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Original Article

Effect of iron deficiency anaemia over glycated hemoglobin in non-diabetic women

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Abstract: Background: Glycated hemoglobin (HbA1c) is a form of hemoglobin bound to glucose and used as an index of glycaemic control reflecting glucose levels of the previous three months. Iron deficiency anemia (IDA) is the commonest form of anemia that affects HbA1c. Reports on the effects of IDA on HbA1c levels are inconsistent in India. Therefore, the study correlated the HbA1c and IDA in non-diabetic female patients. Methods: A correlative study between HbA1c and IDA was carried out at the Department of Biochemistry, A. J. Institute of Medical Sciences, Mangaluru, India. A total of 50 non-diabetic female patients, aged between 20-50 years, with decreased levels of Hb, MCV and MCHC were selected. Their ferritin levels were determined by ELISA method to confirm IDA. Forty confirmed iron-deficient samples whose serum ferritin levels were <90 pg/dL, were tested for HbA1c levels by nephelometry method. Results: HbA1c correlated positively with serum ferritin, Hb, MCV, MCH and MCHC (P<0.05). There was a significant decrease in mean value of HbA1c in those with severe anemia (4.50±0.34) compared to those with moderate anemia (5.18±0.35) (P<0.001). Conclusion: Results showed positive correlation of HbA1c with ferritin and hemoglobin. Therefore, iron status should be considered during the interpretation of the HbA1c concentrations in diabetes mellitus.

Keywords: HbA1c, Iron deficiency anemia, ferritin, India

Introduction

Glycated hemoglobin (HbA1c) is recognized as a potential indicator of glycaemic control and helps monitor glycaemic control in patients with diabetes mellitus (DM). HbA1c reflects the glycaemic status when observed over three months and predicts the risk of long-term complications in diabetes [1]. According to the American Diabetes Association (ADA) and the World Health Organization (WHO), the HbA1c level greater than 6.5% is taken as diagnostic criteria for DM [2]. As per ADA recommendation, testing for HbA1c should be done at least twice a year in patients with stable glycaemic control or four times per year in patients with HbA1c levels above the target value. Estimation of plasma glucose is the simplest test to measure the amount of glucose present in blood at a given point in time [3]. The levels of HbA1c strongly correlate with fasting plasma glucose (FPG). Though, in the absence of HbA1c testing, FPG, two hours post glucose load, plasma glucose, and oral glucose tolerance tests can be considered for the diagnosis of diabetes [4].

Iron deficiency anemia is commonly encountered in urban and rural Indian populations. It is mainly associated with females due to heavy menstrual blood loss, certain diseases, genetic factors, etc. Patients suspected to have IDA should undergo iron studies test. The results determined from this test should be correlated with the red cell indices. The serum ferritin level is the most commonly available and helpful index of iron deficiency [5]. Previous studies have proved that IDA is linked with increase in HbA1c levels [6-8].

Therefore, the present study aimed to correlate the significance of estimating the iron deficiency status and the HbA1c levels in the female, non-diabetic population.

Materials and methods

Study design

Study population: The study was carried out at the Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru (India) from January 2017 to August 2017. Institutional Ethics Committee approval was taken before the study (Reference no. AJEC/REV/14/2016), and informed consent was obtained from all the patients.

The study participants were residents of South India, mainly from in and around Mangaluru. A total of 50 non-diabetic female patients with hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) values below normal were selected (Hb below <10 g/dL, MCV <70 fL, MCH <35 pg/cell).

Serum ferritin levels were estimated by ELISA method. Serum ferritin, being an acute phase protein, can increase as a result of acute phase response. The blood samples with ferritin levels below 90 pg/dL were selected. Forty confirmed iron deficiency samples were tested for HbA1c using the nephelometry method [9].

Patients with a history of acute blood loss, kidney disease and gestational diabetes mellitus were excluded from the study.

The informed consent was obtained, and blood samples were collected in EDTA and plain vacutainer for HbA1c and ferritin estimation respectively. Whole blood was used to assay the HbA1c by nephelometry method using a turbidimetric immunoassay semi-autoanalyzer (Mispa i2, Agappe, India). From the plain tube, serum was separated, and ferritin was estimated using an ELISA kit (Calbiotech, California, USA) and the reading was obtained from LabLife ER2007 microplate ELISA reader.

