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EFFECT OF IRON DEFICIENCY ANEMIA ON HbA1c IN DIABETIC AND NON-DIABETICPATIENTS.

Eman Elsheikh*[1,2], Sereen S Aljohani[3], Munirah M Alshaikhmubarak[3], Alhanouf W Alsubaie[3], Meshari A Alhawl[3], Norah H Alsultan[3], Asmaa F. Sharif [4,5]

[1]Department of Cardiology, Department of Cardiology, College of Medicine, Tanta University, Egypt
[2]Internal Medicine Department, King Faisal University, Alahsa, Saudi Arabia
[3]Collage of Medicine, King Faisal University, Alahsa, Saudi Arabia
[4]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
[5]Clinical Sciences Department, College of Medicine, Dar Al Uloom University, Riyadh, Saudi Arabia.

***Corresponding Author: -** Eman Elsheikh
*Department of Cardiology, Department of Cardiology, College of Medicine, Tanta University, Egypt

Abstract:
Background: The global diabetes mellitus burden is increasing. Developing countries, including the majority of Middle Eastern countries, are experiencing an acceleration in this issue. We investigated the effects of Iron Deficiency Anemia on HbA1c levels in diabetic adults in Saudi Arabia.
Method: A retrospective, cross sectional single-center study was conducted in the period January to April 2023. 445 individuals aged 18 and over received medical care at the polyclinics of King Faisal University are included. Demographic data and clinical condition information were extracted from electronic medical records.
Results: among our patients, 41 patients were diabetic and the other 404 were not. The diabetic patients were significantly older than normal individuals (mean = 57 years among diabetics versus 43.6 years among non-diabetics). Non-diabetic anemic patients showed significantly higher levels of HB A1C compared to the non-diabetic not anemic patients. The median of HBA1C among the non-diabetic anemic patients was 5.46 compared to 5.1 in the non-diabetic not anemic patients ($p < 0.001$). The same finding had been noticed among the diabetic patients, (median of HB A1C = 7.9% in anemic patients versus 6.91 % in non-anemic patients). Nevertheless, this variation didn't reach the level of statistical significance ($p = 0.249$).
Conclusion: iron status must be considered when interpreting HbA1c in both diabetic and non-diabetic individuals our data also imply that clinicians should be careful when identifying both diabetes and prediabetes in anemic patients. In non-diabetics, iron deficiency anemia features are considerably linked with HbA1c. Hence, its variables should be assessed before diagnosing diabetes.

Introduction

The occurrence of diabetes on a global scale is increasing at an alarming rate. In the year 2000, approximately 171 million individuals across the world were affected by the condition. However, it is projected that by 2030, this number will surpass 552 million [1]. In Saudi Arabia, the prevalence

of type 2 diabetes is currently at 32.8%. Nevertheless, it is predicted that this figure will rise to 35.37% in 2020, 40.37% in 2025, and 45.37% in 2030 [2].Therefore, it is crucial to maintain glucose hemostasis to prevent diabetes complications.

Hemoglobin A1C (HbA1c) is the primary form of glycosylated hemoglobin [3]. It is produced when glucose in the bloodstream reacts with the N-terminal amino acid of the hemoglobin's beta chain in the presence of ketamine. The fraction of glycosylated hemoglobin is directly proportional to average plasma glucose levels. For diabetic patients, HbA1c provides an indication of their plasma glucose levels over the preceding three months [4]. The American Diabetes Association (ADA) recommends using HbA1c as a diagnostic tool for diabetes [5].

Previously, it was believed that HbA1c levels were solely influenced by plasma glucose levels [6]. However, recent studies have demonstrated that various other factors, including hemoglobinopathies, hemolytic anemias, chronic kidney disease, and alcoholism, pregnancy, and dietary anemias, can also impact HbA1c levels [7, 8]. Anemia, characterized by a deficiency in essential nutrients, is a widespread health concern in both developed and developing countries [9]. The World Health Organization (WHO) estimates that approximately two billion individuals worldwide suffer from anemia, with iron deficiency anemia accounting for 50% of all cases [10]. In Saudi Arabia, iron deficiency anemia is a significant public health issue, with a prevalence ranging from 30 to 56% [11]. Iron plays a crucial role in numerous vital metabolic processes, including oxygen transport, cell development and differentiation, DNA synthesis, and electron transport. It also influences the presentation of various systemic disorders [12-14]. Changes in iron levels have been associated with an increased risk of diabetes [7]. Research has shown that reduced iron or ferritin levels in the blood are linked to increased glycation of HbA1c [15,16]. Additionally, higher iron levels have been found to affect insulin action and secretion [16], suggesting a bidirectional relationship between iron metabolism and glucose regulation.

