CHAPTER VII

EFFECT OF

GLUCOSE INFUSION ON CVSC COUNT
CHAPTER - VII

7.1. Introduction:

Intravascular sickling due to polymerization of HbS under hypoxic condition has been regarded as the root cause of vaso-occlusion and consequent damage to different organs seen either in vaso-occlusive crisis or as long term sequelae such as in kidney, lungs and spleen. As such during acute episodes of such crisis the number of circulating sickle cells increases sharply. Such sickling phenomenon however is a transient phase in the life of the RBCs because with improved oxygenation the sickled RBCs reverse to normal biconcave discoid shape, although in the process it may lose part of its membrane. Such repeated sickling and unsickling ultimately results in the formation of so called irreversibly sickled cells or ISCs (Paddila et al. 1973) which can't revert back to normal shape any more.

Therefore, in the circulating blood of every patient of sickle cell disease, a number of ISCs are found even in steady state. It has been stated to be fixed for each patient (Diggs and Bibb 1939) though it might vary markedly between different patients depending upon the HbF levels of the patient (Serjeant 1970). To explain the clinical relevance of ISC
formation, the relationship between ISC count and pathological processes has been explored and significant effect of high ISC count with haemolytic rate has been found (Serjeant 1985).

It is a common experience that in venous blood smear of sickle cell disease patients a number of sickle cells and ISCs are encountered even in steady state, though increased greatly during acute vaso-occlusive crisis. Separation of ISC from potentially reversible sickled cells is a cumbersome procedure necessitating sophisticated equipments. ISC count has been relied upon as a research parameter, because, unless special precautions are taken reversible sickled cells are likely to revert back to normal shape on exposure to atmospheric oxygen. However, special technique of fixing the red cells in formal-saline immediately on withdrawal from the vein, may overcome this bottle-neck. The count of deformed red cells from such a preparation will include both ISCs as well as reversible sickled cells in any given patient. In the absence of sophisticated equipment this procedure has been exploited in the present study to assess the effect of raised blood glucose concentration in vivo on the intravascular sickling process. This was done because there was indication from the in vitro studies that glucose consumption of SS red cells is high and it was thought desirable to explore
the effect of high glucose concentration on intravascular sickling \textit{in vivo}.

7.2. Materials and Methods:

Fifteen adult homozygous sickle cell disease cases of both sexes were taken for the study. The patients were all in steady state. The patients had not received any transfusion 3 months prior to the study. After overnight fasting 5 ml of blood samples were collected from each patient for estimation of zero hour glucose, pyruvate and lactate values. The circulating venous blood sickle cell (CVSC) count was also done. After blood collection 500 ml of 5% glucose (dextrose) solution was infused intravenously. The rate of glucose infusion was maintained at 8 to 10 ml per minute. Immediately after the glucose infusion the blood samples were collected for estimation of glucose, pyruvate, lactate and also for CVSC count.

The results were then analysed to see the effect of glucose infusion on the level of glucose, pyruvate, lactate and CVSC count.

7.2.1. Glucose Estimation:

The glucose estimation was done following the method of Trinder (1969) as described by Wootton (1974).
7.2.2. Pyruvate Estimation:

Pyruvate estimation was done following the method of Friedemann and Haugen (1943) as described by Wootton (1974).

7.2.3. Lactate Estimation:

Lactate estimation was done following the method of Barker and Summerson (1941) as described by Varley (1976).

7.2.4. Circulating Venous Sickle Cell (CVSC) Count:

The CVSC count was done as per Charache and Conley (1964) after minor modification. Immediately after blood collection 0.25 ml of whole blood was injected into a solution of 10% formalin in 0.85 percent NaCl solution. The shape of the red cells was unchanged after adding in formalin-saline. After 5 minutes blood was taken out by using pasteur's pipette and smear was made on the microslides. The slides were stained by Leishman's stain. After drying, the slides were observed under the microscope using oil immersion objective. About 1000 red blood cells were counted. All the sickled shaped cells were also counted. The percentage of sickled cells was determined.
7.3. Results:

7.3.1. Glucose:

The mean fasting plasma glucose value of SS patients was 82.2 ± 1.7 mg/dl. The mean glucose values after infusion was 152.4 ± 7.6 mg/dl. The post-infusion glucose value was significantly (p<0.001) higher than the pre-infusion value (Table 7.3.I. Fig.7.3.1).

