CHAPTER 13

MOLECULAR THERAPY

OF

SICKLE CELL DISEASE

13.1. Introduction

Interest in the treatment of sickle cell disease (SCD), by modulation of the level of fetal haemoglobin, has evolved following the reports that SCD patients with an elevated Hb F level frequently have a benign form of the disease. Several agents have been used in animal models, humans or in erythroid cultures to elevate the Hb F level (Warsy & El-Hazmi, 1985). These agents can be classified into several groups based on their mode of action. The first group includes the cytotoxic agents i.e. 5' aza-cytidine, hydroxyurea and methotrexate. The second group includes the agents that interrupt the cell cycle and have an effect on a direct differential gene expression increasing Hb F production. These include the humoral growth factors i.e. erythropoietin. A third group of substances encompass sodium butyrate and its analogues which increase Hb F level probably by a direct action on chromatin altering globin gene expression (Schechter et al, 1987; Rodgers, 1992; Rodgers et al, 1992).

Since the early 1980s several studies have been conducted using these agents either alone or in combination to treat SCD. Some agents have been abandoned due to their serious side effects while others were not acceptable due to their unpleasentness. The most promising of the agents include hydroxyurea, with or without rHuEpo (Rodgers et al, 1992; Dover et al, 1986; Goldberg et al, 1990; Charache et al, 1987; Al-Khatti et al, 1989).

In our laboratory, studies on SCD were initiated in early 1980's. Throughout the course of these investigations the need to try agents for treatment of the severe form of SCD was badly felt (Warsy & El-Hazmi, 1985). In Saudi Arabia the interest in molecular therapy of sickle cell disease evolved during the early 1980's , as part of a second stage of a

At a later stage, combination therapy using HU and rHuEpo was tried. Several protocols have been tried. Briefly our experience using various treatment strategies for Hb SS patients:is presented.

13.2. Treatment of SCD patients with hydroxyurea

The study group included 21 (16-42 years adult) adult patients suffering from Hb SS and 17 Hb S/β°-thalassaemia (15-47 years). The inclusion criteria included an informed

These patients had been regularly attending the outpatient clinic for a minimum of one year. They were clinically assessed by recording symptoms, signs, extent of blood transfusion requirements and hospitalization. The Severity Index (SI) was calculated for each patient by obtaining the sum of all parameters presented in Table 2.2.

13.2.1. Treatment Protocol

Each patient was treated with oral daily doses of hydroxyurea (HU). The HU dose was individually adjusted based on the Area Under a titration Curve (AUC_6) obtained for each patient. The patients were admitted to the hospital and given an oral dose of 25 mg HU/kg body weight.Blood samples were collected immediately and then every hour for 6 hours.The HU level was estimated in the blood sample and a graph was plotted between HU level and time and area under the curve (AUC_{6-}) was calculated for each patient. The dose was selected as follows:

AUC_6 (Conc x time)	HU Dose (mg/kg body wt.):
< 1000	20
1000-1500	15
> 1500	10

All patients had an AUC^6 of < 1000 and were initiated on 20-25 mg HU/Kg body weight. Thereafter, patients were discharged and maintained on the specified oral HU daily dose.Compliance was assessed by estimation of HU level in blood and urine on every follow up visit.

13.2.2. Baseline studies and evaluation protocol

The laboratory investigations conducted on each patient prior to initiation of treatment were used to obtain baseline values (two or more analyses). These included the estimation of haematological parameters and red cell indices using Coulter Counter, haemoglobin electrophoresis at alkaline and acid pH, estimation of Hb F (Helena Hb F Quiplate), Hb A_2 (Helena Sickle-Thal Column) and Hb F cells (Boehringer Mannheim (GmbH). Irreversibly sickled cells, reticulocyte count, platelet count, relevant biochemical parameters (Autoanalyser American Monitor), ferritin (RIA, using kits from Amerlite), Transferrin (Radioimmunodiffusion, using plates from Behring), Vit B12 and folate (Radioimmunoassy).

The DNA, extracted from the buffy coat of each sample, was used to establish the β-globin gene haplotype using the restriction endonucleases Hinc II, Hind III, XmnI, Ava II and Hpa I; α-globin gene arrangements were determined using Bam HI.

For follow-up investigations, conducted every 4-8 weeks, all investigations were carried out except the gene analysis and Hb electrophoresis.

For the group of patient, mean ± SD were calculated prior to and after treatment and paired `t' test was applied to determine the significance of the difference in the results prior to and during treatment. The value $P < 0.05$ was considered statistically significant.

The results of gene analysis showed the presence of Benin haplotype of the β-globin S gene cluster (-----+) both in homozygous and heterozygous state.The Xmn I polymorphic site 5' to Gγ was absent in all patients and α-globin gene arrangements was αα/αα in 18 patients, -α/αα in 6 patients -α/-α in 1 patient and in 13 patients DNA were not

analysed.

13.2.3. Results of HU treatment

The severity index (SI) decreased significantly (P = 0.0001) in patients treated with HU (Figure 13.1). Total haemoglobin (Figure 13.2), the Hb F level (Figure 13.3) and Hb F cells (Figure 13.4) increased in all patients, however, the extent of the increase was different in different individuals. Two of these patients were classified as non-responders since the increase in Hb F level and Hb F cells even after following several months of treatment was not significant (Figure 13.5). No correlation could be demonstrated between the Hb F and Hb F cells prior to treatment and the extent of elevation following treatment. The MCV increased (Figure 13.6), while irreversibly sickled cells (ICS), WBC count (Figure 13.7)), reticulocyte counts (Figure 13.8) and bilirubin levels (Figure 13.9) decreased significantly.

The extent of variations was different in each patients and the improvement continued over a period of one year. The level of iron, transferrin, ferritin, vit B12 and folate did not show any specific changes during or following treatment. No major toxic side effects were noticed. The platelet count decreased (Figure 13.10) but remained within the normal range.

Todate our 80 patients have been treated with HU alone in our laboratory. The follow-up is regular. Most patients show significant improvement in the clinical severity of the SCD, the haematological and biochemical values over the period of time of treatment (Figure 13.11 to 13.18). The extent of haemolysis decreases as judged from the decrease in the level of bilirubin, reticulocyte count and overall anaemic state.

