CHAPTER – V

GLYCOSYLATED HEMOGLOBIN IN
IRON DEFICIENCY ANEMIA (IDA)

Iron is essential for a wide variety of metabolic processes and is required for erythropoietin to effectively stimulate red blood cell production(375). Without sufficient iron available to the RBC precursors, normal erythropoiesis cannot take place and anemia develops(376).

Iron is necessary mineral for the proper function of hemoglobin and for proper muscle and organ function. About three-fourths of the body’s iron is bound to hemoglobin in red blood cells, while the rest (about 25%) of total body iron is stored in the form of ferritin or hemosiderin in the reticuloendothelial system in the liver, spleen and bone marrow or stored in other body tissues such as myoglobin in muscle and iron containing enzymes. When red blood cells die, their iron is released and carried by Transferrin to the bone marrow. In the bone marrow iron is stored and used as needed to make new red blood cells(377). Depending upon iron stores, there are various stages of IDA(378).

Too much iron accumulating in vital structures (especially the heart, pancreas and liver) produces a potentially fatal condition ‘hemochromatosis(376). Iron can damage tissues by catalyzing the
conversion of hydrogen peroxide to free radical ions that attack cellular proteins, DNA and membranes(379).

Food iron can be divided into two types, non-hem iron and hem iron. The absorption of non-hem Iron is facilitated by ascorbic acid and retarded by phytic acid and phosphates, whereas heme iron does not depend on their presence(380).

A well-balanced diet contains sufficient iron to meet body requirements(381).

Most food iron is in ferric state and ascorbic acid converts the ferric iron into ferrous Iron in the gastro-intestinal tract. Iron absorption occurs when the iron is in ferrous state, after absorption the ferrous iron is transported to the subepithelial capillaries possibly by intracellular transferrin and released into the blood stream(380).
There it is oxidized to Fe++ and again taken up by plasma transferrin, most absorbed iron is utilized in bone marrow for erythropoiesis(376). About 10 to 20 % of absorbed iron goes into a storage pool, which is also being recycled into erythropoiesis, so there is a balance of storage and use.

Iron deficiency anemia.

A deficiency of iron causes a reduction in the rate of hemoglobin synthesis and erythropoiesis and can result in iron deficiency anemia. Anemia is a condition characterized by a reduced number of circulating red cells or a reduced amount of hemoglobin in the cells or both. Iron deficiency anemia is the commonest of all single-nutrient deficiencies. The main causes are:
1. Deficient intake, including reduced bioavailability of iron from dietary fiber, phytates, oxalates.

2. Impaired absorption, e.g. abdominal surgery.

3. Excessive loss, e.g. menstrual blood loss in women and in men from gastrointestinal bleeding (in peptic ulcer, diverticulosis or malignancy).

Iron deficiency causes low hemoglobin resulting in hypochromic microcytic anemia in which the size of the red blood cells are much smaller than normal and have much reduced hemoglobin content.

Clinical features of anemia are weakness, fatigue, dizziness, palpitation. Nonspecific symptoms are nausea, anorexia, constipation, and menstrual irregularities(38).

**The interaction of Iron and erythropoietin**

Erythropoiesis involves the close interaction of iron and erythropoietin. In essence, erythropoietin is the accelerator that drives erythropoiesis. Iron is the fuel for the production of new red blood cells. When the two are coupled, red cell production moves briskly and efficiently. If one component is absent e.g. iron, iron deficiency anemia results.
The production of red cells involves the coordinate interaction of two organ systems in the body. The first is the bone marrow which produces red cells. The second is the kidney which produces the hormone erythropoietin. Hypoxia (low oxygen) of the kidney prompts synthesis and release of erythropoietin. The erythropoietin then travels to the bone marrow via the blood circulation where it activates new red blood production. This increases the blood’s oxygen carrying
capacity corrects the hypoxia which was the primary stimulus to erythropoietin production. Erythropoietin therefore is part of a finely-tuned feedback circuit that controls red blood cell levels.

Erythropoietin has been cloned and over the past few years studied in great detail. The hormone is a 165 aminoacid polypeptide chain. **Erythropoietin is heavily glycosylated.** That is, the amino acid backbone has a large number of attached sugar molecules (termed complex carbohydrate chains). The protein has molecular weight of 18 KDa. With the addition of the associated sugars, the apparent molecular weight is 34KDa. The sugars increase the molecule’s stability in the circulation, but not its metabolic activity(382). The carbohydrate chains on the commercially available forms of erythropoietin differ one from the other in patterns of glycosylation, the differences do not affect biological activity(383).

Study of levels of Glycosylated hemoglobin in iron deficiency anaemia suggested that iron metabolism also influences glycosylation of HbAl. Therefore, iron deficiency must be corrected before making any diagnostic or therapeutic decisions based on Glycosylated Hemoglobin levels(384,385,386,387,388). The mechanism leading to increased glycosylated HbAl levels in iron deficiency is not clear. It may be that in iron deficiency the quartenary structure of the hemoglobin molecule is altered, and that glycosylation of the beta-globin chains occur more readily in the relative absence of iron.
Coban et al. reported that before iron treatment the mean Glycosylated Hemoglobin level in patients with IDA was higher than in a healthy group. In patients with IDA Glycosylated Hemoglobin decreased significantly after iron treatment(385).

The high levels of Glycosylated Hemoglobin reported may be due to post-translational modifications of hemoglobin other than glycosylation in iron deficiency, the modified hemoglobin co-eluting with HbA1. It was concluded that when affinity chromatography is used iron deficiency anemia does not produce high glycosylated hemoglobin values(389).

