Original Research Article

Study of Glycosylated Hemoglobin in Iron Deficiency Anemia

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Abstract: The objective of the present study was to determine whether the Glycosylated hemoglobin (HbA1c) levels were increased in some non-diabetic pathological states, like Iron Deficiency Anemia (IDA). 70 non-diabetic, IDA patients and 50 age matched healthy subjects were enrolled in the study. The HbA1c levels were significantly increased among the IDA patients as compared to those in the controls. The mean HbA1c % (6.5 ± 0.42) level in the patients with IDA was higher than that in the control group (5.1 ± 0.13) ($p < 0.001$). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$). Therefore the iron deficiency had to be corrected before any diagnostic or therapeutic decision was made based on the HbA1c level.

Keywords: Glycosylated Hemoglobin, HbA1c, Iron Deficiency Anemia, Diabetes Mellitus.

INTRODUCTION

Iron deficiency is the most common cause of anemia worldwide. Inadequate iron supply for erythropoiesis results in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin [1]. Deficiency of iron, most commonly due to insufficient intake, is prevalent in periods of rapid body growth during infancy and again at puberty.

The definition of iron deficiency was an abnormal value for at least two of the following three indicators: serum ferritin, transferring saturation, and free erythrocyte protoporphyrin [2]. Iron deficiency along with low hemoglobin value leads to the development of iron deficiency anemia (IDA) the most severe stage of iron depletion [3]. IDA reduces the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to national development.

Iron metabolism is controlled by absorption rather than excretion [4]. Iron absorption is only 5 to 10 percent of dietary intake. Absorption can increase three- to fivefold in states of depletion. Dietary iron is available in two forms: heme iron (found in meat and minimally affected by dietary factors) and nonheme iron (found in plant foods) [5]. The bioavailability [6] of nonheme iron requires acid digestion and varies by an order of magnitude depending on the concentration of enhancers (e.g., ascorbate, meat) and inhibitors (e.g., calcium, fiber, tea, coffee, wine) found in the diet [7, 8, 9, 10,11,12,13].

During the initial stages of IDA the clinical manifestations can be so mild that it goes unnoticed. But as the body becomes more deficient in iron and anemia worsens, the signs and symptoms intensify resulting in irreversible delayed psychomotor development. The optimal approach is prevention and early treatment [14].

Glycated hemoglobin A1c (HbA1c) is a major part of HbA1 and comprises approximately 5% of the total hemoglobin in non-diabetic individuals. It provides a better estimate of average glycemia than routine determinations of blood glucose concentration, and is the most widely used index of chronic glycemia [15, 16].

The mechanism of glycation (Fig.1) involves the nonenzymatic binding of glucose to the N-terminal valine and internal lysine amino groups of hemoglobin [17]. The glycation reaction is mostly irreversible, so that the concentration of HbA1c is a function of the concentration of glucose to which the erythrocytes are exposed over their lifespan (120days on average). HbA1c therefore represents a marker of average blood glucose concentration over the previous 2 to 3months [18].
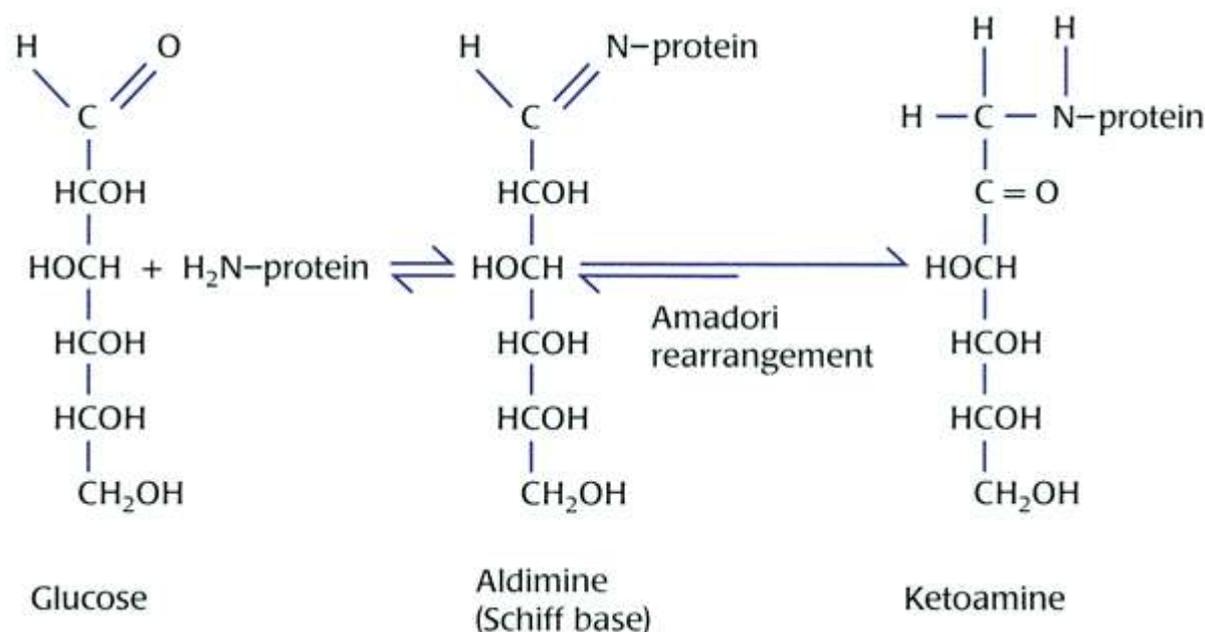
Fig.1. Glucose and protein initially form a labile glycosylamine or Schiff Base, which undergoes an irreversible Amadori rearrangement to produce a more stable ketamine.