Figure 13.1: Effect of HU treatment on the Severity Index in SCD patients

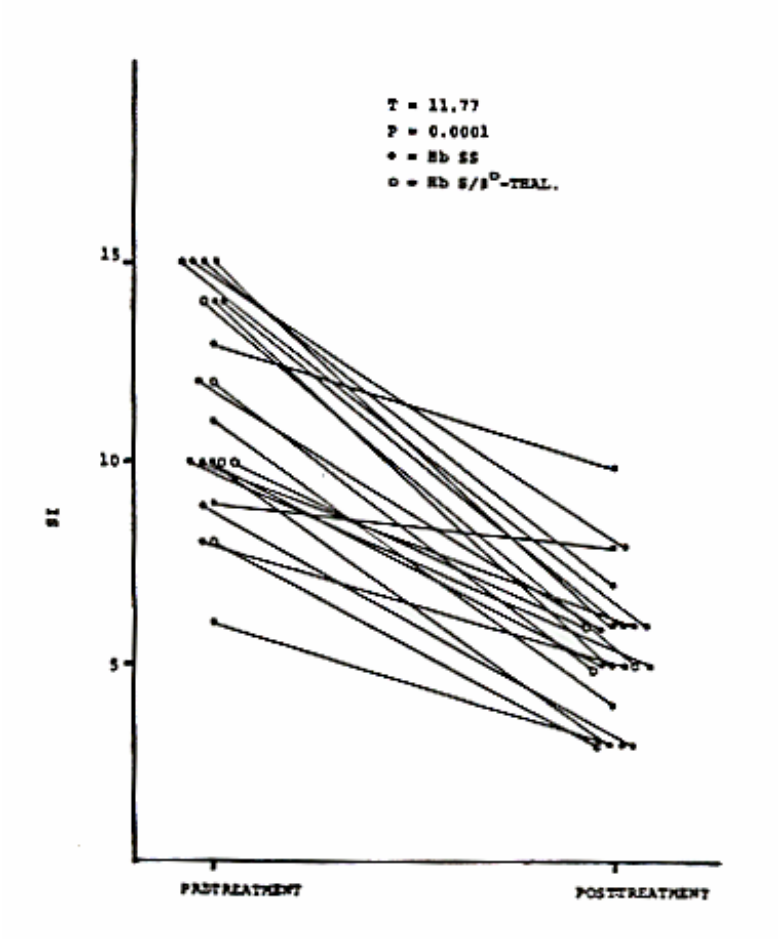


Figure 13.2: Effect of HU treatment on total haemoglobin level in SCD patients

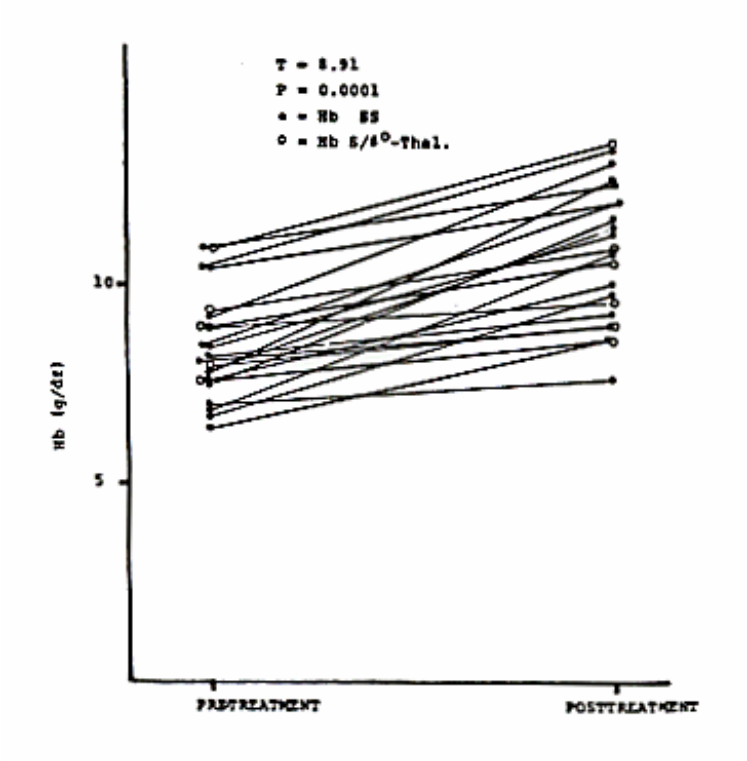


Figure 13.3: Effect of HU treatment on the Hb F level in SCD patients

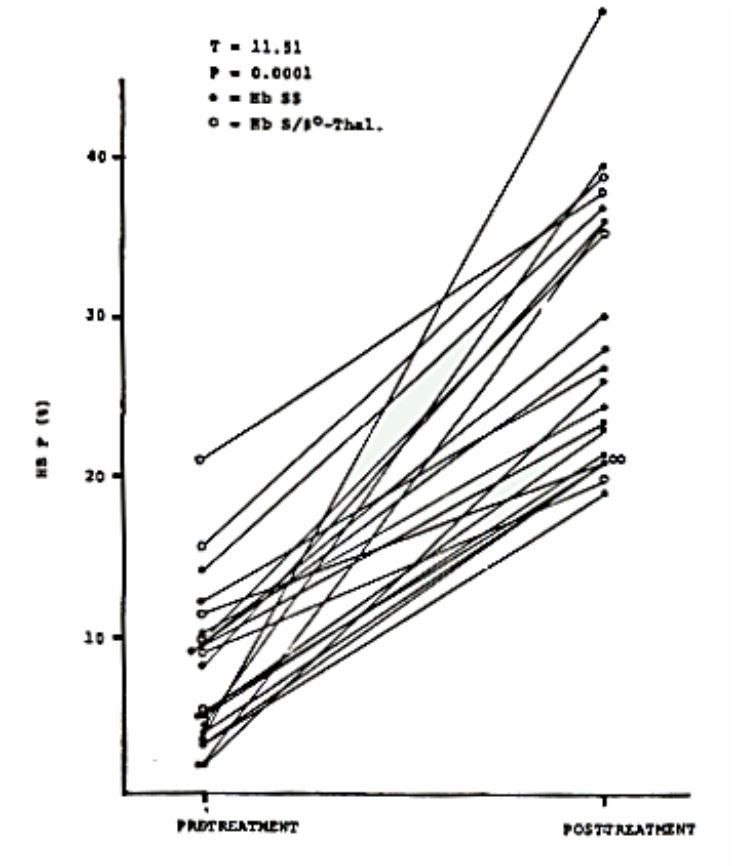


Figure 13.4: Effect of HU treatment on Hb F cells in SCD patients