To address the conflicting evidence, we conducted a study to examine the impact of Iron Deficiency Anemia on HbA1c levels in adults, both with and without diabetes, in Saudi Arabia. The objective of our study was to determine whether individuals with iron-deficiency anemia, with or without diabetes, exhibited higher HbA1c levels.

Subjects and Methods:

A retrospective, single center study was conducted utilizing a descriptive and cross-sectional design. The study population consisted of 445 individuals aged 18 and above who received medical care at the polyclinics of King Faisal University during the period from January to April 2023. Demographic data and clinical condition information were extracted from electronic medical records.

The exclusion criteria for our study included individuals with a documented medical history of acute or chronic blood loss, hemolytic anemias, hemoglobinopathies, kidney disorders, pregnancy, and chronic alcohol consumption. Additionally, patients with pre-diabetes, characterized by fasting blood sugar (FBS) levels ranging from 100mg/dL to 125mg/dL and hemoglobin A1C (HbA1C) levels ranging from 5.7% to 6.4%, were also excluded from the study.All enrolled patients are classified into two categories. The initial group comprises individuals without diabetes, whereas the second group comprises individuals with diabetes. Both categories are further subdivided into subgroups of anemic and non-anemic individuals.

The iron deficiency anaemic patients were diagnosed as per World Health Organization recommendations [17]. We assessed the levels of various parameters using an automated analyzer, including hemoglobin, mean corpuscular hemoglobin (MCH), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelet count. The criteria for defining low levels of hemoglobin were below 12 g/dL in non-pregnant females and below 13 g/dL in males. Additionally, ferritin levels were measured, with values below 9 ng/mL for women and below 15 ng/mL for men indicating low levels. According to the American Diabetes Association's (ADA) Standards of Care recommendations [18], participants were considered non diabetic if FBS is

less than 99 mg/dl or HbA1C less than 5.7 and diabetic if FBS is more than 126mg/dl or HbA1C is 6.5% or higher.

This study was conducted in accordance with the ethical approval obtained from the King Faisal University Ethics Committee and adhered to the principles outlined in the Declaration of Helsinki and its subsequent amendments by the World Medical Association (WMA).

Statistical analysis

The data were analyzed using Statistical Package of Social Sciences (SPSS) software version 28. Data were expressedas numbers and proportions, means and standard deviations (SD), medians and inter-quartile ranges (IQR). Chi-squaretest was used to evaluate the variations in categorical data while t-test and Mann Whitney U test were used for continuous data, according to their distributions. Diabetic and non-diabetic patients were compared and inside each group, all patients were compared according to their anemic status. Box plots were plotted comparing the median of HbA1C level among the studied patients according to their anemic status.

All factors proved their significance in the base line analysis between diabetic and non-diabetic patients were subjected to Spearmen correlations, where the correlation coefficient and p value were calculated. Furthermore, inside each group (nondiabetic and diabetics), the factors proved their significance were exposed to linear regression to ascertaintheir power as predictors of glycosylated hemoglobin level. Binary logistic regression was conducted and Receiver operating characteristic ROC) curve was plotted to investigate the role of HbA1C as a predictor for anemia. Area under the curve, sensitivity, specificity, odds ratio, positive and negative predictive values (PPV, NPV) were calculated for HbA1C at specific cutoff level. The level of 0.05 significance, 95% confidence interval (CI) and 5% margin of error were adopted.

Results

The current study was conducted among 445 individuals. Of them, 41 patients were diabetic and the other 404 were not. As shown in **Table (1),** the diabetic patients were significantly older than normal individuals (mean = 57 years among diabetics versus 43.6 years among non-diabetics). Though the number of male constituted more than half theparticipants (n=295), there was no gender differences between the diabetics and non-diabetic individuals ($p = 0.185$). However, among the non-diabetic participants, the females diagnosed with anemia were significantly higher than males (65.7% female versus 14.4% male, $p < 0.001$).

