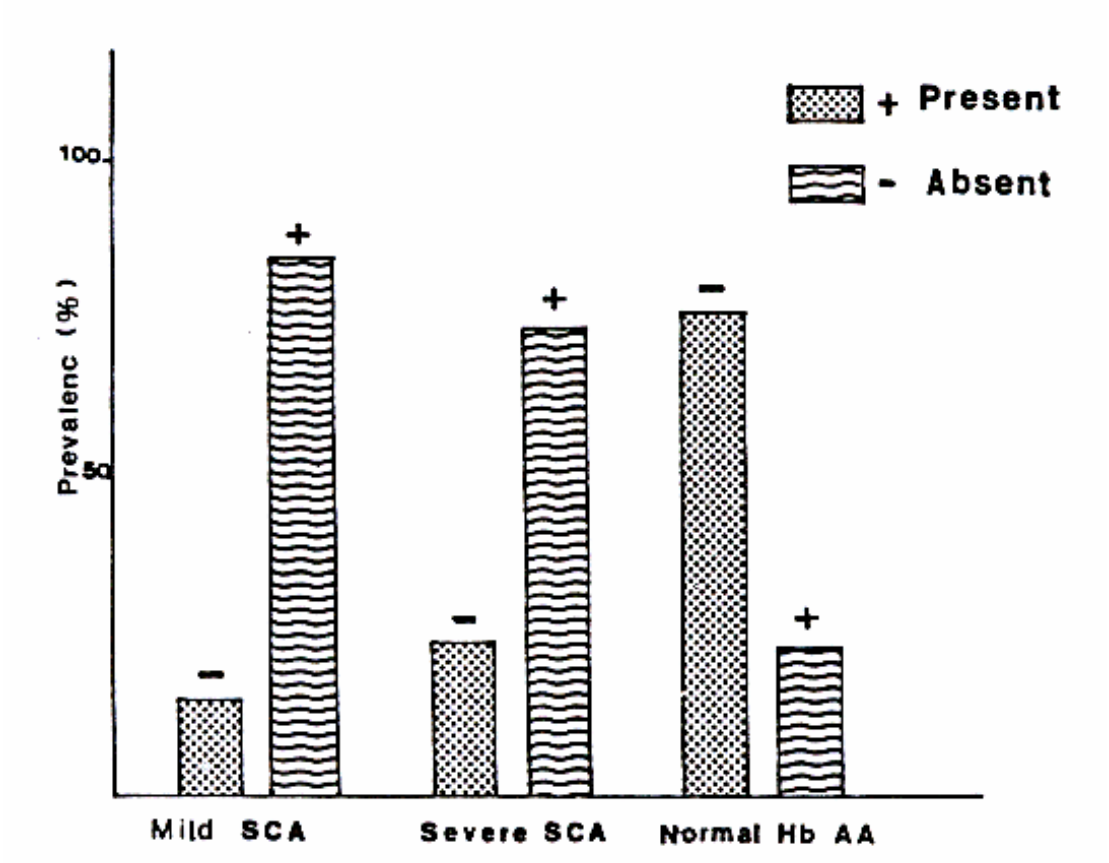
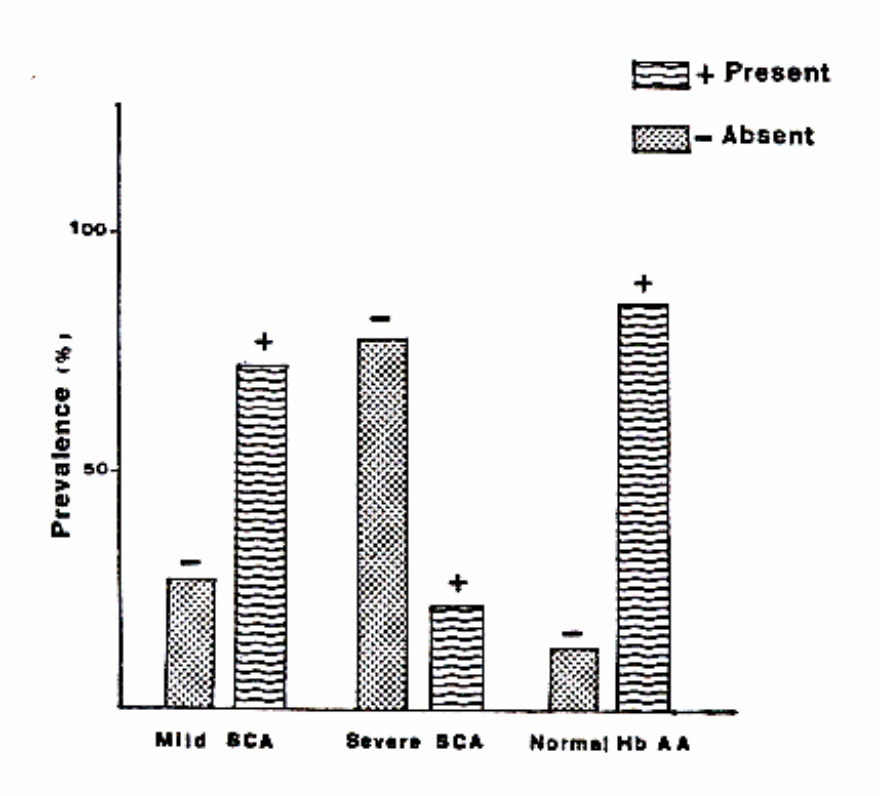