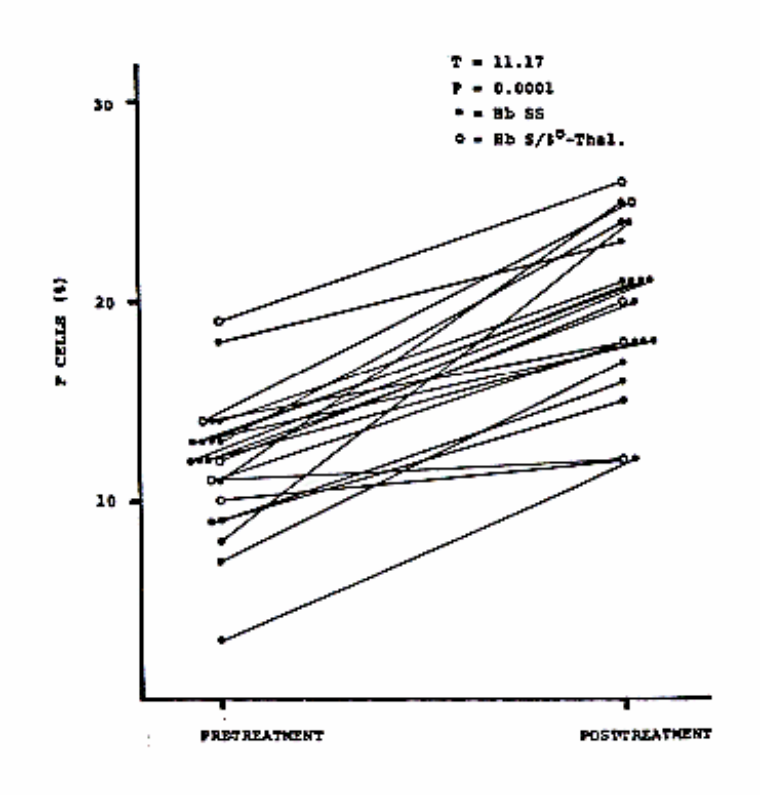


Figure 13.5: Effect of HU therapy on Hb F level

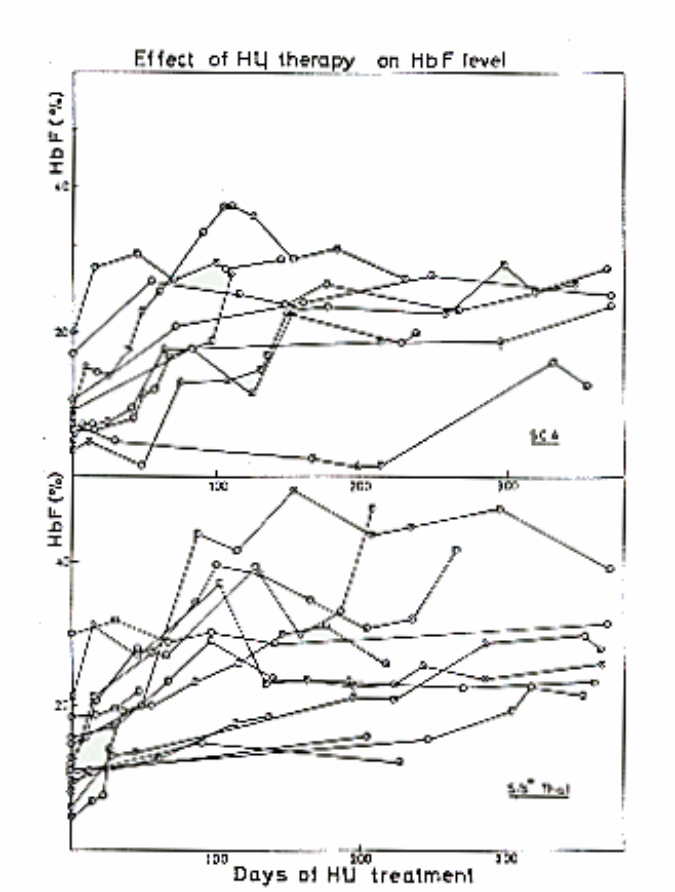


Figure 13.6: Effect of HU treatment on the MCV in SCD patients

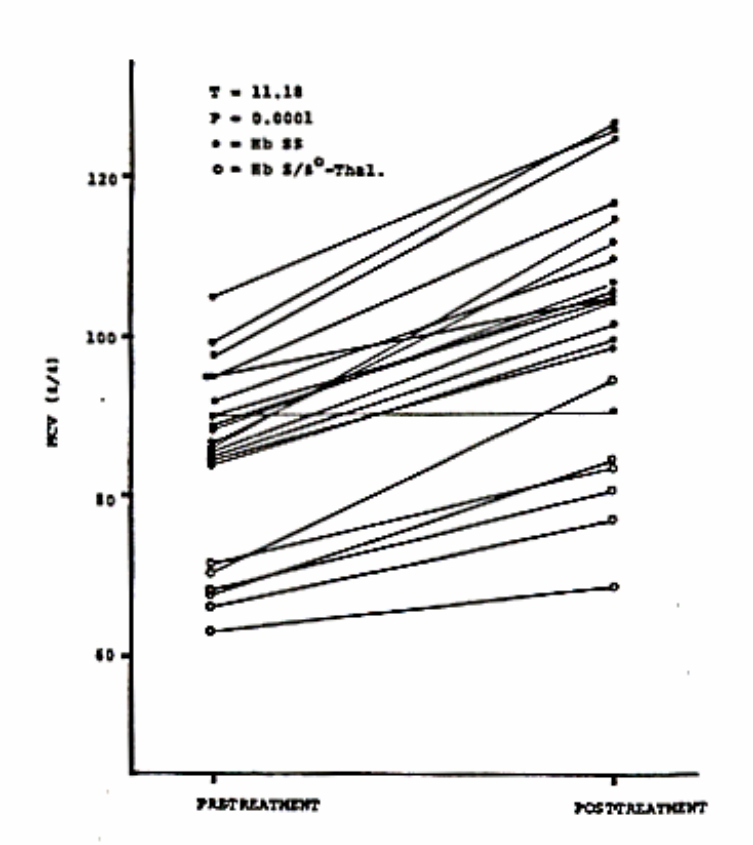


Figure 13.7: Effect of HU treatment on WBC count in sCD patients

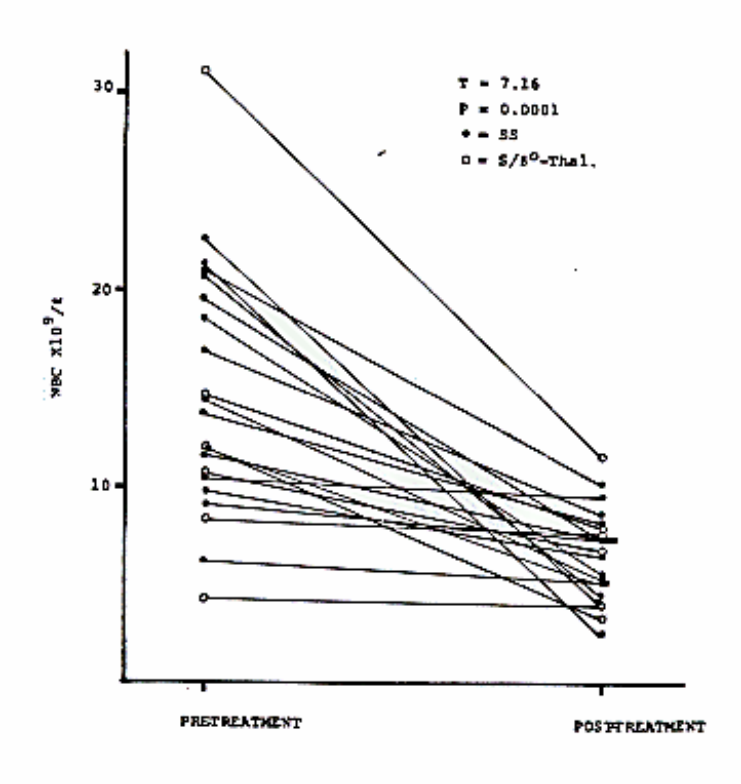


