

Effects of iron deficiency anemia on the level of glycosylated hemoglobin (HbA1c) in non-diabetic patients**Manal Elhabbash¹, Zynab Elfurjani²**¹Oncology department -Tripoli medical center Tripoli /Libya Email : elhabbashm@yahoo.com² Medical technologist in Al Jmail hospital student in Libyan Academy Tripoli /Libya

Abstract: *The major form of glycohemoglobin is hemoglobin A1c (HbA1c). The Hb A1c fraction is elevated in chronic hyperglycemic diabetic patients and correlates positively with glycemic control. Previous studies suggest that iron deficiency anemia (IDA) affects the level of HbA1c.*

Aim of study: To determine the effect of IDA on HbA1c level in non- diabetic patients before and after treatment with iron.

Material and methods: The study included 100 females from Al jmail General Hospital at the west of Tripoli, 50 were with IDA and 50 were not anemic (control group). Age ranged from (20-40) years. Fasting blood glucose, HbA1c , complete blood count , serum iron, total iron binding capacity and serum Ferritin were measured in all females before treatment and after treatment. Patients who had glucose tolerance abnormality (impaired glucose tolerance or diabetes mellitus), hemoglobinopathy ,hemolytic anemia , chronic alcohol ingestion , chronic renal disease , chronic liver disease and rheumatoid arthritis were excluded from study. All patients with IDA were treated with ferrous sulfate as 325mg orally three times a day for 3 months.

Results: The mean percentage of the HbA1c in non anemic patients control group was 5.2% . The mean percentage of HbA1c in patients with mild anemia was (6%) , moderate anemia (5.9 %) . In patients with severe anemia HbA1c was (3.2%) which is significantly low in comparison to normal females. After treatment with iron , significant increase in HbA1c was observed in patients with severe anemia (5.2%) $p < 0.001$ and significant decrease in HbA1c in female with mild and moderate anemia 5.2%,5.3% respectively $P < 0.001$

Conclusion: In patient with iron deficiency anemia , hemoglobin A1c in not reliable parameter for follow up of patients with diabetes mellitus.

Key word: Hemoglobin Hb, glycosylated hemoglobin HbA1c , iron deficiency anemia IDA

INTRODUCTION

Glycosylated hemoglobin (HbA1c) is used as gold standard for monitoring glycemic control and as a predictor of diabetic complications. Conditions that affect erythrocyte turnover affect HbA1c concentration[1]. Anemia is characterized by a decrease in number of red blood cells and hemoglobin levels less than 13g/dl in males and less than 12 g/dl in females [2]. Anemia may results from many causes. It can be acquired or congenital due to hemoglobinopathies. Currently World Health Organization (WHO) accept that 50% of all anemia attributed to iron deficiency [3,4].

Iron is a major component of hemoglobin that carries oxygen to all parts of the body.It plays a role in overall cell function assisting in oxygen utilization, enzymatic system and neural development. Thus, all body function are affected by iron deficiency in general and not only by anemia which appears late in the process of tissue iron deficits [4,5].

In our food two basic iron exist as heme iron which constitute 10% of total dietary iron and non heme iron accounts 90% of our supplement. Most well-nourished adults in industrialized countries contains 3-5 grams of iron in their meals. Iron is mainly absorbed in the duodenum and upper jejunum. Divalent metal transporter 1 DMT1 facilitates transfer of iron across the intestinal cells then iron bound by transport glycoprotein called Transferrin [6]. Normally, about 20-45% of Transferrin binding site are filled with iron, only 0.1% of total body iron is circulating in bound form to Transferrin. About 10-20% of absorbed iron is stored in storage pools in cells of mononuclear phagocyte system particularly macrophage in form of Ferritin [7]. 65% in form of hemoglobin, the remaining iron in the body are in form of myoglobin and other heme compounds that promote intracellular oxidation [5].

Iron deficiency anemia (IDA) is caused by:

1. Dietary: low intake of meat or iron fortified products.
2. Mal absorption of iron: due to gastrointestinal cause
3. pregnancy and menstruation: due to increase demand.
4. blood loss [5,8,9]

Glycated (glycosylated) Hemoglobin is a form of hemoglobin used to identify the average plasma glucose concentration over a prolonged period of time [10]

In 1958, hemoglobin A1c was separated from other form of hemoglobin using a chromate-graphic column (Huisman and Metering).