Table (2) shows that the diabetic patients exhibited significantly higher hemoglobin levels, hematocrit-PCV, and MCV compared to the non-diabetic patients ($p < 0.05$). On the other side the non-diabetic patients showed significantlyhigher MCHC ($p < 0.05$). More closely, examining the non-diabetic patients comparing them regarding their anemic status yielded significant findings. The anemic non-diabetic patients showed significantly less hemoglobin level, hematocrit- PCV, RBCs count, MCV, MCH, and MCHC, but higher RDW-CV ($p < 0.05$). The CBC pattern among the diabetics according to their anemic status was almost similar to the pattern seen inside the non-diabetic group except the insignificant variations in RBCs count, and RDW-CV ($p > 0.05$).

Analysis of platelet indices among the studied patients showed that the diabetic patients exhibited significantly higherMPV, and fasting blood glucose, but lower PCT and ferritin level compared to the non-diabetic patients ($p < 0.05$). Moreover, the ferritin level was significantly less than the non-diabetic patients (mean $=71.3 \pm 62.83$ versus 74.8 ± 48.17 ng/ml). Comparing the non-diabetic patients regarding their anemic status showed that the anemic non-diabetic patients showed significantly less MPV, PDW, P-LCR and ferritin level ($p < 0.001$) than the non-diabetic patients who were not anemic. On the other sides, the anemic non-diabetic patients showed higher fasting blood glucose level compared to the non-diabetic patients not diagnosed with anemia ($p = 0.007$). The mean

fasting blood glucose = 99.1mg/dl versus 94 mg/dl, among the anemic and not anemic non-diabetic patients, respectively **(Table 3).** The variationsbetween diabetic patients according to their anemic status were less obvious. Among the studied parameters, only theRDW-SD, P-LCC and ferritin level were significantly less among anemic diabetic patients compared to diabetic patients not diagnosed with anemia ($p < 0.05$).

Figure (1) illustrates that the non-diabetic anemic patients showed significantly higher levels of HB A1C compared to the non-diabetic not anemic patients. The median of HBA1C among the non-diabetic anemic patients was 5.46 compared to 5.1 in the non-diabetic not anemic patients ($p < 0.001$). The same finding had been noticed among the diabetic patients, (median of HB A1C = 7.9% in anemic patients versus 6.91 % in non-anemic patients). Nevertheless,this variation didn't reach the level of statistical significance ($p = 0.249$) **(Figure 2).**

Table (4) goes beyond the association and displays the correlation between the different factors and Hb A1C level. Among the non-diabetic patients, there was direct weak relationship between the fasting blood glucose level and Hb A1C level ($r = 0.128$, $p < 0.001$). However, indirect correlation had been noticed between the level of Hb A1C and hemoglobin level, hematocrit-PCV, MCV, MCHC, MPV, and Ferritin level. This correlation was weak with MCV ($r = -0.134$), MCHC ($r = -0.153$) , MPV ($r = -0.140$), moderate with hemoglobin level ($r = -0.433$), and hematocrit- PCV($r = -0.301$) and strong with ferritin level ($r = -0.618$). Similarly, among the diabetic patients, indirect moderate correlation existed between MCV and HB A1C level ($r = -0.338$), and indirect strong correlation existed between ferritin and Hb A1C levels ($r = -0.717$). On the other side, the fasting blood glucose level showed direct moderate correlation with Hb A1C level ($r = -0.717$).

Table (5) depicts that the age and some blood indices were significant predictors of Hb A1C level in non-diabetic patients. Among these factors, the ferritin level, being a male, fasting blood glucose level, age and MCHC were the most significant predictor of glycosylated hemoglobin level (regression coefficient = - 6.630, 4.642 , 4.157, 3.613, -3.560 for the five mentioned predictors, respectively), ($p < 0.001$). Moreover, the hemoglobin level, MCV, MCH, and MPV were other less significant predictors of glycosylated hemoglobin level ($p < 0.05$). **Figures (3-6)** displays the correlation between some predictors and level of HbA1C level among non-diabetic patients. On the other sides, **Table (6)** denotes that among the diabetic patients, all studied factors failed to be significant predictors of HbA1C level.

The current study shows that HbA1C was a significant predictor for the anemia (regression coefficient = 5.545, oddsratio = 1.301, 95% CI = 1.045-1.619, $p = 0.019$). **Figure (7)** depicts a ROC curve illustrating that at cutoff higher than 5.32, HbA1C could significantly predict the anemic status with very good AUC (0.774), 731.9 % accuracy (sensitivity = 78.7% and specificity = 68.8%), $p < 0.001$. Though the low PPV (53.9%), the level of HbA1C could correctly exclude 87.4% of patients who were not anemic.