G Vijayan et al. have shown the evidence that concentrations of nonenzymatically glycosylated protein are increased in many non diabetic pathological states(390). Elevated concentrations of Glycosylated Hemoglobin have been found in myocardial infarction, chronic renal failure and nephrotic syndrome patients with normal blood glucose concentrations. Similarly high concentrations of fructosamine are reported in non diabetic chronic renal failure and rheumatic arthritis patients. Increased levels of Glycosylated Hemoglobin have also been documented in iron deficiency anemia patients without any history of Diabetes.

Hiroyuki yamagishi et al. reported reduced hemoglobin and hematocrit values in Iron deficiency rats(391). Serum triacylglycerol and glucose in ID rats were elevated significantly. In addition,
Glycosylated serum proteins and fructosamine were also significantly higher in Iron deficiency rats. Relationships between fructosamine and hemoglobin, hematocrit and relative epididymal adipose weight in all rats displayed significant correlation.

Iron–deficiency anemia increases Glycosylated Hemoglobin because of an **increased percentage of old erythrocytes** (392).

According to some investigators, the increase in Glycosylated Hemoglobin in non–diabetic anemic patients is mainly attributed to the decrease in hemoglobin levels in these patients (393).

K. Beena Rai et al. observed that extent of glucose incorporation was independent of hemoglobin concentration and that non-enzymatic glycosylation of hemoglobin does not decrease with diminution of molar concentration of hemoglobin both in vitro and *in vivo* (394). They concluded that anemia with normal erythrocyte life span will not lead to reduction in glycosylated hemoglobin levels and iron deficiency anemia will not minimize the utility of glycosylated hemoglobin in monitoring hyperglycemia.

Kazumi NOSTU et al. measured HbA1 and Glycosylated Hemoglobin in cases with iron deficiency anemia (I.D.A) (395). HbA1 in cases with IDA was significantly higher than that in control, however, in Glycosylated Hemoglobin components, there was no difference between anemic patients and controls. From these results, they suspected that other components excluding the Glycosylated
Hemoglobin in HbA1 fraction, such as HB A1 + b and HbF and so on might relatively increase in patients with IDA.

**Results and Discussion**

The major form of Glycosylated Hemoglobin, HbA1c fraction is abnormally elevated in chronic hyperglycemic diabetic patients and this correlates positively with glycemic control. Literature review suggests that apart from hyperglycemia, iron metabolism also influences glycosylation of hemoglobin. The aim of the present study was to determine the effect of iron deficiency anemia on Glycosylated Hemoglobin level. The study was carried out in forty patients of iron deficiency anemia with mean age of $38.39 \pm 15.13$ years. Patients who suffered from glucose tolerance abnormalities (impaired glucose tolerance or Diabetes mellitus), chronic renal failure, hemolytic anemia, hemoglobinopathies and chronic alcohol ingestion were excluded from the study. Glycosylated Hemoglobin, random blood glucose, hemoglobin, hematocrit and red blood cell indices were measured in all subjects for comparison with control.
Table No.I and II summarises the results of iron deficiency anemia.

Table No. I depict the results of hemoglobin, hematocrit and red blood cell indices in iron deficiency anemia patients.

In IDA, the levels of Hemoglobin and Hematocrit were significantly decreased as compared to the control (Table I) which is similar to the findings of Hiroyuki et al. (391). The levels of mean cell volume (MCV) and mean cell Hemoglobin concentration (MCHC) were significantly reduced as compared to the control (Table I) confirming the findings of Brooks et al. (384).

The decreased levels of Hemoglobin, Hematocrit, Mean cell volume, Mean cell hemoglobin and Mean cell hemoglobin concentration in the subjects were suggestive of iron deficiency anemia (IDA).

Table No.II shows the results of glycemic indices in iron deficiency anemia patients. The mean values of Glycosylated Hemoglobin in the subjects were significantly higher as compared to the control (Table II). This increased may either be due to iron metabolism influences glycosylation of hemoglobin (384-388) or increased percentage of old erythrocytes in circulation (392).

There was no significant difference in the levels of random plasma glucose in iron deficiency anemia patients as compared to the control (Table II). The increased levels of Glycosylated Hemoglobin
have also been documented in iron deficiency anemia patients without any history of Diabetes and with normal glucose concentrations(390). This suggests that with normal glucose concentrations in iron deficiency anemia patients and control group, iron deficiency anemia is associated with higher concentrations of Glycosylated Hemoglobin.

The present results suggest that iron status of the patient must be considered during the interpretation of Glycosylated Hemoglobin concentrations in Diabetes mellitus.

**VARIOUS PARAMETERS IN IRON DEFICIENCY ANAEMIA**

**TABLE – I**

Hemoglobin, Hematocrit and Red Blood cell indices

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IDA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>8.29±2.6**</td>
<td>13.06±1.00</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>24.8±5.6*</td>
<td>49.0±6.30</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>74.0±13.8**</td>
<td>90.2±4.8</td>
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<tr>
<td>Mean Cell Hemoglobin (pg)</td>
<td>22.26±4.5*</td>
<td>29.52±4.5</td>
</tr>
<tr>
<td>Mean Cell Hemoglobin Concentration (gm/dl)</td>
<td>29.30±2.12*</td>
<td>33.48±1.52</td>
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</table>

**TABLE – II**

Random Sugar and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IDA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random plasma Glucose (mg/dl)</td>
<td>115.41±79.01</td>
<td>106.93±19.15</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>5.5±1.0*</td>
<td>4.6±0.82</td>
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</tbody>
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All values are mean with ±standard deviation * P < 0.01 **P < 0.05