Figure 13.8: Effect of HU treatment on reticulocytic count in SCD patients

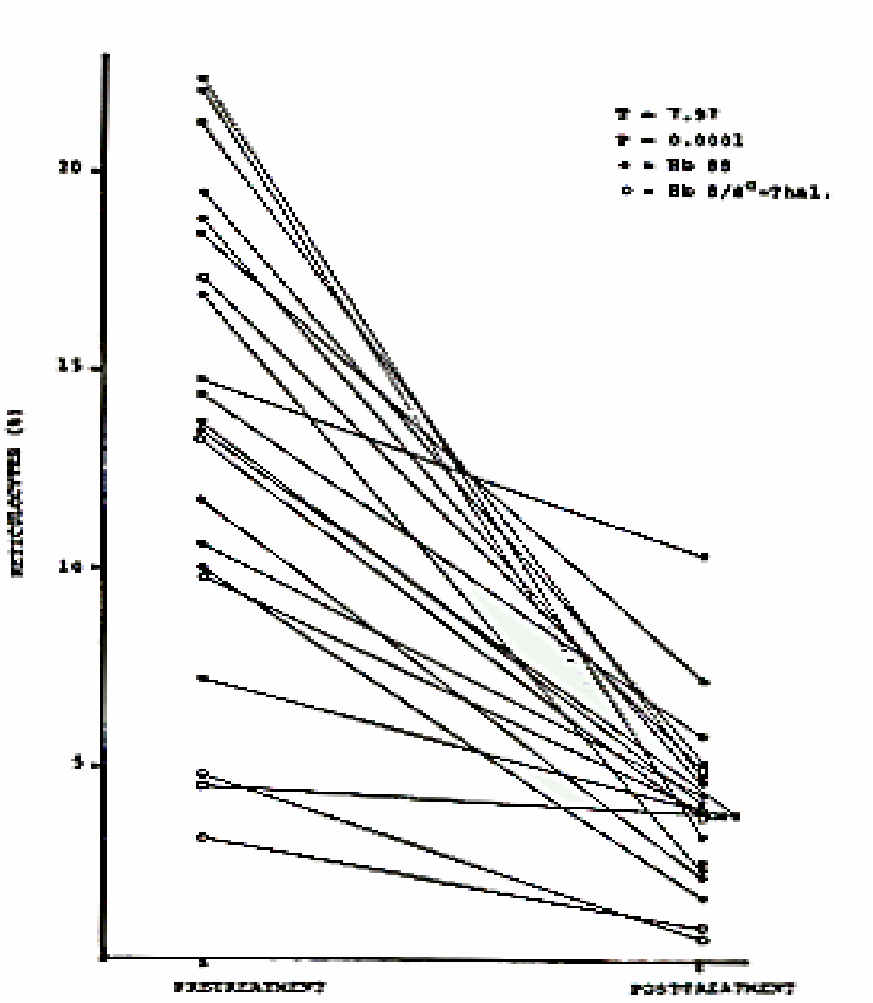


Figure 13.9: Effect of HU treatment on total bilirubin level in SCD patients

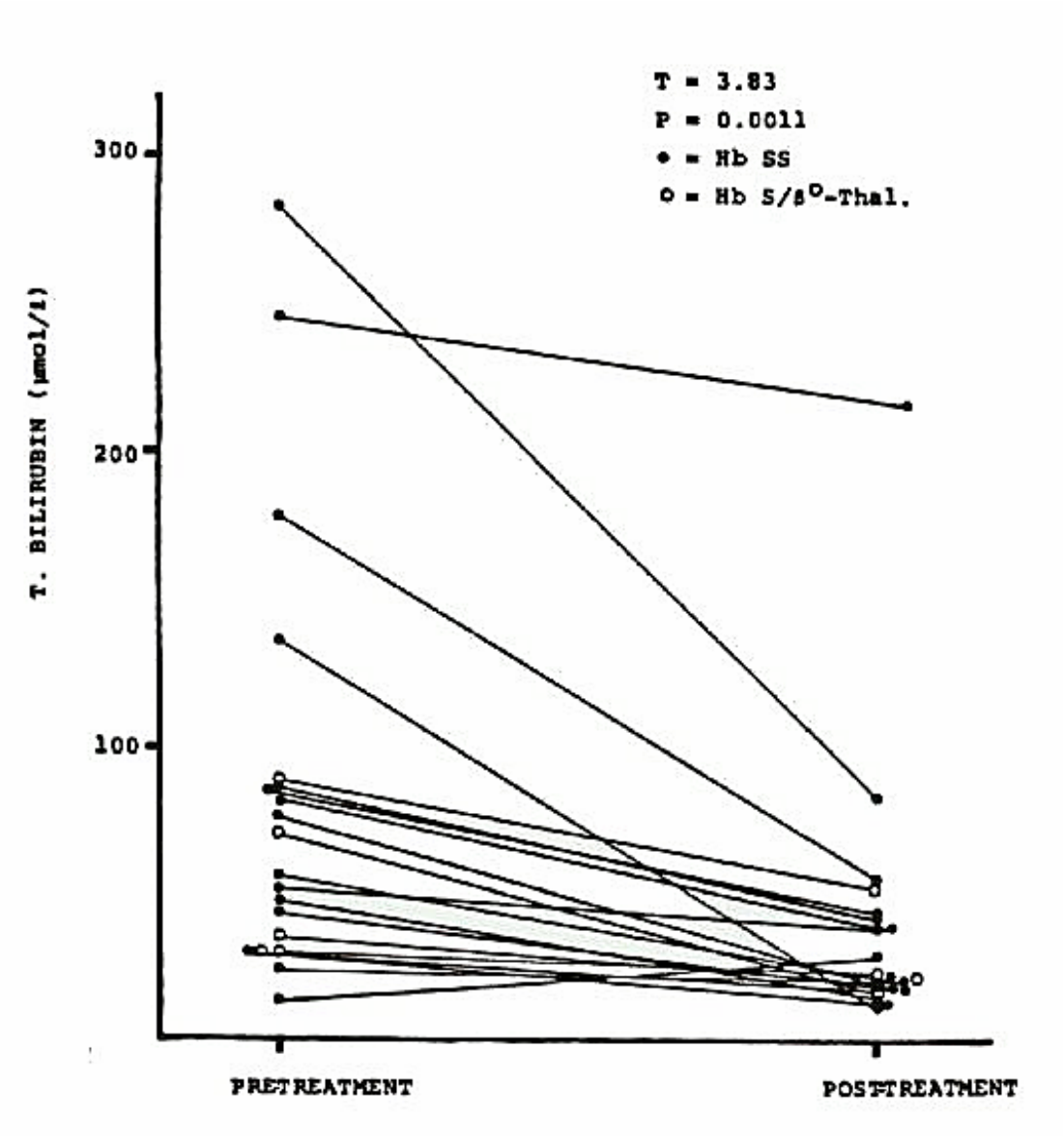


Figure 13.10: Effect of HU treatment on platelet count in SCD patients

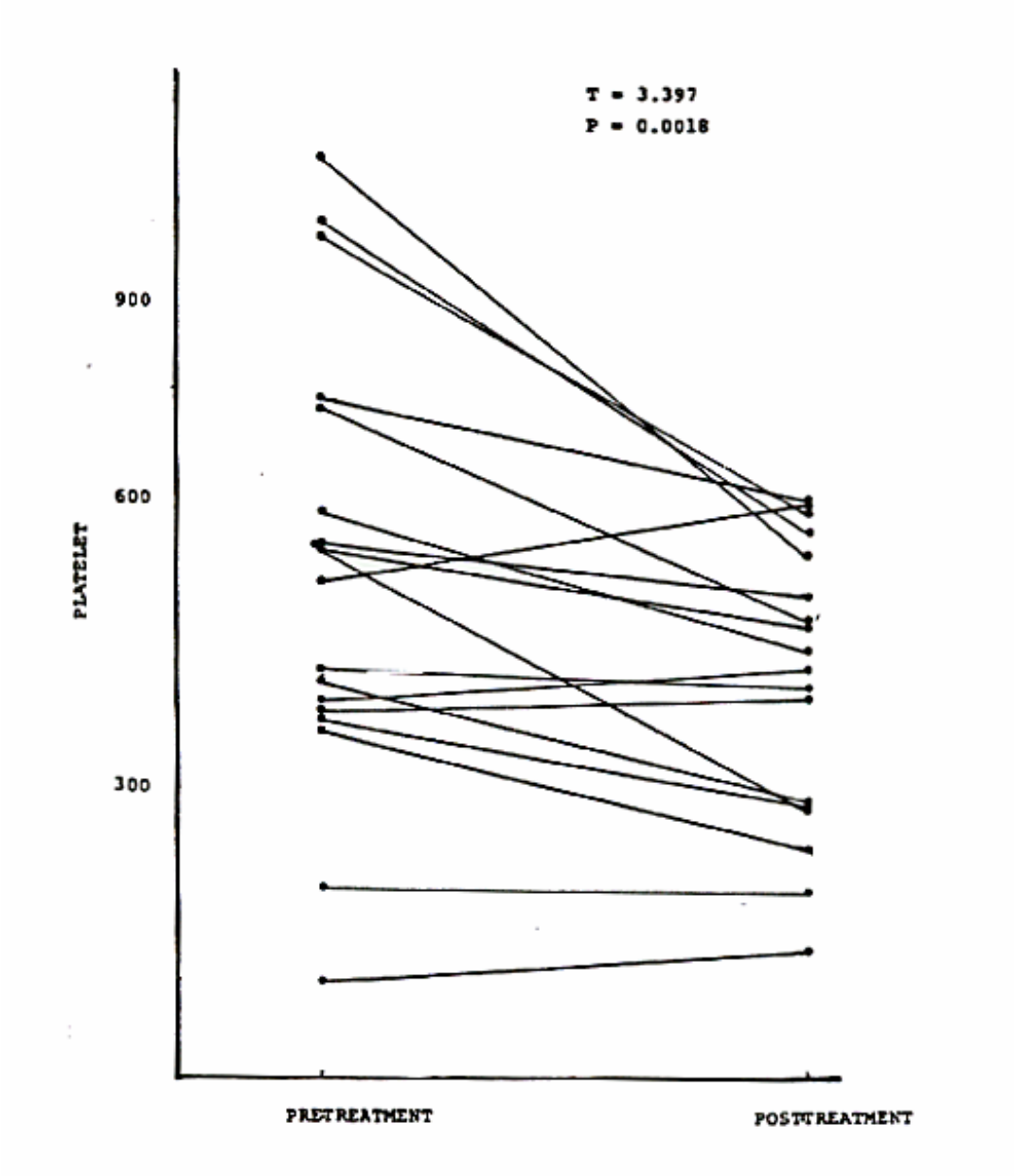


Figure 13.11: Effect of hydroxyurea treatment –
Haemoglobin level in SCD patients