Statistical analysis: Data was analyzed by using SPSS version 17. Continuous variables were represented as mean ± SD and categorical variables as frequency and percentage. Comparison of continuous variables between two groups was done using independent t test. Correlation between continuous variables was done using Karl Pearson's correlation analysis. Statistical significance was considered at P<0.05.

Results

Out of 40 confirmed IDA study participants, 37.5% (n=15) were in the age group of 41-50 years, 32.5% (n=13) were between 20-30 years, and the remaining 30% (n=12) were in the group of 31-40 years. The mean age was 36.05±10.75 years. The baseline parameters of the study subjects are shown in **Table 1**. Mean HbA1c and ferritin value was found to be 5.04±0.44% and 18.17±16.14 pg/dL, respectively.

Based on hemoglobin values, the confirmed IDA study participants were classified into mild (11-11.9 g/dL), moderate (8-10 g/dL), and severe (<8 g/dL) anemia groups. The percentages of the study participants with mild, moderate and severe anemia were 2.5% (n=1), 77.5% (n=31) and 20.0% (n=8) respectively. The biochemical and haematological parameters were compared between the moderate and severe anemia groups as shown in **Table 2**. Comparison with mild anemia was excluded as there was only one subject in the group. The levels of Hb, MCV, MCH, MCHC, serum ferritin and HbA1c were significantly low among individuals with severe anemia when compared to individuals with moderate anemia (P<0.001).

The Pearson correlation test showed a positive correlation between HbA1c and ferritin (r= 0.351, P=0.027) (**Figure 1**). Also, the HbA1c showed significant correction with Hb (r=0.839, P<0.001), MCV (r=0.504, P=0.001), MCH (r= 0.607, P<0.001) and MCH (r=0.523, P=0.001).

Discussion

IDA is the most common cause of anemia and is accounted as the most prevalent nutritional deficiency in the world [10]. Decrease in serum ferritin, indicating a depletion in the body iron stores, is the hallmark of IDA. Reduction in Hb and blood indices such MCV and MCH is also seen in IDA [11].

IDA is said to be prevalent in individuals with diabetes mellitus. The exact mechanism by which iron deficiency influences glucose homeostasis is not well understood. However, it has been postulated that IDA is associated with alterations in Hb, delayed turnover and increased lifespan of red blood cells (RBCs).

Table 1. The biochemical profile of the study subjects (n=40)

Parameters	Mean ± SD	Range Minimum-Maximum	Pearson correlation	*p*-value
Age (in years)	36.05±10.75	20-50	-	-
HbA1c (%)	5.04±0.44	4.01-5.96	-	-
Ferritin (pg/dL)	18.17±16.14	1.16-69.32	0.351	0.023
Hb (g/dL)	8.69±1.79	3.4-11.9	0.839	<0.001
MCV	64.00±4.01	53.3-69.4	0.504	<0.001
MCH	18.91±1.96	13-21.7	0.606	<0.001
MCHC	29.49±1.92	24.1-32.4	0.523	<0.001

Table 2. Comparison of biochemical and haematological parameters between moderate and severe anemia groups

Parameters	Mild Anemia (n=1)	Moderate Anemia (n=31)	Severe Anemia# (n=8)
Age (in years)	48.00	35.03±10.39	38.50±12.24
HbA1c (%)	5.12	5.18±0.35	4.50±0.34**
Ferritin (pg/dL)	62.77	20.03±14.81	5.38±5.03**
Hb (g/dL)	11.90	9.28±0.90	6.03±1.81**
MCV (fL)	53.30	65.36±2.79	60.08±3.72**
MCH (pg/cell)	15.70	19.65±1.28	16.44±1.87**
MCHC (g/dL)	29.50	30.06±1.58	27.28±1.63**

#Comparison between severe anemia and moderate anemia. **P<0.001.

