Discrepancy of Glycosylated Haemoglobin and Fructosamine in Egyptian Patients with Prediabetes and Iron deficiency

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ABSTRACT

Background: the controversies concerning the influence of iron deficiency on glycaemic control parameters in patients with prediabetes prompted us to conduct this research.

Objective: Our aim was to get a delicate estimation of these changes that may alter the management plan in this clinical situation.

Patients and methods: a case control study included 197 Egyptian patients with prediabetes; 113 patients with iron deficiency versus 84 patients without iron deficiency (control group) during the period (May 2019 - October 2019). History, physical examination and laboratory tests of fasting and postprandial plasma glucose (FPG and PPPG), glycosylated haemoglobin (HBA1c), fructosamine, serum creatinine, ALT, AST, CBC, ferritin, serum iron and total iron binding capacity (TIBC) were done.

Results: HBA1c was significantly higher in the iron deficiency group than the control group with non-significant difference between the studied groups regarding FPG and PPPG. HBA1c level was positively correlated with fructosamine, exclusively in the healthy group indicating the lack of credibility of HBA1c in iron deficient subjects with prediabetes. HBA1C level was inversely correlated with serum iron level in both study groups. **Conclusion:** there is discordance in glycaemic control parameters in patients with prediabetes and iron deficiency. Diagnosis of deranged glucose homeostasis in patients with co-existent iron deficiency and prediabetes should not be with HBA1c solely.

Keywords: HBA1c, Iron deficiency anaemia, Prediabetes.

INTRODUCTION

Fasting and post prandial plasma glucose (FPG and PPPG) tests are the main tools for diagnosis of deranged glucose homeostasis, however the American Diabetes Association (ADA) and World Health Organisation (WHO) recommended glycosylated haemoglobin (HBA1c) for diagnosis of prediabetes and diabetes [(1)]. Blood glucose is the main determinant of HBA1c but other factors can affect HBA1c status as haemolytic anaemia, haemoglobin disorders, pregnancy, malnutrition (iron, vitamin B12 and folic acid deficiency), race and ethnicity [(2)]. HBA1c was influenced by disordered erythrocyte turn over conditions therefore HBA1c level in these disorders is doubtful [(3)]. Iron deficiency and iron deficiency anaemia (IDA) represent a common global health challenge. People with iron deficiency are more vulnerable to disordered glucose homeostasis and subsequent complications. The precise interrelation between iron deficiency, serum glucose and HBA1c is still controversial [(4)].

In case of iron deficiency, there is persistent doubt concerning the credibility of HbA1c in diagnosis of prediabetes and diabetes [(5)]. Contradictory data concerning HBA1c measurement in iron deficiency states were found in the literature [(6)]. **Madhu *et al.*** [(7)] reported elevated HBA1c level in patients with iron deficiency with reversible decrease after iron therapy.

However, **Kim *et al.*** [(8)] found a non-significant relationship between iron deficiency and HBA1c level in non-diabetic subjects. On the other hand, **Sinha *et al.*** [(9)] found a significant lowering of HBA1c in iron deficient patients.

To our knowledge, there is a lack of data concerning blood glucose control parameters alterations in Egyptian patients suffering from prediabetes with iron deficiency. Our aim was to get a delicate estimation of these changes that may alter the management plan in this clinical situation.

PATIENTS AND METHODS

We conducted this case control study at the outpatient department of our university hospital during the period from May 2019 to October 2019. This study included two groups of patients with impaired glucose regulation; the case group comprised 113 patients; 41 male (36%) and 72 female (64%) with iron deficiency versus the control group that comprised 84 subjects; 34 male (40%) and 50 female (60%) without iron deficiency.

Patients were subjected to a detailed medical history including age, socioeconomic status, marital status, smoking, alcohol consumption, exercise, infectious and tropical diseases, hereditary and familial diseases with therapeutic history of previous iron therapy or history of blood transfusion as well as

Received:8 /10 /2020
Accepted:26 /11 /2020

surgical history including bariatric surgery and physical examination.