Figure 11.26: Frequency of Hpa I polymorphic site 3' to β gene in sickle cell disease with mild or severe disease compared to normal controls



polymorphic sites. Though the sickle cell mutations are the same in all these patients i.e. GAG → GTG but the disease presentation is extremely variable and genetic polymorphism seem to influence this significantly.

11.3.6. Influence of the β-globin gene clusters on sickle cell disease presentation

Using the combination of the sites, the β-globin gene haplotypes were constructed. In an attempt to investigate the influence of β-globin gene haplotypes in association with the β^S gene on the haematological and clinical presentation of sickle cell disease, the patients were grouped into two groups i.e. whether they had the Benin (----+) or the Saudi-Indian haplotype. The clinical presentation and haematological parameters were determined in the two groups. The results showed that the frequency of crises was significantly more in the patients with the benin haplotype and also had significantly higher blood transfusion requirements. They had to be hospitalised more frequently due to various complications compared to the patients with Saudi-Indian haplotype (Figure 11.27). Abdominal pain, pallor, jaundice and hand-foot syndrome occurred in a higher percentage of the patients with the benin haplotypes (Figures 11.28 and 11.29). Though pain in bone and joints, osteomyelitis and skeletal deformities in these patients did not differ significantly in the two groups (Figure 11.29).

Haematologically significant differences were found in the value of total haemoglobin (Figure 11.30), RBC count (Figure 11.31), WBC count (Figure 11.32) and PCV (Figure 11.33) in the two groups of patients, where the benin haplotype patients had more severe haematological abnormalities compared to the patients with Saudi-Indian haplotype. When the Hb F level was determined in the two groups, the

Figure 11.27: Frequency of crises, blood transfusion requirements and hospitalization in sickle cell disease patients with Benin and Saudi-Indian haplotype