7.3.2. Pyruvate:

The table 7.3.I and Fig.7.3.2 shows that the pre-infusion mean pyruvate value was 1.21 ± 0.08 mg/dl blood, whereas the post-infusion value was 1.31 ± 0.09 mg/dl. There was no significant difference between these two mean values.

7.3.3. Lactate:

The pre-infusion mean lactate value was 13.07 ± 1.50 mg/dl and the post-infusion mean value for lactate was 12.2 ± 1.50 mg/dl. There was no significant difference between the two mean values (Table 7.3.I and Fig.7.3.2).

7.3.4. Circulating Venous Sickle Cell Count:

The pre and post-infusion CVSC count percentages were 19.9 ± 0.44 and 12.48 ± 0.31. The post-infusion circulating venous sickle cell count was
<table>
<thead>
<tr>
<th></th>
<th>Pre-infusion Mean ± SE (Range)</th>
<th>Post-infusion Mean ± SE (Range)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>82.2 ± 1.7 (73-90)</td>
<td>152.4 ± 7.6 (115-193)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pyruvate (mg/dl)</td>
<td>1.21 ± 0.08 (0.96-1.7)</td>
<td>1.31 ± 0.09 (0.96-1.81)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Lactate (mg/dl)</td>
<td>13.07 ± 1.50 (5.0-20.9)</td>
<td>12.2 ± 1.50 (3.9-19.7)</td>
<td>N.S.</td>
</tr>
<tr>
<td>CVSC Count (Percentage)</td>
<td>19.9 ± 0.44 (18.2-22.4)</td>
<td>12.48 ± 0.31 (10.4-13.0)</td>
<td>p&lt;0.001</td>
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</table>
FIG. 7.3.1 BLOOD GLUCOSE AND CVSC COUNT IN SCD PATIENT.
FIG. 7.3.2 BLOOD PYRUVATE AND LACTATE LEVELS IN SCD PATIENTS.
significantly lower (p<0.001) than the pre-infusion value (Table 7.3.1 and Fig.7.3.1).

7.4. Discussion:

In 15 SS patients in steady state, by raising the blood glucose level about 2 folds (82.2 ± 1.7 mg/dl to 152.4 ± 7.6 mg/dl) by glucose infusion, excess supply of glucose for RBC metabolism has been ensured artificially. Absence of any significant change in pyruvate and lactate level (Table 7.3.1) suggest no change in anaerobic metabolism. Simultaneous count of circulating venous blood sickled cells, prior to glucose infusion and at the height of raised blood glucose, showed a significant decrease (p<0.001), from 19.9 ± 0.44 to 12.48±. 0.31 per hundred RBCs. In absence of any other factor which might increase the blood oxygenation level or decrease the tendency for intravascular sickling within such a short span of time (1 hour), the decrease in sickled cell count can reasonably be attributed to increased blood glucose level and adequate supply of energy source for RBCs.

There has been suggestions that ATP dependant cation pumps in the RBC membrane might have some role in the deformability of RBC seen in the sickling process (Hebbel 1991). In the present experiment adequate energy supply might be a factor eliminating any deficiency in ATP dependant mechanism responsible
for sickling process. If the results are confirmed by further and more sophisticated studies of energy measurement, it might provide an easy and harmless procedure of glucose infusion in reducing the number of intravascular sickled cells especially during acute vaso-occlusive episodes.

There are reports of improvement in pain following rapid infusion of intravenous fluids (Jenkins et al. 1956; Sharpstein 1957; Scott and Ferguson 1960) with or without Pentoxiphylline (Khosla and Chintu 1984; Tripathy, et al. 1990). In most situations the intravenous fluids contain glucose. It is possible that the beneficial effect has been due to glucose rather than the fluid. However, there has been no controlled trial in this regard (Serjeant 1985).

Thus the present study may serve as a preliminary observation on the beneficial effect of glucose on elevation of painful crisis by reducing the intravascular sickle cell count subject to control clinical trial.