In the morning following at least 8 hours fasting, blood samples were withdrawn for assessment of fasting plasma glucose (FPG) and serum creatinine levels measurement via Hitachi Automatic Analyzer 7600 (Hitachi, Tokyo, Japan). Two hour postprandial plasma glucose (PPPG) was measured after 75 g glucose load.

High-performance liquid chromatography (HLC-723G7; Tosoh, Tokyo, Japan) was used to measure HbA1c level. Cumulative pulse height detection was the tool to measure haematocrit via an XE-2100D haematology analyser (Sysmex, Kobe, Japan). Meanwhile serum ferritin level was measured by an immuneradiometric assay.

Diabetes and prediabetes were defined according to American Diabetes Association diagnostic criteria [(1)]. World Health Organization criteria were considered in diagnosis of anaemia as well as iron deficiency terms. IDA was defined as anaemia with iron deficiency [(10)].

We excluded patients with pregnancy, diabetes, infectious diseases, malignancy, endocrine disorders, nutritional disorders, hematologic diseases, liver, renal, cardiovascular and/or pulmonary diseases as well as autoimmune disorders, hemochromatosis and patients administrating haematinic or immunosuppressant medications.

Ethical Approval and consent to participate:

This study was approved by the Institutional Review Board for Clinical Research Committee of Mansoura University with approval number (No.R.20.02.742). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was approved by the Institutional Review Board for Clinical Research Committee of Mansoura University and obtained from all participants.

Statistical method:

We used R language (R-studio Version 0.99.484 – © 2009-2015) for statistical analysis of data. Continuous and categorical variables are expressed as mean±SD and frequency with percentage respectively. Differences in frequency of characteristics were assessed by independent sample student's t-test for continuous variables and Chi-square statistics for discrete variables. Variables, which had not fulfilled the normal distribution frequency conditions, were treated by non-parametric tests (Mann Witney test). Linear regression analysis was done to model the dependent and independent variables with univariate analysis. P-value of 0.05 expressed a statistically significant result.

RESULTS

Table (1) shows the iron status and glycaemic control parameters between the study groups. Glycosylated haemoglobin (HBA1c) was significantly elevated in the case group than the control group (Table 1 and figure 1). Haemoglobin concentration, ferritin and serum iron levels were significantly lower in the case group versus the control group. However, total iron binding capacity (TIBC) was significantly higher in the case group than the control group (Table 1).

Table (1): Iron and glycaemic control parameters in the study groups

	Non-anaemic N=84 (mean ± SD)	**Anaemic N=113 (mean ± SD)**	**P value**
HB (g/dL)	14.24±1.34	9.81±1.33	P < 0.0001*
Ferritin (µg/L)	31.58± 3.31	6.92± 1.19	P < 0.001*
Iron (µg/Dl)	66.88± 4.56	26.8± 5.05	P < 0.001*
TIBC (µg/dL)	340.9± 57.28906	419.4± 73.16	P < 0.001*
HBA1c%	6.29±0.36	7.34±0.5	P < 0.001*
FPG (mg/dL)	113.4± 6.8	111.5± 6.7	P = 0.0613
PPPG (mg/dL)	160.4± 14.8	156.9± 12.5	P = 0.0821

[a]* P-value < 0.05 =significant.