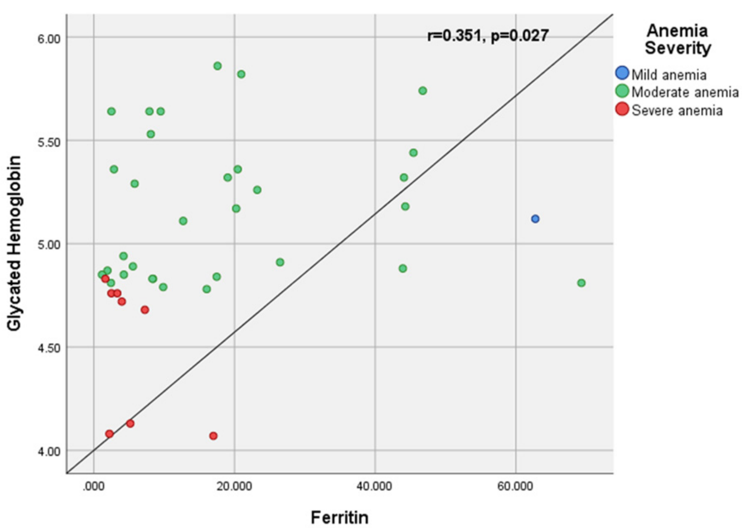

Figure 1. Correlation between glycated haemoglobin and ferritin.

These factors contribute towards increase in glycation rate of Hb [3].

Glycated hemoglobin (HbA1c) is a traditional tool to assess glycemic control over the last three months, among diabetic individuals. Besides blood sugar, conditions such as haemolytic anaemias, hemoglobinopathies, acute and chronic blood loss, pregnancy, and uremia can also affect HbA1c levels [8, 12].

Various studies have been carried out to delineate the influence of IDA over HbA1c. The study done by Sinha N. and colleagues, revealed that the glycated haemoglobin levels were significantly lower among patients with IDA than the healthy control group. There was a subsequent rise in HbA1c with iron supplementation within 2 months of initiation of treatment. [6] On the contrary, Brooks et al. reported increased levels of HbA1c in non-diabetic individuals with IDA, which reduced to near normal levels after iron supplementation [8]. Another study done by van Heyningen and Dalton R.G. showed no difference in HbA1c levels while comparing nondiabetic patients with IDA before and after iron treatment to healthy controls. Authors opined that the reported difference in HbA1c concentrations before and after iron supplementation was due to the difference in the laboratory methods used to measure HbA1c [13]. However, when Rai et al., used different biochemical techniques like colorimetric assays, ion-exchange chromatography, and affinity chromatography for measuring HbA1c, and these methods did not make any significant difference in HbA1c values [14].

Our study suggests that HbA1c has a positive correlation with serum ferritin, Hb, MCV, MCH and MCHC in non-diabetic women with IDA, indicating an inverse relationship with IDA. Serum ferritin level is closely related to hyperglycemia and correlates positively with increased HbA1c reflecting poor glycaemic control.

Several reports have shown a strong association between elevated serum ferritin concentration and increased risk for diabetes [5].

On further evaluation, HbA1c was also found to be significantly reduced in women with severe anemia compared to those with moderate anemia. Low serum ferritin, Hb, MCV, MCH and MCHC levels are indicative of IDA. Based on these findings, we suggest that HbA1c decreases with severity of IDA. There is limited literature available showing the relationship of HbA1c with anemia severity among non-diabetic women. One study carried out by Bindayel IA in Saudi Arabia comprised of non-diabetic women with and without IDA. The IDA group consisting of 21 women were further divided into two groups according to severity of anemia, mild (n=9) and moderate-severe (n=12). The HbA1c levels did not differ between the two severity groups. However, the absolute HbA1c concentrations did show significant difference in mean values, between the two groups [15].

Iron supplementation, the mainstay treatment of IDA, is known to help cells fight against oxidative stress [16]. However, iron reduces malondialdehyde levels, resulting in hemoglobin reduction, thereby limiting the HbA1c measurements [17]. Therefore, caution should be taken while diagnosing prediabetes and diabetes in individuals with anemia, while IDA must be considered for monitoring the patients [18].

Conclusion

This study found a positive correlation of HbA1c with ferritin and hemoglobin and inversely correlated with iron deficiency anemia. Therefore, parallel measurement of iron, hemoglobin, and HbA1c in anemic patients can be considered for proper interpretation of glycaemic status as it would also increase the reliability of the HbA1c determinations.

Disclosure of conflict of interest

None.

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