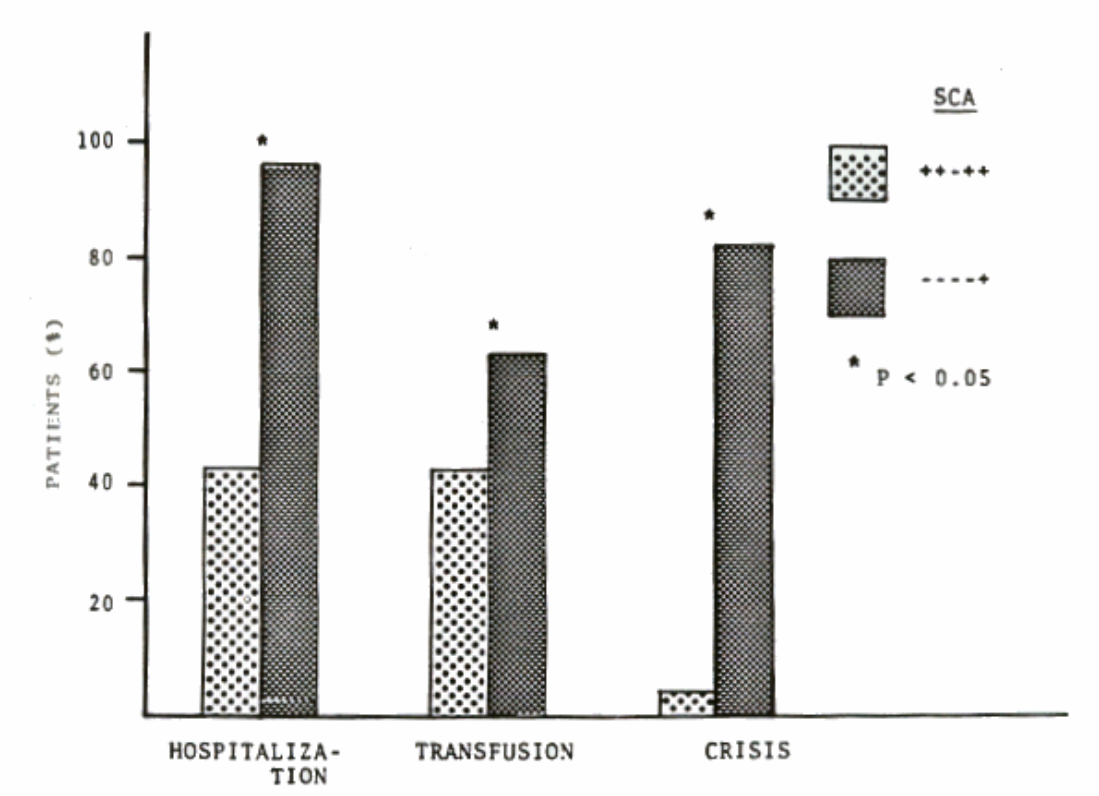


Figure 11.28: Frequency of abdominal pain, pallor and jaundice in sickle cell disease patients with Benin and Saudi-Indian haplotype

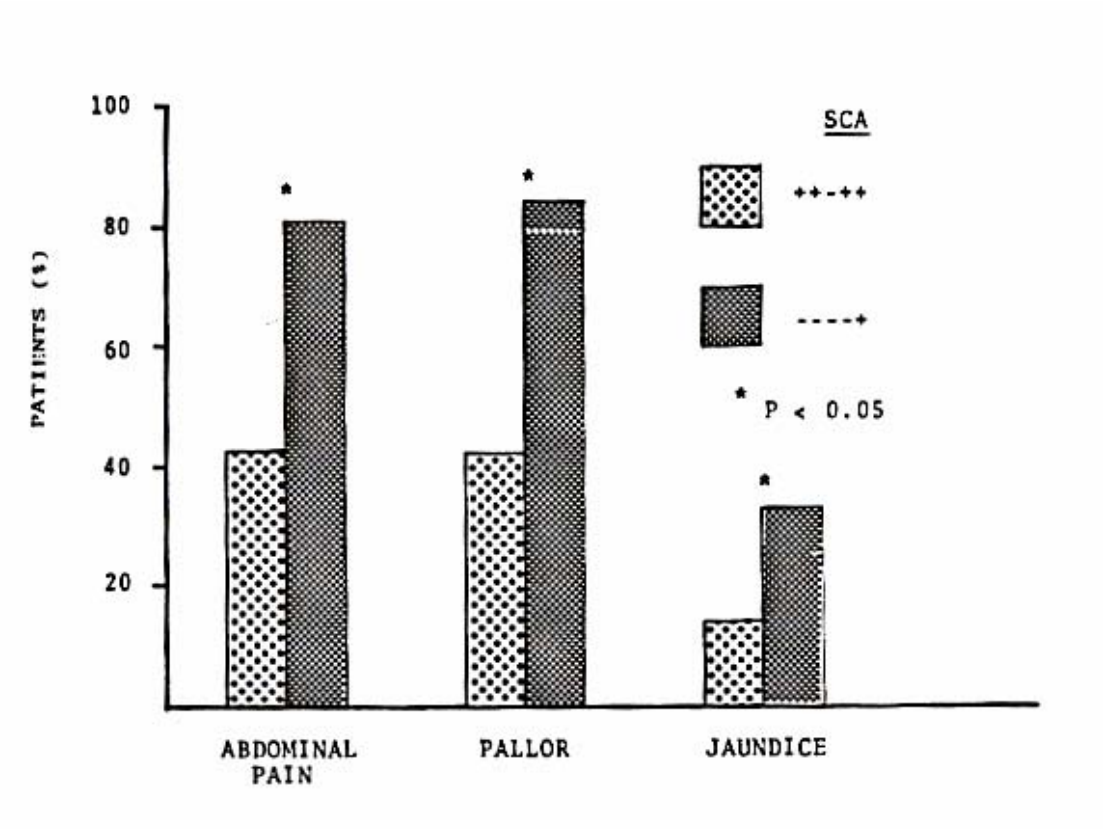


Figure 11.29: Frequency of pain in bones and joints, hand-foot syndrome oesteomyelitis and skeletal deformity in sickle cell disease patients with Benin and Saudi-Indian haplotype