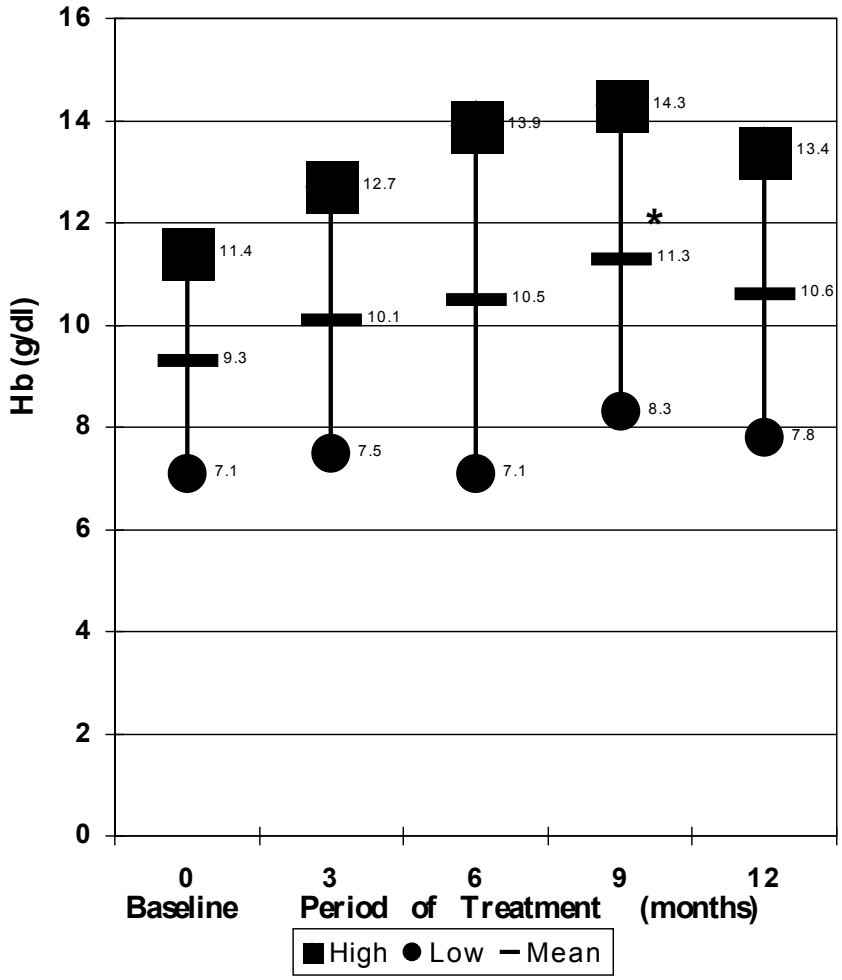


Figure 13.12: Effect of hydroxyurea treatment –
Hb F level in SCD patients

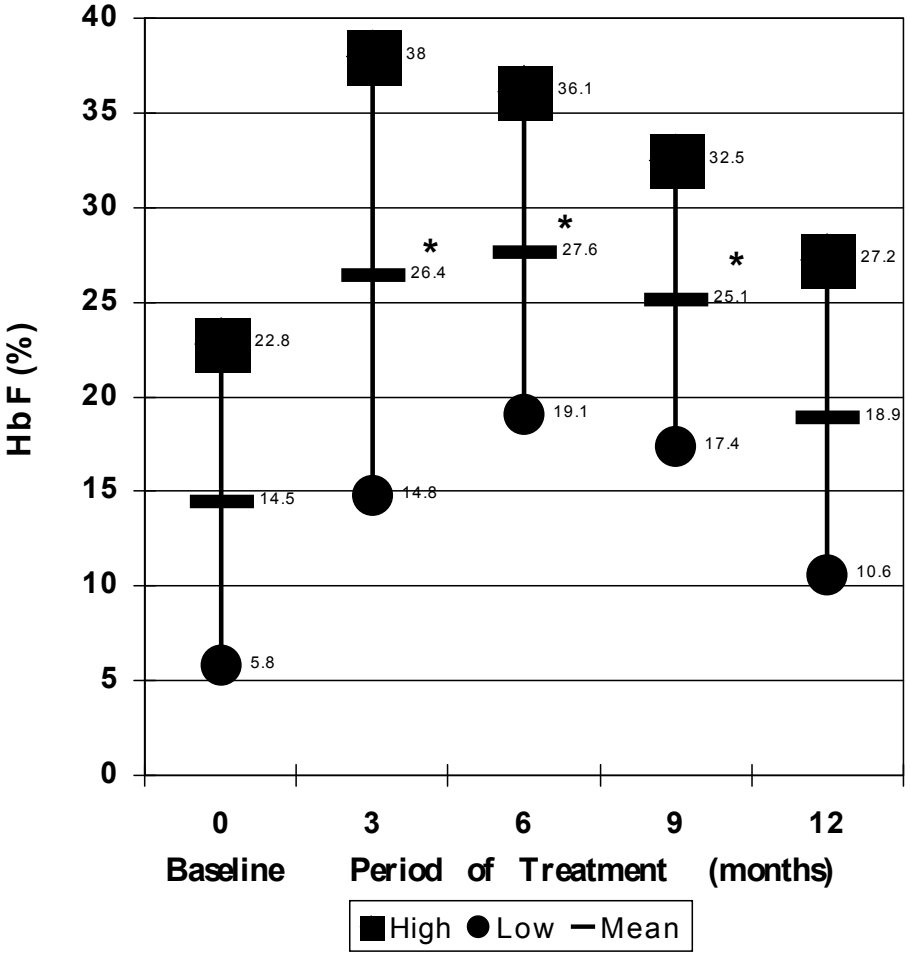


Figure 13.13: Effect of hydroxyurea treatment –
Hb F cells in SCD patients

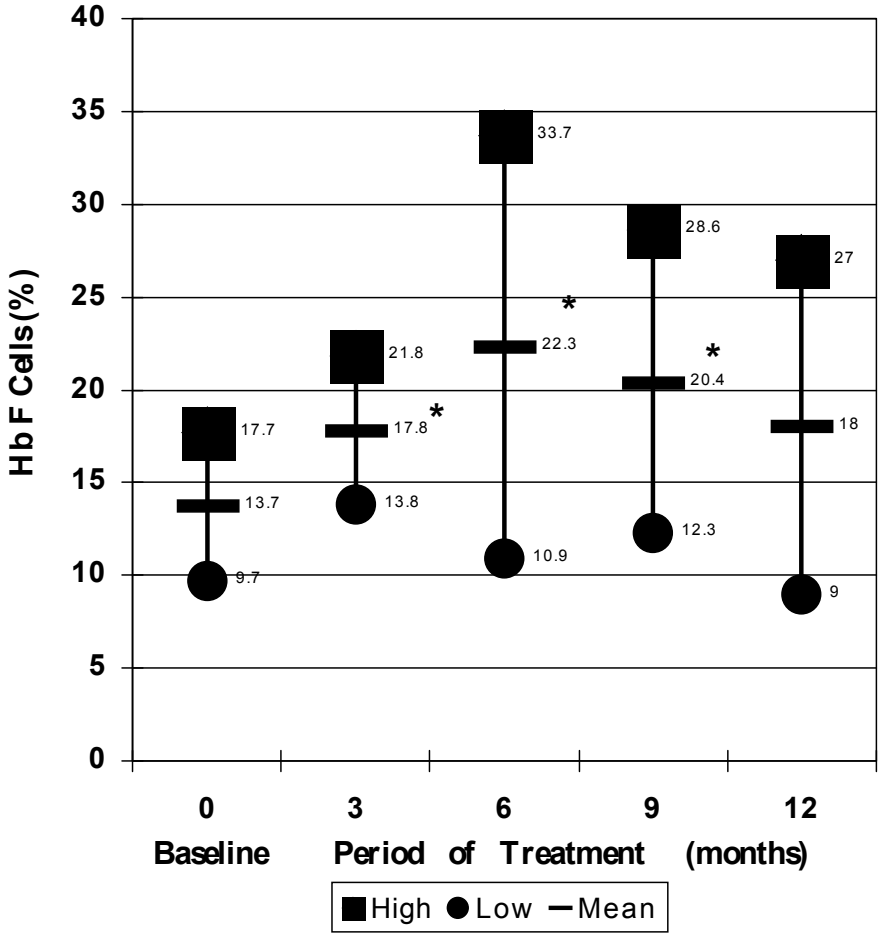


Figure 13.14: Effect of hydroxyurea treatment –
MCV in SCD patients

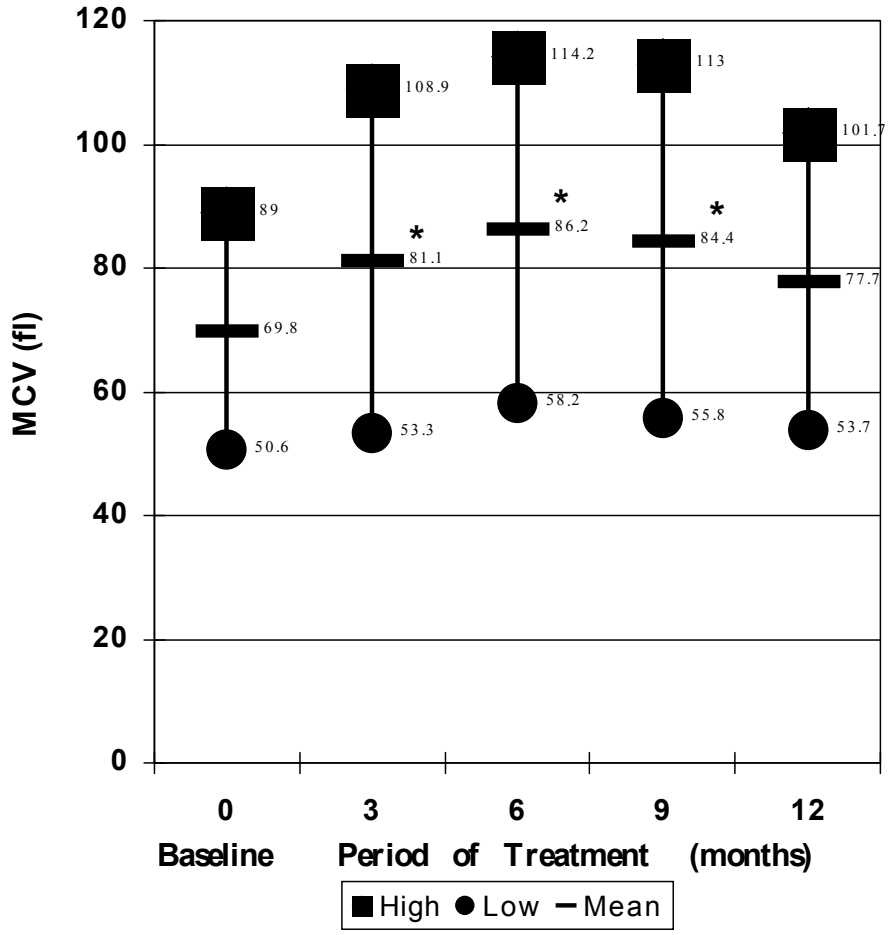


Figure 13.15: Effect of hydroxyurea treatment –
ISC level in SCD patients

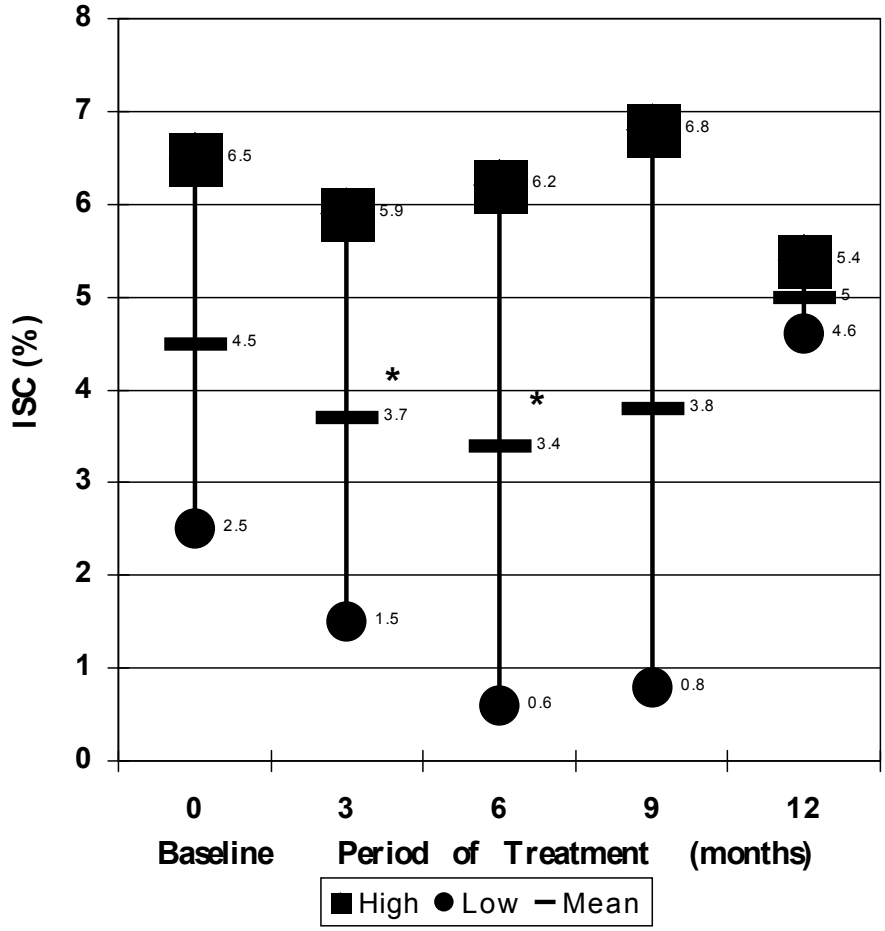


Figure 13.16: Effect of hydroxyurea treatment –
WBC level in SCD patients