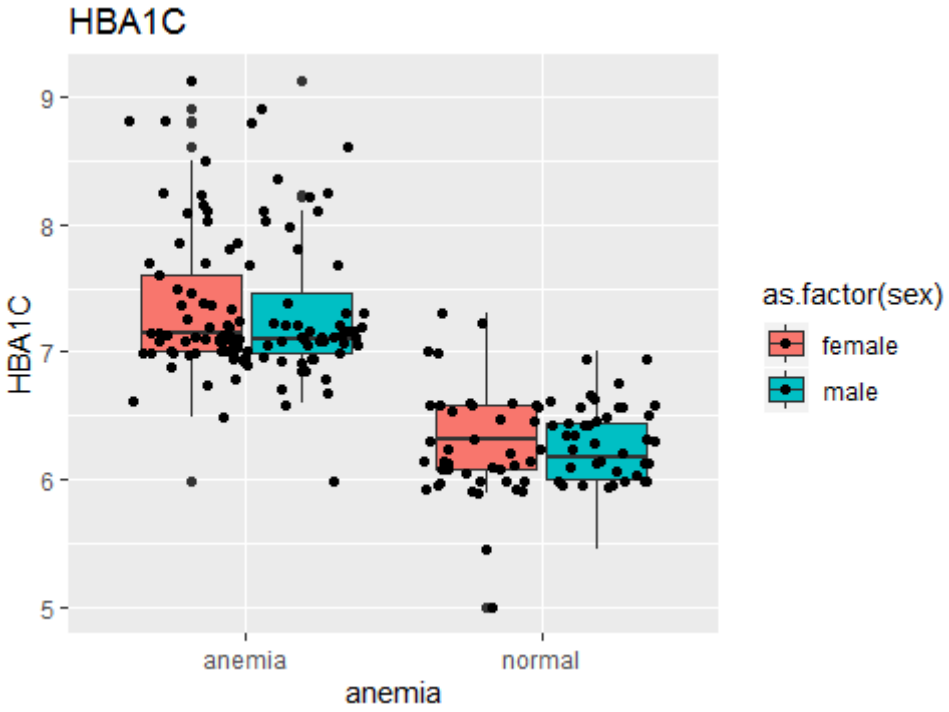

Fig (1): Glycosylated haemoglobin (HBA1c) in the study groups

Table (2) shows the correlations between HBA1c with fructosamine and serum iron in the study groups. We found a significant positive correlation between HBA1c and fructosamine in the control group. HBA1c was inversely correlated with serum Iron in the anaemic group as well as the control group (Table 2 and figure 2).

Table (2): Correlation between HBA1c with fructosamine and serum iron in the study groups.

Variable	**HBA1c**	
Fructosamine	**r**	**P-value**
Control group	0.54	P ≤ 0.001*
Anaemia group	0.103	0.274
Serum iron	**r**	**P-value**
Control group	-0.22	0.03*
Anaemia group	-0.21	0.01*