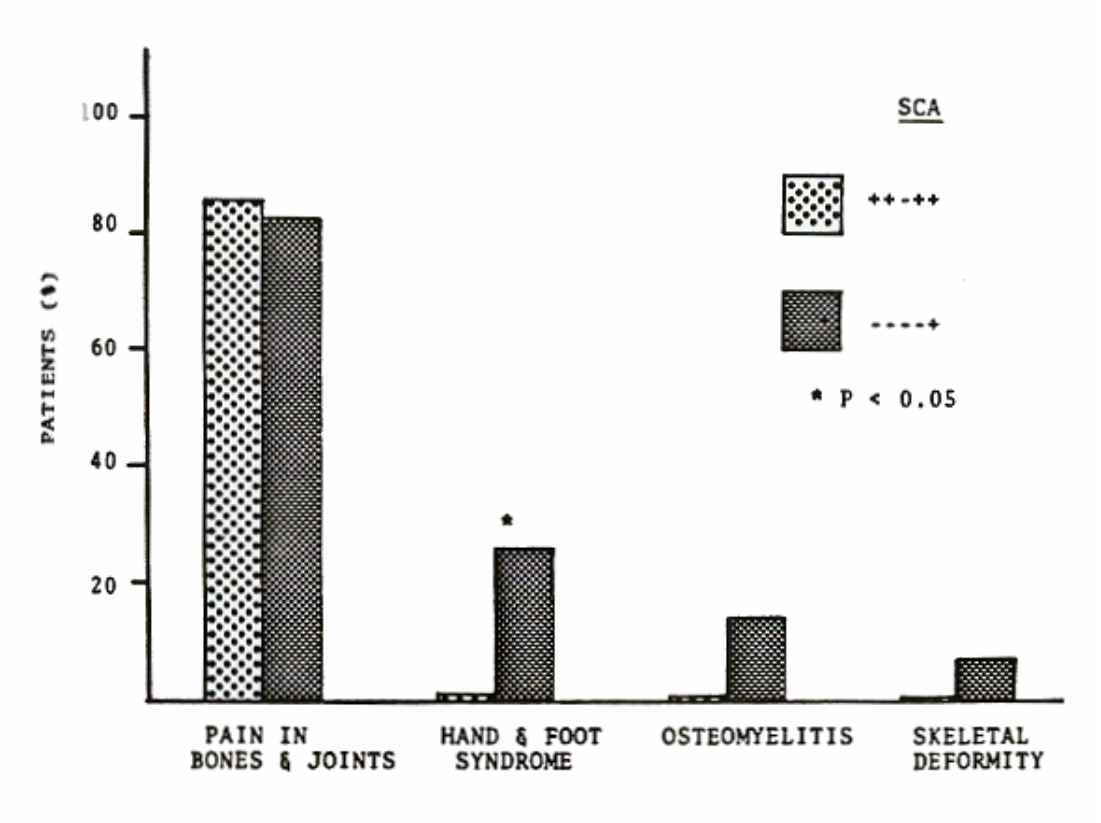


Figure 11.30: Haemoglobin level in sickle cell disease patients
with different β-globin haplotype