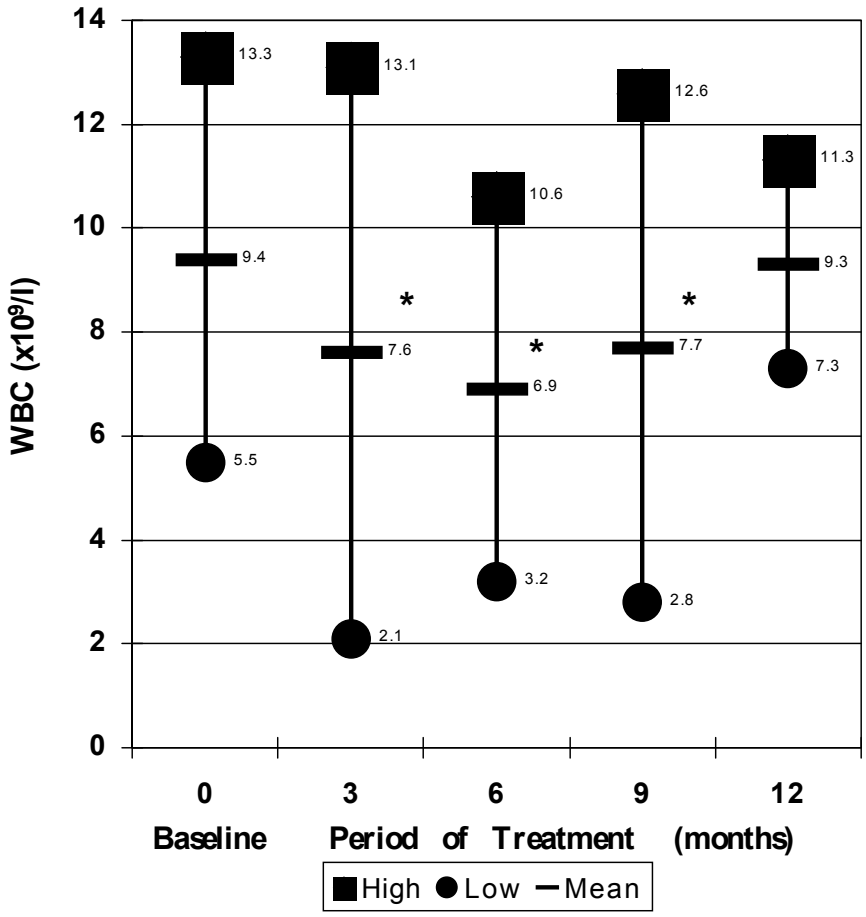


Figure 13.17: Effect of hydroxyurea treatment –
Retic counts in SCD patients

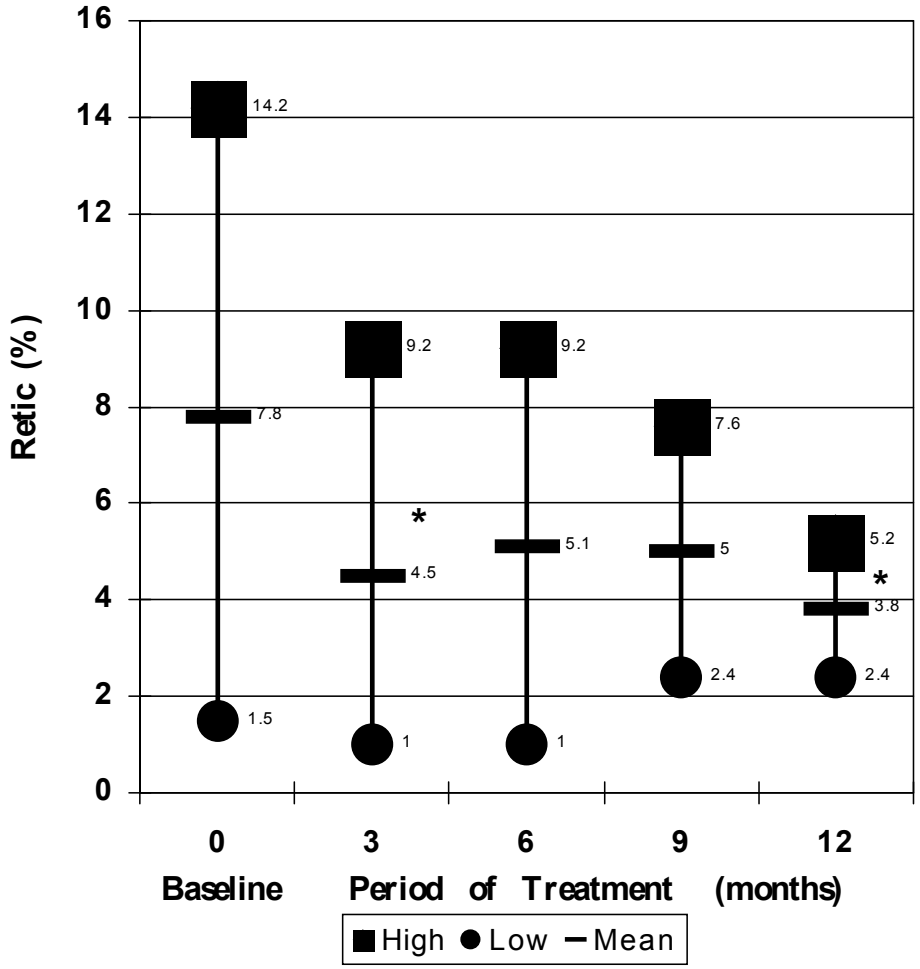
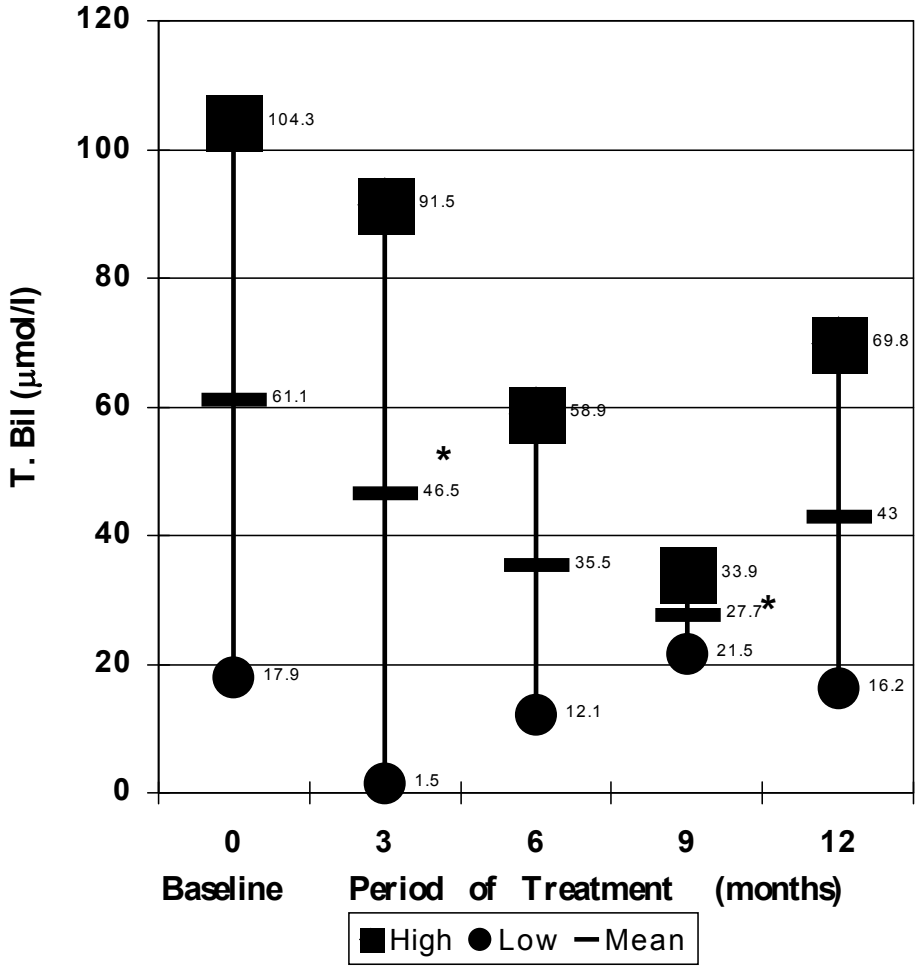


Figure 13.18: Effect of hydroxyurea treatment –
Total bilirubin in SCD patients



Though the beneficial effect of HU treatment is through elevation of the Hb F level and number of Hb F containing cells. However, a significant part is played by elevation of the mean cell volume. As the red cell volume increases it may further dilute the Hb S level, thus inhibiting gelation and the complications which follow.

13.2.4. Effect of incorporation of recombinent human erythropoietin in patients on HU treatment

Patients who were being treated with hydroxyurea alone for upto a period of 1 year were given rHuEpo in doses of 270-900 U/Kg week i.v. (once a week) along with the oral HU. In both Hb SS and Hb S/β°-thalassaemia patients significant improvement was observed (Table 13.1, Figures 13.19 and 13.20).

This was particularly obvious in patients who were non-responders to HU alone. No side effects were seen an the patients showed improvement in clinical, haematological and biochemical presentation.