Ten years later, HbA1c was characterized as a glycoprotein (non-enzymatic attachment of glucose to protein) by (Bookchin and Gallop). Blood level of HbA1c is found to be elevated in diabetics and seven years later formally recommend its use to monitor the control of blood glucose in diabetic patients (Ralibar and coworker) [11].

During the normal life span of red blood cells , glucose molecules react with hemoglobin forming glycated hemoglobin. In normal non diabetic people ,about 4-6% of their hemoglobin is glycosylated. Glycated hemoglobin is produced by a ketoamine reaction between glucose and the N-terminal valine of both β chains of hemoglobin.

The glycation of hemoglobin occur over the entire 120 day life span of erythrocyte [12,13]. HbA1c is used as a key for monitoring glycemic control in people with diabetes and more recently it has been proposed as the preferred test for the diagnosis of diabetes mellitus. It does not need a fasting state for sample collection and can be measured in a single sample collected at any time of the day[14,15,16].

MATERIAL AND METHODS:

The study was prospective randomized study which included 100 non diabetic females, 50 were anemic female and 50 were not anemic female as control group. The study was done in the period between July to November 2016 and included the females who visit the outpatient clinic at Jmail General Hospital. The collected data were included personal data, drug history, nutritional habits, history of blood loss, and previous illnesses. Informed medical consents were taken from all participant, and consent from medical ethics committee in Al Jmail Hospital was also taken. After that blood samples were taken for Complete blood count CBC, fasting blood sugar FBS , glycoslayted Hemoglobin (HbA1c), serum Iron , Total iron binding capacity (TIBC) and serum Ferritin.

Normal values for Serum iron in female, Normal TIBC, Serum Ferritin were 37-145 μ g/dl, 250-400 μ g /dl, 20-250ng /dl respectively (5). Ferrous sulfate was given as treatment for anemic patients one tablet 325mg three times a day for three months. CBC, Serum Ferritin and HbA1c were repeated after treatment course .

exclusion criteria: patients who had impaired glucose tolerance or diabetes mellitus

hemoglobinopathy, hemolytic anemia, chronic alcohol ingestion, chronic liver disease, chronic renal failure and rheumatoid arthritis were excluded from study.

STATISTICAL ANALYSIS:

Statistical analysis was done using the SPSS software package version 20.0 to obtain the mean and standard deviation. For comparison between the different groups involved in this study ONE WAY test and student t-test was used.

RESULTS:

This study was carried out on 100 female divided into two groups, 50 females with iron deficiency anemia and 50 female were not anemic. The age range between 20-40 years. According to classification of anemia[5], the mean Hb concentration was 10.3g/dl in 25% of females which classified as mild anemia, 8.9g/dl (moderate anemia) in 15% of females and 5.8g/dl (severe anemia) in 10% of females. The mean hemoglobin concentration in the control group was 12.8g/dl in 50% of females (P <0.001). FBS was normal in all female. The mean percentage of HbA1c with mild anemia,

moderate anemia ,severe anemia and for control group were 6.0%,5.9%,3.2%, and 5.2% respectively $P < 0.001$. Table -1 showed analysis of different variance for serum iron, TIBC and serum Ferritin of anemic and control group.

Table 1- analysis of variance for IDA and control group

Parameters	Mild anemia	Moderate anemia	Severe anemia	Control	P value
Number of cases	25	15	10	50	
Mean Hemoglobin before treatment	10.3g/dl	8.9g/dl	5.8g/dl	12.8g/dl	0.000
Serum iron 37-145µg/dl	32.9	30.6	30.1	102	0.000
TIBC 250-400µg/dl	436.7	448	471	305.6	0.000
Mean Serum Ferritin20-250ng/dl	15.2	14.2	11.2	147.7	0.000
Mean Hemogloin after treatment	15.2g/dl	14.2g/dl	11.2g/dl		0.001
Mean HbA1c before treatment	6.0%	5.9%	3.2%		0.000
Mean HbA1c after treatment	5.2%	5.3%	5.2%		
	$P < 0.000$	$P < 0.000$	$P < 0.000$		

