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Effect of treatment of iron deficiency anemia on hemoglobin A1c in type 2 diabetic patients

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Background/aim: Iron deficiency anemia (IDA) affects hemoglobin A1c (HbA1c) levels. This study aimed to evaluate the effect of treatment of iron deficiency anemia on hemoglobin A1c in type 2 diabetic patients.

Materials and methods: Ninety type 2 diabetes mellitus (T2DM) patients with IDA were included in a randomized, placebo-controlled, single-blind clinical trial. The intervention group (n = 45) received 200 mg/day oral iron for 3 months and the control group (n = 45) received an oral placebo for the same period. Fasting blood sugar, complete blood count, and HbA1c were measured for all subjects at the beginning and the end of the trial.

Results: The mean age of the treatment and control group was 51.47 ± 1.05 and 52 ± 1.1 years, respectively. The two groups were not statistically significantly different with regard to diabetes duration (P = 0.436) and age (P = 0.617). Hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, serum iron, ferritin, total iron-binding capacity, and HbA1c were significantly improved in the intervention group in comparison with the control group (P = 0.005).

Conclusion: Iron status should be considered during the interpretation of the HbA1c concentrations in diabetes mellitus. Iron replacement therapy can decrease HbA1c in anemic patients with IDA and T2DM.

Key words: Type 2 diabetes mellitus, hemoglobin A1c, iron deficiency anemia

1. Introduction

Anemia is a common problem in the world. Approximately one-third of patients with anemia have iron deficiency. Previous studies suggested that iron deficiency anemia (IDA) affects hemoglobin A1c (HbA1c) levels (1).

The most important factor that determines HbA1c concentration is the blood glucose level. HbA1c is the standard for monitoring long-term glycemic control and estimating complications in diabetes mellitus (2,3). When plasma glucose is consistently raised, nonenzymatic glycation of hemoglobin is increased. Erythrocytes have an average lifespan of 120 days; this alteration reflects the glycemic history over the previous 2–3 months (4).

HbA1c is increased in patients with diabetes (5). Several studies have shown that some trace elements such as zinc and chromium are involved in the management of the secretion and function of insulin (6–8).

Iron is an important cation in many metabolic actions, and it plays a key role in many physiological functions and especially in the tricarboxylic acid cycle. Rising Fe levels inhibit the metabolism of glucose and lead to increases in blood glucose (9). There have been more reports on the effects of Fe on blood glucose regulation in diabetes focused on excessive Fe supply than on lower dosages of Fe (10).

The World Health Organization has defined anemia as a hemoglobin level of <13 g/dL for men and postmenopausal women and <12 g/dL for premenopausal women, mean corpuscular volume (MCV) of <80 fL, and mean corpuscular hemoglobin (MCH) of <26 pg/cell and

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based on peripheral blood smears (mostly microcytic hypochromic) (11,12).

HbA1c level is not affected only by blood glucose levels. It is also altered in some conditions such as hemolytic anemia, hemoglobinopathies, acute and chronic blood loss, pregnancy, and uremia (13). Vitamin B_{12}, folate, and IDA have also been shown to affect HbA1c levels. Several studies suggest that IDA affects the levels of HbA1c. However, reports on the effects of IDA on HbA1c levels are inconsistent (14–16).

We conducted this study to evaluate the effects of treatment of IDA on HbA1c in type 2 diabetes mellitus (T2DM) patients.

2. Materials and methods

2.1. Study population

Ninety diabetic patients (aged 18–65 years) participated in this single-blind, randomized clinical trial study. All of the participants had confirmed IDA (Hb of <12 g/dL in women and Hb of <13 g/dL in men, or ferritin levels of <15 ng/mL for women and ferritin levels of <9 ng/mL for men). Patients with a history of erythropoietin treatment, oral iron treatment, or blood transfusions within the 1 or 2 past weeks or Hb of >9 mg/dL, hemolytic anemia, hemoglobinopathies, renal failure, heart failure, uremia, pregnancy, lactation, or antibiotic consumption of quinolone or tetracycline were excluded. Further exclusion criteria were chronic alcohol abuse, chronic liver disease, an increase of transaminase levels more than 3 times above the upper limit of normal, portal hypertension with esophageal varices, known hypersensitivity to ferrous sulfate, history of acquired iron overload, myelodysplastic syndrome, known active infection, clinically significant overt bleeding, active malignancy, surgery with relevant blood loss (Hb decrease of >2 mg/dL) within 3 months before screening or planned surgery within the following 3 months, known infection with human immunodeficiency virus or hepatitis B or C, and significant cardiovascular disease. All patients were screened to rule out these conditions.

Subjects were assigned randomly using a 1:1 randomization ratio to the intervention and placebo groups. All patients in the intervention group were treated with oral ferrous sulfate at 200 mg/day for 3 months and patients in the control group were treated with an oral placebo (dried breadcrumbs) at 200 mg/day for 3 months.