13.2.5. Effect of HU + HuEpo combination therapy

Patients were initiated on combination therapy using fixed daily doses of HU and variable doses of rHuEpo once/week. The analysis of data showed improvement in the clinical presentations and the number of crises were significantly reduced. Hb F level and Hb F cells increased (Figures 13.21 and 13.22) and the increase was greater as the level of rHuEpo was increased. Haematological parameters improved as shown in Figures 13.23 and 13.24 for total haemoglobin and MCV level, with the:various HU + rHuEPo combinations. However, with rHuEpo doses of 300 or more U/mg body weight gave more significant improvements. WBC count (Figure 13.25), ICS, reticulocyte count and total

bilirubin level decreased. The decrease was more significant as the rHuEpo dose was

Table 13.1: Effect of HU + rHuEpo combination therapy in SCD patients treated with HU.

A. Sickle cell anaemia patients = 4

Parameters	Before Treatment	After HU alone	After HU + rHuEpo combination therapy	After HU maintenance therapy
Hb (g/dl)	8.4 ± 1.4	9.7 ± 1.5*	10.1 ± 1.1*	10.2 ± 1.2*
Retic. (%)	15.4± 2.5	9.6 ± 9.2*	4.9 ± 2.7*	6.3 ± 4.6*
MCV (fl)	90.0±14.0	102.3±14.0*	106.6±11.6*	104.3± 12.4
Hb F (%)	9.5 ± 7.0	19.2 ±10.3*	21.5 ± 5.1	25.0 ± 9.7*
HbF cells (%)	10.7± 7.6	18.0 ± 8.9*	17.5 ± 5.0*	16.5 ± 1.7*
T.Bil (mmol/l)	67.3±36.7	51.0 ±27.7*	42.3 ± 20.1	38.3 ± 17.0

B. Sickle Cell β°-thalassaemia patients = 3

Parameters	Before Treatment	After HU alone	After HU + rHuEpo combination therapy	After HU maintenance therapy
Hb (g/dl)	7.5± 0.7*	7.6 ± 1.4	:7.8 ± 1.1*	:7.7 ± 1.6
Retic. (%)	13.4± 4.0	6.9 ± 7.9*	:1.9 ± 1.6*	:4.5 ± 3.4*
MCV (fl)	68.3±4.8*	89.5 ±20.5*	91.3±22.5*	89.5±20.9*
Hb F (%)	10.3± 2.2	30.5 ±13.3*	27.4 ± 9.3	35.4±20.8*
HbF cells (%)	16.0± 7.2	18.7 ± 8.6*	20.7±10.1*	28.7±13.3*
T.Bil (mmol/l)	65.3±32.6	23.0 ±14.9*	21.7±14.5*	23.7 ± 17.2

*Statistically significant ($P < 0.05$) compared to the baseline values.

Figure 13.19

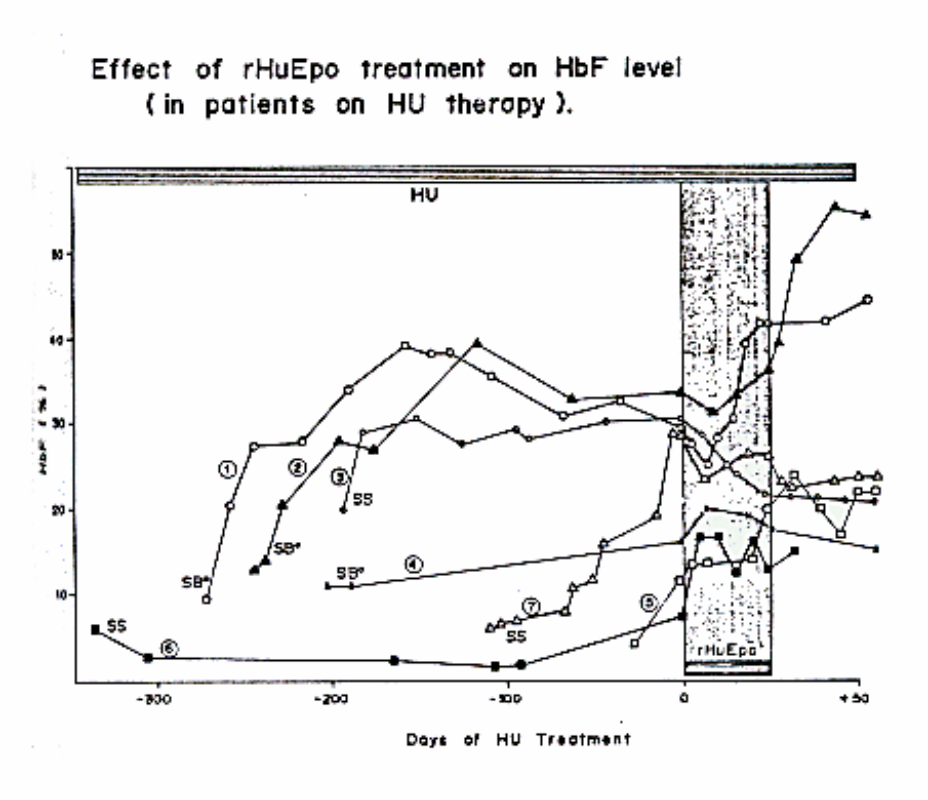
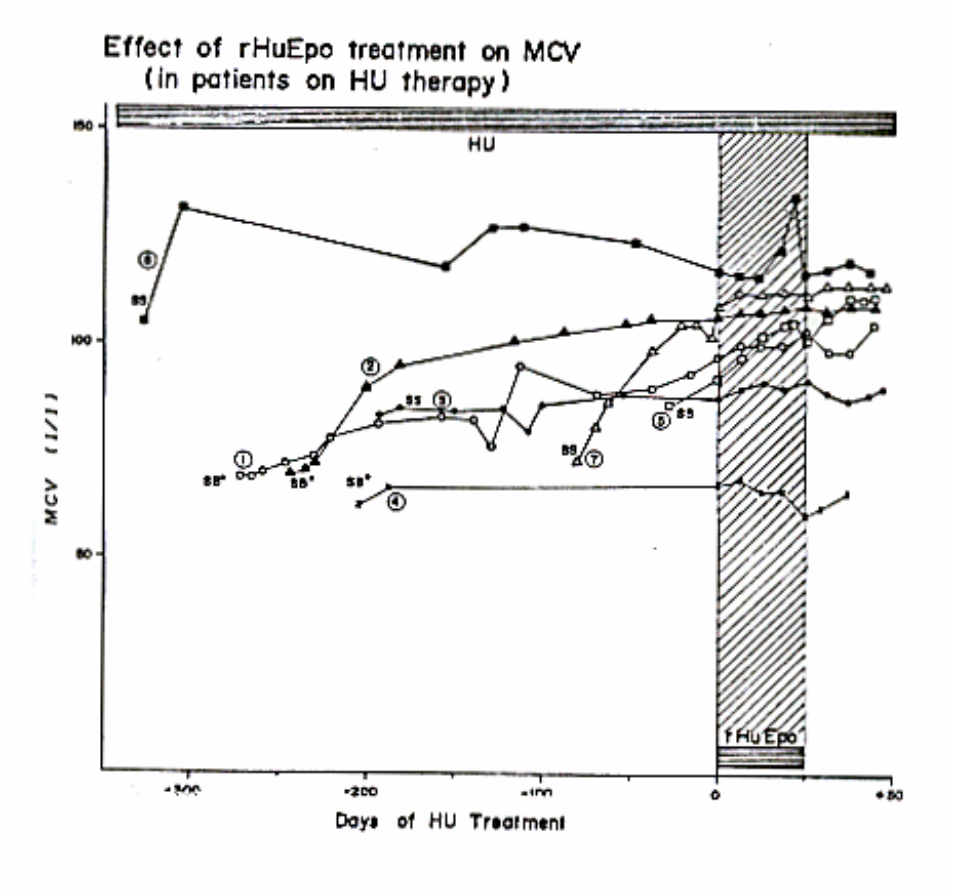

Effect of rHuEpo treatment on HbF level
(in patients on HU therapy).
HU
rHuEpo
SS
SB°
Days of HU Treatment

Figure 13.20


Effect of rHuEpo treatment on MCV
(in patients on HU therapy)
HU
rHuEpo
MCV (fl)
Days of HU Treatment