CHAPTER VII

STUDIES ON GLUTATHIONE REDUCTASE
Erythrocytic Glutathione Reductase

Glutathione reductase activity of the same red cells suspension was measured using the technique of Dawson and co-worker. Red cells obtained from the heparinised blood and suspended in saline as mentioned before were used for this purpose. From the red cell suspension 0.5 ml was taken and to that 3.3 ml of distilled water was added, mixed well and haemolysate was prepared. To that haemolysate 0.5 ml of tris buffer 0.036 M pH 7.4, 0.5 ml of GSSG 1 x 10^{-4} M was added and mixed well. Thereafter 0.2 ml of NADPH 1.5 x 10^{-4} was added to that mixture and incubated at 37°C for 15 minutes and then the reaction was stopped by adding 3 ml of 3% metaphosphoric acid. Then to that mixture 2 gm of sodium chloride was added and then it was filtered. From that filtrate 4 ml was taken and to that 0.5 ml of 2% sodium nitroprusside and then 0.5 ml of sodium cyanide carbonate solution as mentioned before was added and read in a photoelectric colorimeter at 515 nm wave length against distilled water as blank. The activity of the enzyme GR was estimated by measuring the rate of oxidation of NADPH to NADP in the presence of haemolysate and GSSG.

The calculation was done like this

\[
\text{GR} = \frac{U - B}{S - B} \times 50 \times 100
\]

When u is the density of unknown, B is blank.
S is the value of standard in mg%.
The values per minute were calculated and expressed as mg. of GSH formed/100 ml RBC/min.

In 30 normal subjects the value of GR varied from 4.0 to 8.0 mg/100 ml RBC/min with a mean of 4.8 mg.

The results of the activity of Glutathione reductase expressed as mg. of GSH formed/100 ml RBC/min in 147 patients with different types of leukaemia shown in table No. 37.

Table - 37
Level of GR in 146 cases of leukaemia

<table>
<thead>
<tr>
<th>Types of leukaemia</th>
<th>No. of cases</th>
<th>Level of Glutathione reductase activity expressed as mg. of GSH formed/100 ml RBC/min.</th>
<th>No. of cases with</th>
<th>Range</th>
<th>Mean</th>
<th>Less than 4 mg.</th>
<th>More than 8 mg.</th>
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</table>
In 58 patients the erythrocytic GR activity was measured. The value ranged from 2.2 - 8.5 mg with a mean value of 4.2. One patient, case No.53 was with low value of GR of 2.2 mg. This patient had low level of G-6-PD and it was 1.5 units and the GSH value was 36 mg in preincubated erythrocytes and 20 mg in post incubated erythrocytes. This patient with unstable GSH had Hb 7.0 Gm\% and reticulocyte count 29,000 per cmm of blood. Of these 58 patients with CML, only 1, case No.36 had GR value above normal range and it was 8.5 mg. This patient had normal value of G-6-PD 4.8 units, preincubation GSH 110.0 mg, post incubation GSH of 104 mg, Hb 12.7 Gm\% and reticulocyte count 74,000 per cmm of blood.

In 25 ALL patients the value of erythrocytic GR varied from 4 - 9.2 mg with a mean of 6.18 mg. Only two patients, case Nos. 12 and 13 had GR value 3.2 mg. and 8.2 mg. respectively. These two patients with GR value more than normal were with normal G-6-PD of 6.1 and 5.2 units respectively. The Hb and reticulocyte count of case No. 12 was 4.5 Gm\% and 96,650 per cmm of blood respectively. This patient was also with normal GSH and its stability of 78.2 mg and 75.1 mg respectively. In case No. 13 the GSH was 80.1 mg and its post incubation value was 75.2 mg. The Hb was 3.2 Gm\% and reticulocyte was 7,000 per cmm of blood. One patient case No. 6 who had low G-6-PD of 1.2 units, low GSH of 26.2 mg, with its unstable post incubation GSH value of 10.2 mg, was with normal level of GR value of 4.2 mg. The Hb of this patient was 3.0 Gm\%, reticulocyte count was 6,200 per cmm of blood.
The GR value of 32 AML patients varied from 4.2 - 6.8 mg with a mean value of 5.36 mg.

In 19 patients with CLL the GR value ranged from 4.1 - 8.5 mg with a mean value of 6.6 mg. One patient, case No. 1 had GR value more than normal and it was 8.5 mg. This patient had a G-6-PD activity of 4.1 units and Hb 7.5 Gm% and reticulocytes 200,000 per mm of blood. The GSH and its stability was within normal range.

In 5 AML patients the value of GR ranged from 4.8 to 8.0 mg with a mean value of 6.8 mg. The GR value of 4 Ery.L. patients varied from 4.2 - 6.2 mg with a mean value of 5.2 mg. In total 146 patients with different type of leukaemia only 1 was with low level of GR and 4 had GR value more than normal range.