*Significant P-value< 0.05. r=correlation coefficient

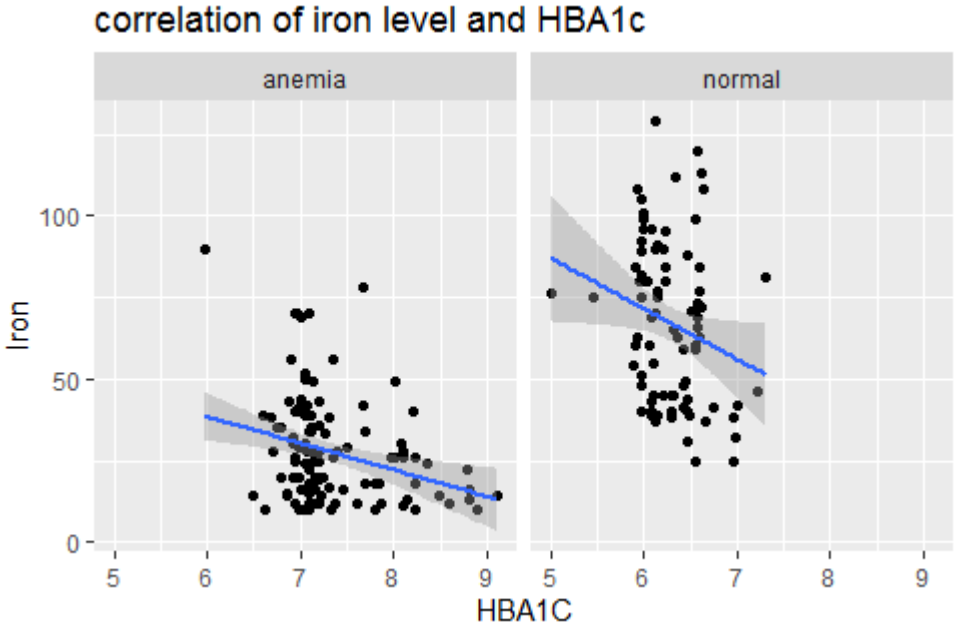

Fig (2): Correlation between HBA1c and serum iron level in the study groups.

Table (3) shows the model of linear regression analysis of HBA1c as a dependent variable and other haematological parameters as independent variables in the anaemia group. Haemoglobin concentration was the significant negative independent predictor of HBA1c (Table 3). Meanwhile, ferritin was the only independent predictor of HBA1c changes in both studied groups as shown in table (4) which represents the linear regression analysis of HBA1c as a dependent variable and other haematological parameters as independent variables in all study participants.

Table (3): Model of linear regression analysis of HBA1c with haematological and iron status parameters in the anaemia group

Variable	B (estimate)	S.E	P-value
Intercept			
RBCS	0.00006975	0.00108551	0.94889
HB%	-0.18812238	0.0482237	< 0.001*
Ferritin	-0.0064433	0.0184770	0.7280
Iron	-0.00024953	0.00412033	0.9518
TIBC	-0.0001353	0.00077045	0.86091
Iron/TIBC	-0.00002247	0.00047792	0.96259
MCV	-0.00278845	0.01048611	0.79083
MCH	0.00027867	0.02283808	0.99029

¶Adjusted R-squared: 0.1758 *Significant P-value< 0.05.

Table (4): Model of linear regression analysis of HBA1c with haematological and iron status parameters in the study groups

Variable	B (estimate)	S.E	P-value
Intercept			
RBCS	0.0965292	0.1163810	0.409496
HB	-0.0757330	0.0496146	0.131110
ferritin	-0.0109004	0.0030000	< 0.001*
Iron	0.0008770	0.0034689	0.801100
TIBC	0.0001640	0.000873	0.851640
Iron/TIBC	-0.0049763	0.0111480	0.65660
MCV	0.0026991	0.0119405	0.821780
MCH	-0.0024418	0.0058114	0.675552

Adjusted R-squared: 0.1744 *Significant P-value< 0.05.

DISCUSSION

In our study, despite the non-significant difference in FPG and PPPG between the case and the control groups, HBA1c was significantly elevated in the iron deficiency group that exceeded the upper limit of the prediabetes stage to lie within DM diagnostic range.

These findings agreed with previous epidemiological studies that showed abnormal high HBA1c in patients with IDA regardless serum plasma glucose levels even with different study designs and methodologies. **Rajagopal *et al.* [11] and Hardikar *et al.* [12]** studied HBA1c status in iron deficient non-diabetic population and doubted the reliability of HBA1c use in diagnosis of diabetes in these populations.

Our results of the iron deficient patients showed that HB concentration exhibited an independent negative effect on HBA1c meanwhile ferritin showed a similar effect on HBA1c in all studied participants. In accordance with **Ford *et al.* [2]** who found an independent relationship between iron deficiency and increased HBA1c regardless serum glucose level with subsequent reduction of HBA1c level following iron therapy.

Our results agreed with **Bhardwaj *et al.* [13]** who found a significant difference in the mean of HBA1c level in iron deficiency anaemic (HBA1c: 6.6 g/dl) than non-anaemic patients (HBA1c: 5.4%) with a significant decline of HBA1c (from 6.6 to 5.7%) with anaemia treatment. Also, **Madhu *et al.* [7]** found a significant negative correlation between HBA1c and iron deficiency indices (serum ferritin, haemoglobin, haematocrit and erythrocyte count) in IDA subjects. In alignment with **Attard *et al.* [14]** who doubted the durability of the diagnosis of prediabetes in iron deficient Chinese men with use of HBA1c alone without fasting plasma glucose.