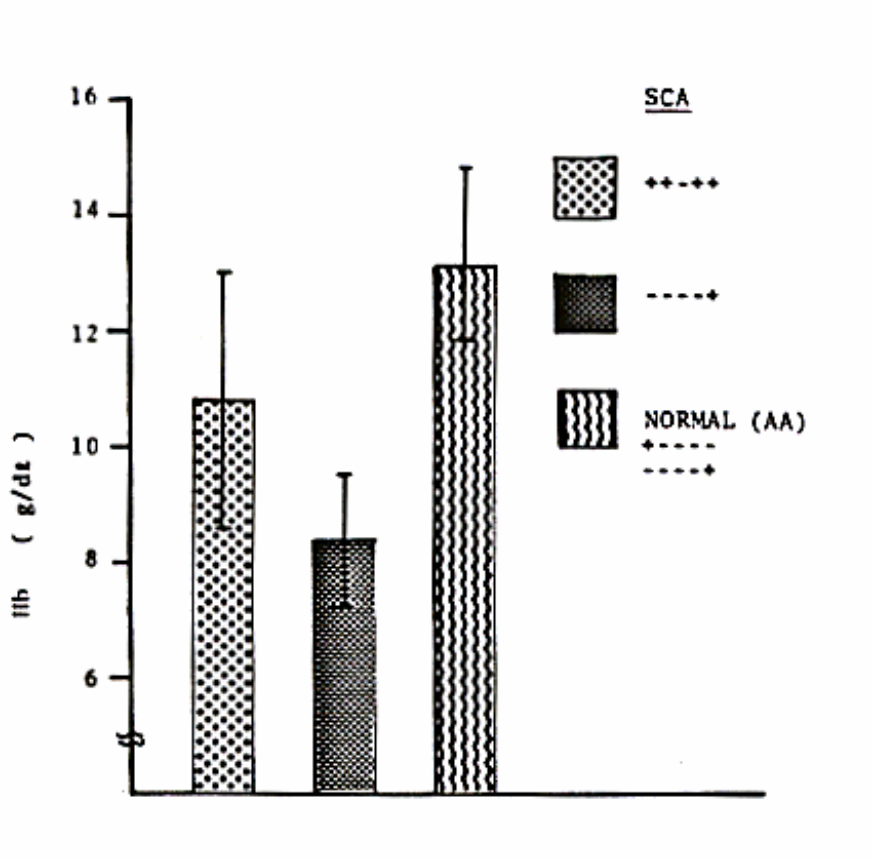


Figure 11.31: Red blood cell count in sickle cell disease patients
with different β-globin gene haplotype

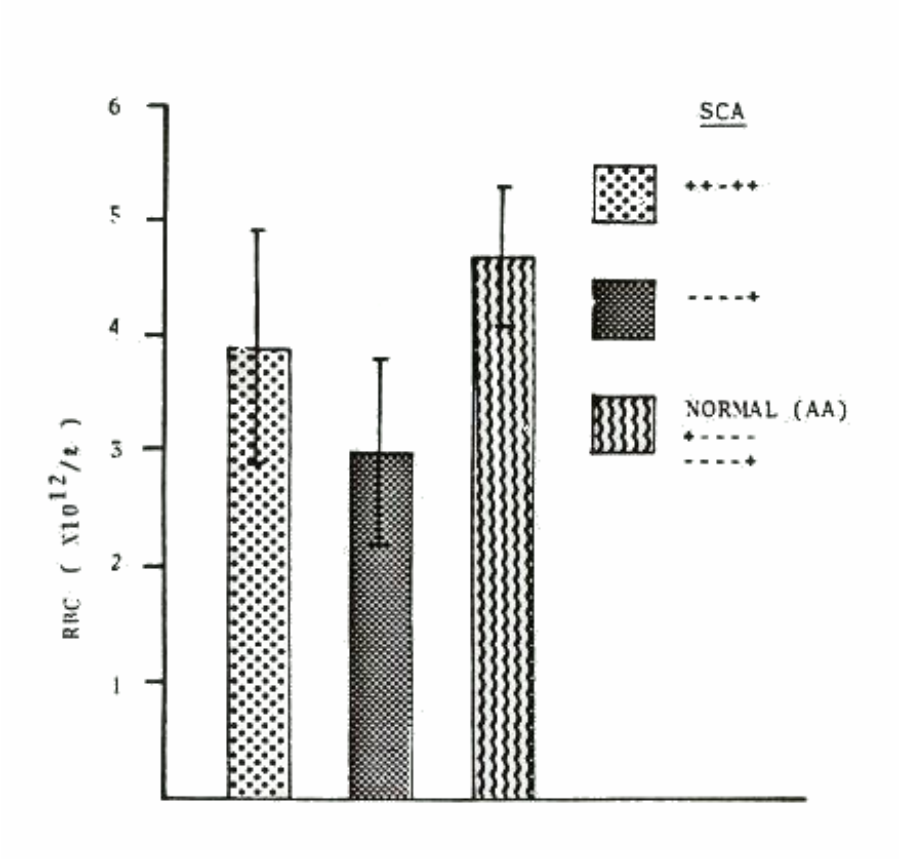


Figure 11.32: White blood cell count in sickle cell disease patients
with different β-globin haplotype