Table (1). Demographic data of the participants including non-diabetic and diabetic patients

	Non- diabetic			Diabetic			Total (n= 445)	Testof sig.	p
	Not anemic (n=274)	Anemic (n=130)	Total (n=404)	Not anemic (n=30)	Anemic (n=11)	Total (n=41)			
Age								U 3189.0	p1 <0.001*
Mean ± SD	45.2 ± 11.01	40.2 ± 14.03	43.6 ± 12.27	56.0 ± 9.97	59.7 ± 19.83	57.0 ± 13.16	44.8 ± 12.94		
Min. – Max.	21 – 70	15 – 70	15.0 – 70.0	21 – 74	21 – 74	21.0 – 74.0	15.0 – 74.0		
Median(IQR)	47 (39 – 53)	39 (30 – 48)	46.0 (36.0 –52.75)	55 (50 – 59)	70 (61 – 74)	55.0 (50.0 –65.5)	47.0 (36.0 –55.0)		
	p2 <0.001*			p3 0.025*					
Sex								χ^2 1.755	p1 0.185
Male (n)	226	38	264	25	6	31	295		
%	85.6%	14.4%	100.0%	80.6%	19.4%	100.0%	100.0%		
Female (n)	48	92	140	5	5	10	150		
%	34.3%	65.7%	100.0%	50.0%	50.0%	100.0%	100.0%		
	p2 <0.001*			p3 0.098					

IQR: Inter quartile range, SD: standard deviation, χ^2: Chi square test, *p ≤ 0.05 (Statistically significant), U: Mann Whitney U test

p1 (Between non-diabetic and diabetics), p2 (Between anemic & not anemic in non-diabetic group) p3 (Between anemic & not anemic in diabetic group)

Table (2). Complete Blood Count showing some red blood cells indices of the participants including non-diabetic and diabetic patients

	Non- diabetic			Diabetic			Total (n = 445)	Test of sig.	p
	Not anemic(n=274)	Anemic (n=130)	Total (n=404)	Not anemic(n=30)	Anemic(n=11)	Total (n=41)			
Hemoglobin								U 6369.0	p1 0.015*
Mean ± SD	15.1 ± 1.20	11.2 ± 1.01	13.8 ± 2.13	15.4 ± 1.12	12.0 ± 0.68	14.5 ± 1.82	13.8 ± 2.11		
Min. – Max.	12.1 – 17.6	8.5 – 12.9	8.5 – 17.6	12.5 – 16	11.2 – 12.8	11.2 – 16.0	8.5 – 17.6		
Median (IQR)	15 (14.1 – 15.75)	11.3 (10.675 –11.8)	14.15 (11.8 – 15.2)	16 (15.5 – 16)	12.3 (11.2 – 12.6)	15.5 (12.7 – 16.0)	14.2 (12.15 –15.3)		
	p2 <0.001*			p3 <0.001*					
Hematocrit - PCV								U 6504.0	p1 0.023*
Mean ± SD	44.9 ± 3.61	38.5 ± 4.52	42.9 ± 4.93	45.4 ± 5.03	41.1 ± 5.25	44.2 ± 5.39	42.9 ± 4.98		
Min. – Max.	36.6 – 53.2	29.2 – 49.4	29.2 – 53.2	34.2 – 49.1	34.2 – 47.4	34.2 – 49.1	29.2 – 53.2		
Median (IQR)	45.2 (41.9 – 46.5)	39.1 (35.7 – 39.6)	42.8 (39.6 – 45.9)	48.2 (44.5 – 49.1)	42.7 (34.2 – 47.4)	47.4 (41.0 – 48.2)	43.6 (39.6 –46.0)		
	p2 <0.001*			p3 0.003*					
RBCs Count								t 0.337	p1 0.737
Mean ± SD	5.2 ± 0.52	4.8 ± 0.51	5.1 ± 0.55	5.2 ± 0.70	5.1 ± 1.25	5.1 ± 0.86	5.1 ± 0.58		
Min. – Max.	4.14 – 6.48	4.06 – 5.89	4.06 – 6.48	3.68 – 5.95	3.68 – 6.85	3.68 – 6.85	3.68 – 6.85		
Median (IQR)	5.11 (4.8525 –5.58)	4.78 (4.34 – 5.18)	5.1 (4.705 – 5.4)	5.17 (5.14 – 5.95)	5.15 (3.68 – 6.85)	5.15 (4.87 – 5.95)	5.11 (4.72 –5.4)		