2.2. Blood measurements

All of the laboratory investigations were performed before treatment with the iron or placebo and 3 months after that. The blood specimens were drawn after overnight fasting. A Sysmex automated hematology analyzer (Japan) was used for the whole blood counts (hemoglobin (Hb), haematocrit (Hct), MCV, and MCH). Serum ferritin levels were measured using a Hyperion ELISA reader (Germany), while fasting blood sugar (FBS), total iron-binding capacity (TIBC), and serum iron (SI) were measured using the BioLife 24i Premium (Boeki Medical System, Japan). HbA1c levels were determined by high-performance liquid chromatography with a TOSOH-G8 (Japan). Peripheral blood smears were examined for all patients.

2.3. Statistics

Data are expressed as mean ± SD for continuous variables and frequency and percent for categorical variables. Independent t-tests and chi-square tests were used to compare baseline characteristics. We used repeated measure ANOVA to compare changes of responses between the study groups. $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS 21 for Windows (IBM Corp., Armonk, NY, USA).

2.4. Ethics

This study was approved by the Ethics Committee of Tehran University of Medical Sciences, and written consent was obtained from all of the patients after describing the study in detail.

3. Result

A total of 45 people were assigned to each group (intervention and control); mean age was 51.47 ± 1.05 years in the intervention group and 52 ± 1.1 years in the control group. The two groups were not statistically significant with regards to diabetes duration ($P = 0.436$) or age ($P = 0.617$).

Thirty percent of participants were male and 70% were female; the sex ratio was not statistically significant between the two groups ($P = 0.490$).

Baseline characteristics of subjects are shown in Table 1. Descriptive statistics of all the parameters that were tested for both groups are reported in Table 2.

At the start of the study, the two groups were similar in all indices ($P > 0.05$) except in red blood cell (RBC) count, which was significantly greater in the intervention group than the control group (4.70 ± 0.55 vs 4.32 ± 0.42 with $P < 0.001$). After treatment there were no significant differences between the MCV, TIBC, FBS, and HbA1c values of the two groups ($P > 0.05$ for mean comparison of the two groups). In repeated measure ANOVA, time as a within-subject effect was significant for all indices, and the change of index between two time points was not the same among the two groups ($P < 0.001$, based on time × group interaction) for all indices with $P = 0.005$ for HbA1c, but for RBC count and FBS the changes during the study period were not statistically significant. As is obvious from the Figure, the mean reduction of HbA1c from the beginning to the end of the study was greater among intervention group than the controls (Table 2).

Table 1. Baseline characteristics of patients.

		Control group (n = 45)	Intervention group (n = 45)	P-value
Sex	Male	15 (33.3%)	12 (26.7%)	0.490
	Female	30 (66.7%)	33 (73.3%)	
Age		51.47 ± 7.07	52 ± 7.35	0.617
Diabetes duration		10.77 ± 5.69	9.71 ± 5.28	0.436

Table 2. Comparison of anemia indices and glycemic control between intervention and control groups.

Variable	Test point	Group		P*	Factor	F (P)
		Intervention (mean ± SD)	Control (mean ± SD)			
RBC Count	Pre	4.70 ± 0.55	4.32 ± 0.42	<0.001	Group	19.24 (<0.001)
	Post	4.86 ± 0.41	4.41 ± 0.52	<0.001	Time	13.11 (<0.001)
					Time × group	1.11 (0.269)
Hb	Pre	11.52 ± 0.86	11.3 ± 0.73	0.287	Group	31.04 (<0.001)
	Post	13.71 ± 1.37	11.6 ± 1.24	<0.001	Time	0.014 (<0.001)
					Time × group	88.79 (<0.001)
MCV	Pre	79.94 ± 6.04	82.08 ± 6.7	0.115	Group	0.041 (0.84)
	Post	84.62 ± 4.37	82.99 ± 7.34	0.203	Time	47.75 (<0.001)
					Time × group	21.69 (<0.001)
MCH	Pre	26.98 ± 3.27	27.65 ± 3.02	0.316	Group	0.69 (0.405)
	Post	28.66 ± 2.66	26.98 ± 3.23	0.009	Time	4.74 (0.032)
					Time × group	25.70 (<0.001)
MCHC	Pre	31.84 ± 1.9	31.81 ± 1.73	0.942	Group	4.14 (0.045)
	Post	33.38 ± 1.41	32.04 ± 1.98	<0.001	Time	29.05 (<0.001)
					Time × group	15.76 (<0.001)
Serum Iron	Pre	45.46 ± 29.77	47.35 ± 36.74	0.789	Group	2.76 (0.100)
	Post	73.26 ± 34.27	48.56 ± 34.28	0.001	Time	55.61 (<0.001)
					Time × group	46.69 (<0.001)
Ferritin	Pre	32.33 ± 39.23	39.37 ± 42.46	0.417	Group	0.82 (0.367)
	Post	64.10 ± 56.82	40.56 ± 38.87	0.024	Time	38.27 (<0.001)
					Time × group	32.89 (<0.001)
TIBC	Pre	227.59 ± 116.27	238.37 ± 108.01	0.650	Group	0.300 (0.583)
	Post	280.47 ± 85.30	245.94 ± 111.79	0.103	Time	25.56 (<0.001)
					Time × group	14.19 (<0.001)
FBS	Pre	146.95 ± 42.69	152.62 ± 38.68	0.511	Group	1.63 (0.204)
	Post	131.76 ± 41.75	145.60 ± 33.35	0.086	Time	11.88 (0.001)
					Time × group	1.61 (0.208)
Hb A1C	Pre	7.59 ± 1.16	7.40 ± 1.01	0.419	Group	0.18 (0.676)
	Post	6.80 ± 0.85	7.14 ± 0.95	0.740	Time	32.39 (<0.001)
					Time × group	8.44 (0.005)