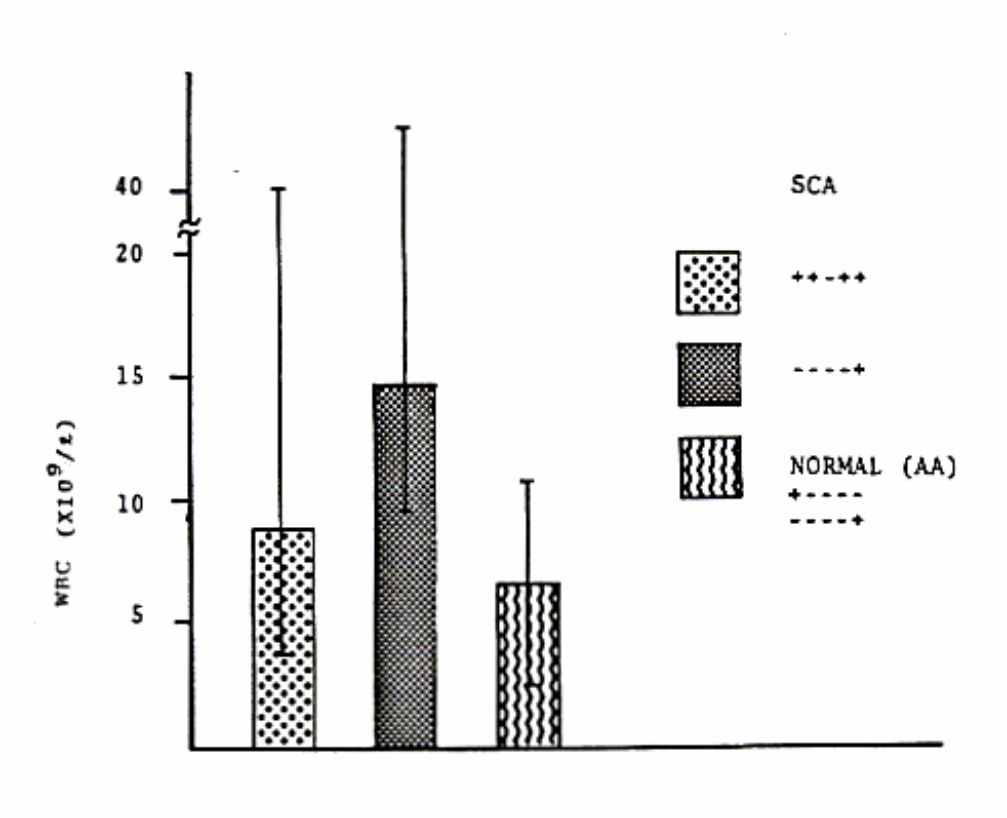


Figure 11.33: Packed cell volume in sickle cell disease patients
with different β-globin haplotype