Scientific literature has documented that HbA1c levels increases in some non-diabetic pathological states, like IDA. Some authors have reported a significant decrease in the levels of HbA1c after therapy including iron supplementation [19]. Evidence has accumulated, which supports the hypothesis that the glycation reaction, apart from the traditional chronic hyperglycaemia, can be modulated by the iron status of the patient. Such glycation reactions (as observed in the formation of glycated haemoglobin) may also occur in several other proteins in anemic patients that could have important clinical implications.

Thus, the objective of the present study was to determine whether the HbA1c levels were increased among the patients suffering from iron deficiency anemia without diabetes. If such phenomenon exists, the iron deficiency had to be corrected before taking any significant diagnostic decision based on the increased HbA1c levels.

METHODOLOGY

The present study was designed as a case control study. This study has been conducted on 70 clinically established cases of Iron Deficiency Anemia of both sex and varying age groups attending the outpatient clinics in the Department of Medicine associated with Dr. S. N. Medical College and its associated group of hospitals, Jodhpur. 50 age matched healthy subjects of either sex were included as control group and the results compared.

To find out the influence of body iron stores on various biochemical parameters the subjects underwent the following investigations: blood haemoglobin (Hb) concentration [20], total red blood cell count, serum iron and TIBC [21], percent saturation, fasting & postprandial blood glucose [22] and glycosylated hemoglobin [23].

The patients with impaired glucose tolerance, diabetes mellitus, chronic renal failure, haemoglobinopathies, haemolytic anaemia and chronic alcohol ingestion were excluded from the study. All the results were presented as mean ± S.D. Significant differences were evaluated using Student's t-test when $P \leq 0.05$.

RESULTS

The results of the present study are shown in Table No.1. The fasting and the postprandial blood glucose levels confirmed the nondiabetic status. In the present study hemoglobin concentration in healthy controls and IDA subjects is 12.81+0.66 gm/dl and 9.38+1.74 gm/dl respectively. Statistical evaluation reveals highly significant difference (p<0.0001).

Total red blood cell counts and iron levels were found to be decreased in IDA subjects as compared to the controls showing a significant difference (p<0.001). TIBC is significantly higher (p<0.001) in IDA subjects in comparison to healthy controls.

The percent saturation of transferring was significantly low (p<0.001) in IDA subjects in comparison to healthy control subjects. The HbA1c levels were significantly increased among the IDA patients as compared to those in the controls. The mean HbA1c % (6.5 ± 0.42) level in the patients with IDA was higher than that in the control group (5.1 ± 0.13) (p < 0.001). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups (p > 0.05).

DISCUSSION

Iron deficiency is the most common and widespread nutritional disorder in the world. It continues to be the top ranking cause of anemia [24]. IDA is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic) [25].

The earliest study to investigate the effects of iron deficiency anemia on HbA1c levels was conducted by Brooks *et al.;* who assessed HbA1 levels in 35 non-diabetic patients having iron deficiency anemia both before and after treatment with iron. They observed that HbA1 levels were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. The mechanisms leading to increased glycated HbA1 levels were not clear. It was proposed that, in iron deficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycation of the globin chain occurred more readily in the relative absence of iron [26]. Sluiter *et al.;* tried to provide an explanation for the above findings. They proposed that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1 in the erythrocyte will increase linearly with the cell's age. For example, they found that in patients with normal blood glucose levels, but with very young red cells, as would be found after treatment of iron deficiency anemia, HbA1 concentration was reduced. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, leading not only to anemia but also to a higher-than-normal average age of circulating erythrocytes and, therefore, increased HbA1 levels [27].

Further research by various group of scientists concluded that if serum glucose remains constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction [28, 29]. These arguments were provided to explain the fact why HbA1c levels were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron.

The above explanations are merely hypotheses, and further studies are needed to confirm and elucidate the exact mechanisms underlying this phenomenon.

CONCLUSION

Iron deficiency is the most prevalent nutritional deficiency and the most common cause of anemia in India. Previous studies have showed effects of iron therapy on glycated hemoglobin and found a significant reduction in HbA1c levels after iron therapy in non-diabetic population [29].In this study, the results shown in Table No.1 has associated iron deficiency anemia with higher levels of HbA1c. Therefore problems could occur during the diagnosis of Diabetes Mellitus in the patients suffering from Iron Deficiency Anemia. The iron status must be considered during the interpretation / diagnosis of Diabetes mellitus based on the concentration of HbA1c. The iron replacement therapy is thus especially important in diabetic patients with iron deficiency, as it would also increase the reliability of the HbA1c determinations.

Table -1: Comparison of various biochemical parameters between the control group and the IDA subjects

S.No.	Parameters	Normal subjects (n=50) Mean + SD	IDA subjects (n=70) Mean + SD	Significance
1.	Blood hemoglobin (gm/dl)	12.81+0.66	9.38+1.74	p < 0.001
2.	TRBC (million/cumm)	5.13+0.30	4.10+0.47	p < 0.001
3.	Serum Iron (mg/dl)	0.09+0.02	0.05+0.02	p < 0.001
4.	Serum TIBC (mg/dl)	0.34+0.04	0.48+0.03	p < 0.01
5.	Percent saturation (%)	26.61+4.48	11.01+4.01	p < 0.001
6.	Fasting Blood Glucose (mg/dl)	81.35+7.23	82.73+5.25	p > 0.05
7.	Postprandial Blood Glucose (mg/dl)	119.23+8.26	117.11+7.74	p > 0.05
8.	Glycosylated Hb (%)	5.1+0.13	6.5+0.42	p < 0.0001

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