*P-value for independent t-test.

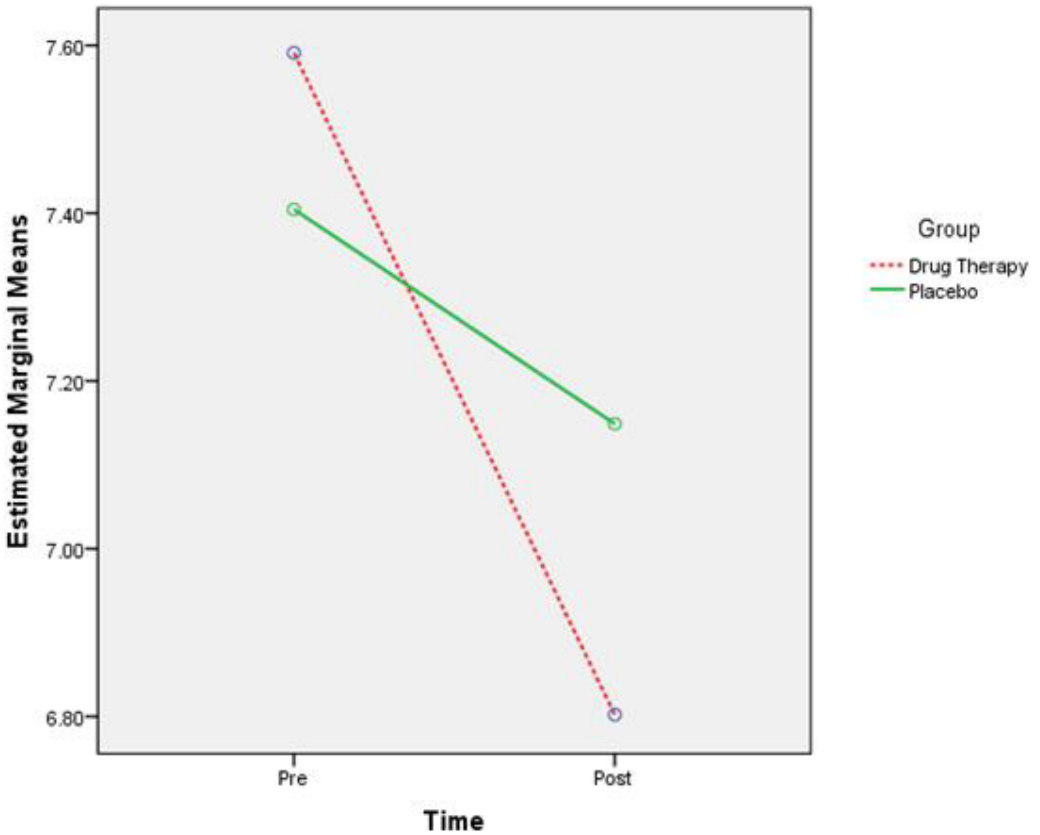

Figure. Mean change of HbA1c in intervention and control groups.

4. Discussion

The first study to investigate the effects of IDA on HbA1c levels was by Horton and Huisman (15), who demonstrated the mean HbA1c concentration for four patients with IDA as 4.9% compared with a mean HbA1c concentration of 5.3% among 14 healthy people.

Several researchers suggested a potential role of Fe in the pathogenesis of T2DM, obesity, and metabolic syndrome. Fe is capable of generating reactive oxygen species and is elevated in oxidative stress (17,18).

In animal models, its excess might result in pancreatic oxidative stress and elevated insulin secretary (19). In the presence of hyperglycemia, Fe may contribute to the development and progression of oxidative injury and may also negatively impact glucose control. However, iron indices are strongly correlated with Hb, which represents an important risk factor for morbidity and mortality in patients with diabetes.