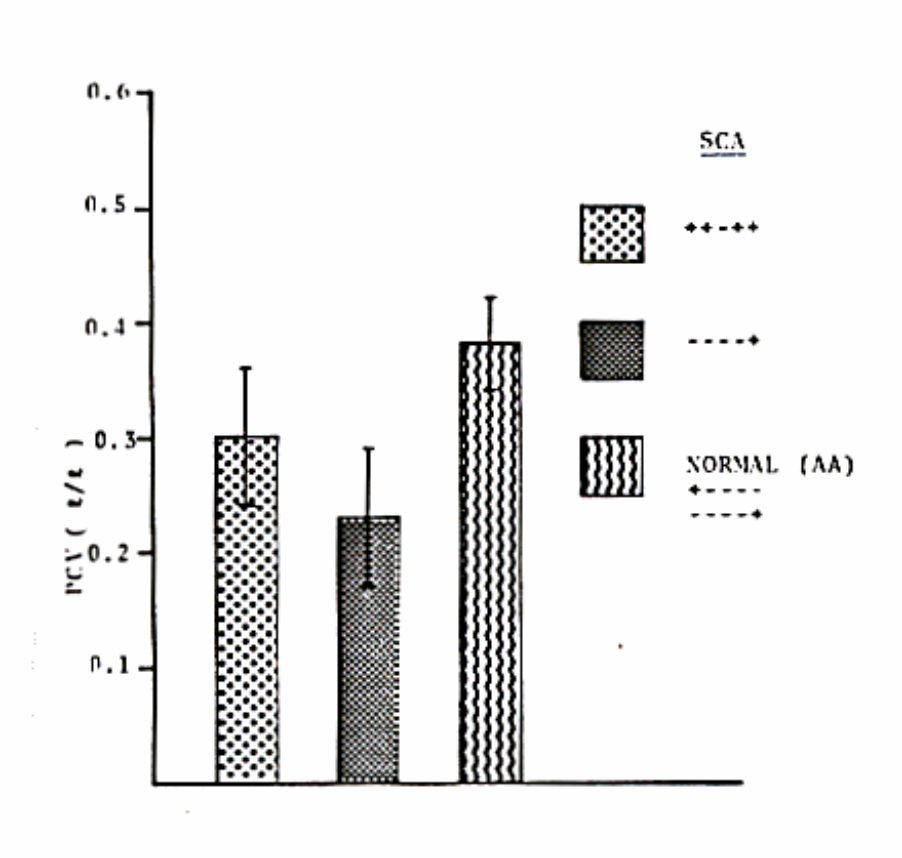


Figure 11.34: Haemoglobin F level in sickle cell disease patients
with different β-globin haplotype

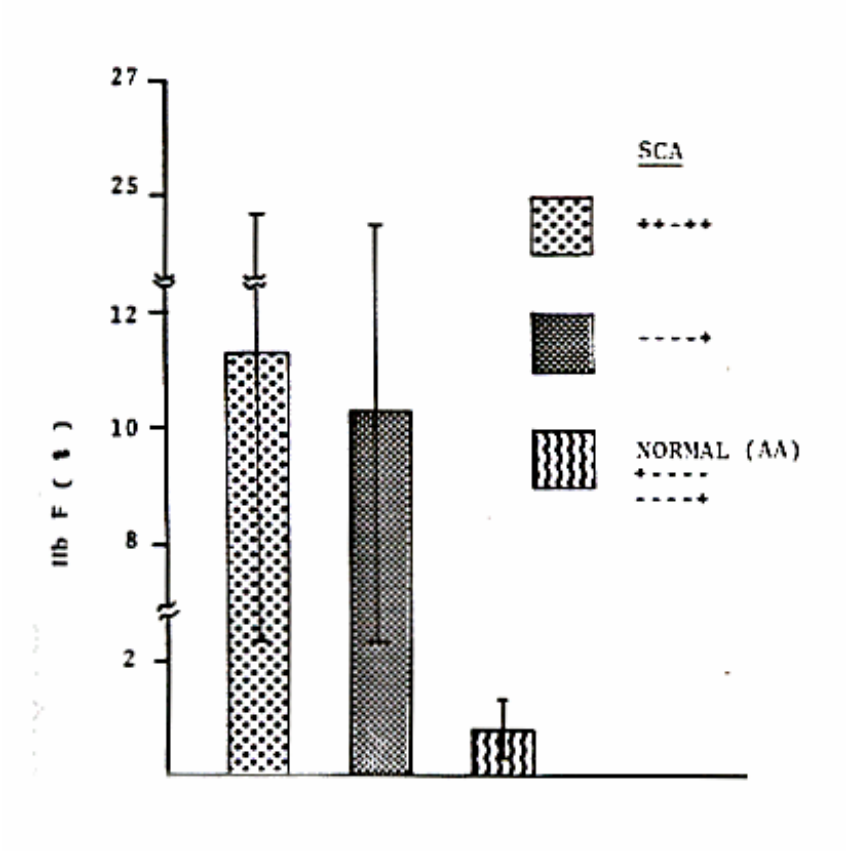
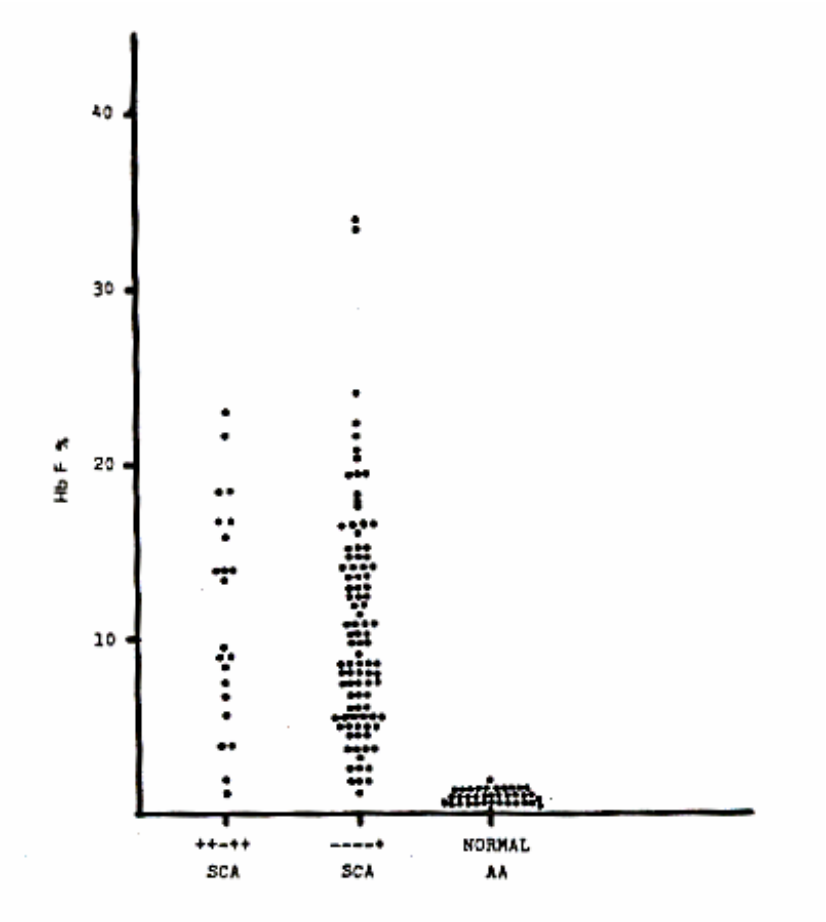