However, **Kim *et al.* [8]** analysed data of the US National Health and Nutrition Survey (1999–2006) and found a slight increase of HBA1c in iron deficient non-diabetic women regardless fasting plasma glucose level, in contrast to our findings in iron deficient cases that showed a significant elevation of HBA1c values that catch the diagnostic range of DM despite concomitant pre-diabetic values of FPG and PPPG. However, this inconsistency can be explained by the variation in the baseline HBA1c, ethnicity and gender of the participants. Therefore, diagnosis of DM in patients with prior prediabetes necessitates accurate estimation of all glycaemic control parameters along with iron status evaluation.

In vitro, metabolic alterations such as hyperinsulinemia, hyperglycemia, hyperlipidemia, decreased oxidative capacity and increased glucose utilization due to cellular hypoxia were found in iron deficient rats **[15].** Also, **Davis *et al.* [16]** reported heterogeneity in expression of gene controlling glucose metabolism and increased lipogenic gene expression with decreased β-oxidation gene expression inducing hyperlipidaemia and hyperglycaemia in iron deficient rodents. **Hagiwara *et al.* [17] and Iynedjian *et al.* [18]** attributed the iron deficiency induced metabolic changes to overexpression of glucokinase (Gck) with relative hyperinsulinemia that enhance the rate of glucose phosphorylation as well as hepatic denovo lipogenesis. Meanwhile, **Davis *et al.* [16]** attributed the increased gluconeogenesis in iron-deficient rats to increased lactate accumulation due to the hypoxic state accompanying iron-deficiency. **Yamagishi *et al.* [19]** suggested the existence of certain threshold of haemoglobin reduction for this metabolic disruption. However, **Saad *et al.* [20]** found moderate iron deficiency is sufficient to disrupt normal glucose homeostasis regardless the diet formula or cortisol level.

Our results disagreed with **Sinha *et al.* [9]** who found lower HBA1c levels with iron deficiency but their trial included only fifty patients with a different design. On the other hand, **Gram-Hansen *et al.* [21]** found normal HbA1c level in iron deficiency state; they noticed increased HbA1c after iron supplementation and attributed these findings to nutritional deficiencies and unknown variables.

Cavagnolli *et al.* [22], Grossman *et al.* [23] and Akkermans *et al.* [24] found no association between HBA1c and HB levels in non-diabetic subjects that contradicts our findings due to variation in study design, ethnicity and population criteria. Also, **Christy *et al.* [25]** found non-significant correlation between ferritin and haemoglobin with HBA1c level in well controlled diabetic state. This inconsistency regarding the relation between IDA and HBA1c might be attributed to different methods of HbA1c assessment as well as patient heterogeneity. Thirty methods are used for HbA1c measurement like ion-exchange High Performance Liquid Chromatography (HPLC), affinity HPLC, immunoassay, and enzymatic methods with a constant controversy in between **[24].** The cut-off value of (HbA1c) for the diagnosis of prediabetes and diabetes in patients with iron deficiency is still controversial that may lead to poor handling and subsequent adverse outcomes **[26].**

Our strength point was the patients' selection in specific clinical situation of concomitant pre-diabetics and iron deficiency. Our limitations were the single centre, single ethnicity and lack of the traditional oral glucose tolerance test use. Multicentre trials with larger scale and different ethnicities are

needed in the future to delineate the diagnostic cut-off values of HBA1c for accurate diagnosis of DM and prediabetes in subjects with iron deficiency.

CONCLUSION

The clinician should be cautious in diagnosing DM in patients suffering from iron deficiency with impaired glucose regulation. Interpretation of HBA1c values without synchronous evaluation of iron status and other glycaemic control parameters will be misleading.

Funding: by authors.

Conflict of interest: authors declare no conflict of interest.

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