IDA is one the most common anemias among the nutritional anemias in Iran. Brooks et al. (20), Gram-Hansen et al. (21), and Coban et al. (22) previously demonstrated the effects of treatment of IDA in patients with T2DM and found a significant reduction in HbA1c levels after iron therapy.

Anemia is caused by decreased or faulty RBC production, and the consequently increased mean age of circulating RBCs leads to elevated HbA1c levels (23).

Comparing several studies, the different changes in HbA1c levels were due to different laboratory methods, control of blood glucose, pregnancy, metabolic status and chronic kidney disease, or very little positive correlation with age (14,24–26).

Hashimoto et al. found that HbA1c levels were significantly raised in the third trimester, but serum glycated albumin did not change; HbA1c was not correlated with serum ferritin, and it was suggested that HbA1c was affected by iron stores. Furthermore, iron therapy was associated with decreases in HbA1c, independent of changes in blood glucose (27,28).

Arredondo et al. explained a similar relation among iron indices in diabetic patients or patients with metabolic syndrome. However, increasing iron store is not necessarily a usual factor for diabetes. Their results supported the idea that patients with increased iron stores have a higher predisposition to develop noninsulin-dependent diabetes (29).

Rafat et al. showed that Hb concentrations were positively correlated with HbA1c concentrations, whereby

HbA1c concentrations tended to be increased in the presence of iron deficiency. The positive correlation between HbA1c concentrations and Hb may have offset the small inverse association between HbA1c concentrations and iron status. A potential caveat lies in the inclusion of serum ferritin as a component in our diagnosis of iron deficiency, because ferritin has been recognized as an inflammatory marker associated with insulin resistance (24). In contrast, Saudek et al. explained measurements of HbA1c to be invalid in the presence of anemia (30).

IDA not only increases HbA1c levels in nondiabetic patients; it can also show the glycemic status in patients with T2DM. Our results demonstrate that there is a relation between iron therapy and HbA1c in patients with T2DM. This is in agreement with the studies of Brooks et al. and Davis et al. (20,31). They defined a relationship between IDA and HbA1c levels and tried to explain the alteration in HbA1c levels in IDA on the basis of both alterations in the form of hemoglobin and levels of HbA1c in old and new RBCs.

Koga et al. showed that erythrocyte indices with glycated hemoglobin in premenopausal women revealed RBC count to be positively and Hb, MCH, and MCV to be negatively associated with HbA1c, but none of them showed any associations with HbA1c in postmenopausal women (32).

Our findings show that anemia might play a role in rising HbA1c levels in the presence of T2DM; consequently, care should be taken before making any change to the treatment regimen. Our observations also demonstrate that, in repeated measure ANOVA, HbA1c levels were significantly higher for anemic patients who had uncontrolled FBS. As a result, anemia may exaggerate the picture of glycemic status in this group of patients.

Serum ferritin is a marker of iron status and an acute phase reactant; it reflects body iron stores in healthy people. The Third National Health and Nutrition Examination Survey reported that serum ferritin was associated with abdominal obesity and insulin resistance (33). One American study provided evidence that obese patients with diabetes have significantly higher ferritin levels than obese patients without diabetes (34).

HbA1c is not affected by blood glucose levels alone and there are different factors when it is measured, especially in terms of iron deficiency status. In IDA the ferritin level is reduced and the red cell lifespan is increased (22), both of them changing HbA1c levels. Two prospective studies reported that ferritin concentrations were independently related to the development of T2DM and another study showed that elevated ferritin levels in T2DM are mainly a result of an inflammatory mechanism rather than iron overload (11,23,35).

Our study did not show any significant correlations between the MCV, TIBC, FBS, and HbA1c levels of the two groups, but we had a decreasing average of HbA1c after treatment of anemia among the intervention group.

HbA1c is commonly used to assess long-term blood glucose control in patients with diabetes mellitus, because the HbA1c value has been shown to predict the risk for the development of many of the chronic complications in diabetes. Iron deficiency should be corrected before making any diagnostic or therapeutic decisions based on HbA1c.

Some limitations should be noted here. The results from the present study may not be fully generalizable to individuals with severe degrees of IDA or non-IDA because the sample size was small. Furthermore, since the patients in this study had different socioeconomic statuses, they had different dietary regimes, which could have affected the results.

In conclusion, our results showed that iron deficiency was associated with higher relative levels of HbA1c, which could cause problems in the diagnosis of uncontrolled diabetes mellitus in IDA patients. The iron status should be considered during the interpretation of the HbA1c concentrations in diabetes mellitus. Iron supplementation is important in diabetic patients with IDA, as it would also increase the reliability of the HbA1c results.

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