Figure 11.35:Distribution of Hb F in sickle cell disease patients
with different β-globin gene haplotype



difference was not significant in the two groups (Figure 11.34) where a whole range of Hb F levels from as low as 2% to higher than 300% were encountered in the patients groups investigated (Figure 11.35).

Thus, it may be concluded that β-globin gene haplotypes play a role in modulating the clinical presentation of sickle cell disease where a mild disease is encountered in patients with Saudi-Indian haplotypes and the severe disease in patients with benin haplotypes. However, this modulation is not through the level of Hb F, which does not show significant differences in the patients with different haplotypes.

11.3.7 Influence of the locus control region (LCR) on the clinical presentation of sickle cell disease

In the β LCR the tandemly arranged 5' HS2 and 5' HS3 are shown to be the most important sites. The 5' HS3 fragment contains NF-E2 and GATA-1 binding sites as well as GGTGG motifs while the 5' HS2 fragment contains similar sequences plus a tendem repeat for activating protein 1 (AP1) binding sites. A 46 bp enhancer, containing two binding sites, is both necessary and sufficient for the increased γ-globin gene expression (Sorrentino et al, 1981). This region in 5' HS2 also contains a sequence repeat of (AT) x N_{12} $(AT)_y$ motif which could have some negative regulatory functions. Since ethnic and geographical variations were reported in the sickle cell disease patients, we investigated this motif in a few patients from the Eastern and Western provinces of the country to correlate it to Hb F expression and disease severity in these patients sequences of amplified DNA in this region showed that the motif in the patients from the Eastern province with Saudi-Indian haplotype, both with low and high Hb F level was TGA $(AT)_{10}$ A $(CA)_2$ $(AT)_2$ CGT $(AT)_{12}$ TTG, while the motif in the

These results clearly indicate that patients with a mild disease have different motif than those with a severe disease, though the mechanism by which the disease presentation does not seen to be through modulating Hb F level.

Other factors

There may be other unknown genetic factors involved in the significant heterogeneity of sickle cell disease presentation in the Saudi population. Further, indepth studies at the DNA level may provide more evidence to the existance of other genetic markers of sickle cell disease severity.

Conclusion

It is evident from our studies that the sickle cell mutation despite being a monogenic disorder, behaves very much like a multifactorial and polygenic disorder, where environmental and genetic factors contribute to the nature of the sickle cell disease presentation. It may be summarized that the sickle cell disease is mild in the eastern Saudi Arabia and severe in western Saudi Arabia. The former is linked to Saudi-Indian haplotype, presence of Hpa I and Xmn I polymorphic sites and as high Gγ/Aγ ratio, while the latter is associated with the benin haplotype, absence of Xmn I and Hpa I polymorphic sites and a lower Gγ/Aγ ratio as summarized in Figure 11.36.

Figure 11.36: a summary of the major genetic factors in association with a mild or severe sickle cell disease in Eastern and Western Saudi Arabia