Patients with iron deficiency anemia were given ferrous sulfate as 325mg three times a day. Comparison before and after ferrous sulfate treatment was done using the two sample. t–test which showed a highly significant increase in Hb levels after treatment among mild, moderately and severe iron deficiency ($P < 0.001$). There was a significant increase in the mean concentration of HbA1c in severely anemic patients from 3.2% before treatment to 5.2% after treatment ($p < 0.001$). There was significant decrease in HbA1c level in patients with mild anemia from 6% to 5.2% after treatment, and also significant decrease in HbA1c in patients with moderate IDA from 5.9% to 5.3% $p < 0.001$.

DISCUSSION:

The Hb levels of iron deficiency anemia patients without diabetes before treatment were low compared to normal ranges of Hb for healthy adult female. Mean Hemoglobin concentration was 10.3g/dl in mildly anemic patients , 8.9g/dl for moderately anemic patients, 5.8g/dl for severely anemic patients and 12.8g/dl in non- anemic female. The statistical analysis showed the mean percentage of HbA1c in iron deficiency anemia without diabetes before treatment with iron tab were 6% in mildly anemic patients, 5.9% in moderately anemic patients, and 3.2% in severely anemic patients. In control non anemic group, the mean HbA1c concentration was 5.2%. Comparison before and after ferrous sulfate showed a highly significant increase in

Hemoglobin levels after treatment. There was a significant decrease of HbA1c concentration from 6% before treatment to 5.2% after treatment in mildly anemic patients and from 5.9% before treatment to 5.3% after treatment in moderately anemic patients. On the other hand, HbA1c concentration was significantly increased from 3.2% before treatment to 5.2% after treatment in severely anemic patients.

According to World Health Organization (WHO) iron deficiency anemia is the most common type of anemia worldwide [17].

The concentration of HbA1c in one erythrocyte will increase linearly with cell's age, however, if iron deficiency anemia persisted for long time , the red blood cells production would fall leading to higher than normal average age of circulating erythrocyte and therefore increase in HbA1c concentration[18]. The non-enzymatic glycation of protein has pronounced effects on the structure and the function of protein.

The two known factors which modulate the glycation are the prevailing concentration of glucose and the half life of the protein [19].

Evidence in the literature have documented increase in glycated protein level in some non diabetic iron deficiency patients , some authors have found that on supplementation with iron therapy, there was a significant decrease in the level of glycated hemoglobin [20].

In one study, in non-diabetic iron deficiency anemia patients, a fall in HbA1c from 7.2% pretreated IDA to 6.2% after 3 months of ferrous tab treatment, these patients were followed for another 20 weeks

and HbA1c fell steadily over that period from 6.2% to 5.25% ($p < 0.01$) [21,22].

In other studies, in IDA, values of HbA1c were elevated before iron treatment and decreased after treatment [23]. It was proposed that in IDA, the quaternary structure of the hemoglobin molecules was altered and the glycation of the globin chain occurred more readily in relative absence of iron [24]. Also it was observed if IDA has persisted for long time the red cell production rate would fall, leading to higher than normal average age of circulating erythrocyte and therefore increased HbA1c levels [25].

Interestingly, another study showed converse patterns of results in patients with moderate to severe IDA, the mean HbA1c was significantly low in anemic group 4.6% compared to non-anemic group 5.5%, after two months of treatment for IDA, the HbA1c were significantly higher than the control [26]. Another study showed, in 40 patients with IDA, HbA1c were found to be significantly lower in comparison with control [27,28].

In other studies, there were no difference between HbA1c levels of anemia patients and control [27].

CONCLUSION:

In patient with iron deficiency anemia, hemoglobin A1c is not a reliable parameter for follow up of patients with diabetes mellitus.

Further researches should be done for HbA1c level in IDA which will include sufficient participants to differentiate between the effects of type and severity of erythrocyte abnormalities and anemia on HbA1c values.

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