	p2 <0.001*			p3 0.674					
MCV								**U 6699.0**	**p1 0.044***
Mean ± SD	86.2 ± 5.23	80.1 ± 8.76	84.2 ± 7.16	88.2 ± 4.88	81.9 ± 9.33	86.5 ± 6.84	84.5 ± 7.15		
Min. – Max.	70.9 – 95	61 – 95	61.0 – 95.0	82.5 – 93.3	69 – 92.9	69.0 – 93.3	61.0 – 95.0		
Median (IQR)	87.4 (82 – 91)	78.8 (74.825 – 88)	86.0 (79.8 – 89.1)	85.2 (82.5 – 93.3)	82.8 (69 – 92.9)	85.2 (82.5 – 93.1)	85.9 (80.0 –90.2)		
	p2 <0.001*			p3 0.040*					
MCH								**U 7532.0**	**p1 0.339**
Mean ± SD	29.0 ± 2.26	26.3 ± 3.74	28.1 ± 3.09	29.4 ± 1.64	26.1 ± 3.87	28.5 ± 2.80	28.2 ± 3.06		
Min. – Max.	22 – 32.4	18 – 32	18.0 – 32.4	26.9 – 30.9	20.9 – 30.4	20.9 – 30.9	18.0 – 32.4		
Median (IQR)	29.7 (27.2 – 30.6)	25.9 (24.15 – 29)	28.7 (26.0 – 30.4)	29.7 (26.9 – 30.9)	25.5 (20.9 – 30.4)	29.7 (26.9 – 30.65)	28.7 (26.5 –30.4)		
	p2 <0.001*			p3 0.005*					
MCHC								**t 2.091**	**p1 0.037***
Mean ± SD	33.6 ± 0.95	32.7 ± 1.41	33.3 ± 1.20	33.3 ± 0.88	31.7 ± 1.63	32.9 ± 1.33	33.3 ± 1.22		
Min. – Max.	30.7 – 35	29.1 – 34.9	29.1 – 35.0	32.6 – 34.8	30.2 – 34.2	30.2 – 34.8	29.1 – 35.0		
Median (IQR)	33.7 (33.1 – 34.4)	32.8 (31.9 – 33.5)	33.4 (32.8 – 34.2)	33.1 (32.6 –34.35)	30.5 (30.2 – 32.7)	32.7 (32.6 – 33.65)	33.3 (32.7 –34.2)		
	p2 <0.001*			p3 0.007*					
RDW-CV									
Mean ± SD	13.7 ± 0.82	14.5 ± 3.17	13.9 ± 1.96	13.7 ± 0.49	14.1 ± 1.14	13.8 ± 0.73	13.9 ± 1.88	t 0.421	p1 0.674
Min. – Max.	12 – 15.3	11.8 – 31.1	11.8 – 31.1	12.9 – 14.2	13.4 – 15.9	12.9 – 15.9	11.8 – 31.1		
Median (IQR)	13.6 (13 – 14.3)	13.8 (13.1 –14.775)	13.6 (13.1 – 14.3)	13.9 (13.275 –14.2)	13.6 (13.4 – 15.9)	13.9 (13.4 – 14.2)	13.6 (13.1 –14.3)		
	p2 0.044*			p3 0.828					

IQR: Inter quartile range, SD: standard deviation, *p ≤ 0.05 (Statistically significant), U: Mann Whitney U test, t: Independent t test

p1 (Between non-diabetic and diabetics), p2 (Between anemic & not anemic in non-diabetic group) p3 (Between anemic & not anemic in diabetic group)

Table (3): Some platelete indices, fasting blood glucose and ferritina level among the participants including non-diabetic and diabetic patients

	Non- diabetic			**Diabetic**			**Total (n = 445)**	**Test ofsig.**	**p**
	Not anemic(n=274)	**Anemic (n=130)**	**Total (n=404)**	**Not anemic(n=30)**	**Anemic (n=11)**	**Total (n=41)**			
MPV								**t 5.937**	**p1 <0.001***
Mean ± SD	9.5 ± 0.89	9.1 ± 0.76	9.4 ± 0.87	10.2 ± 1.82	31.3 ± 39.66	15.9 ± 22.01	9.9 ± 6.92		
Min. – Max.	7.3 – 11.3	8 – 10.5	7.3 – 11.3	8.1 – 12.7	7.5 – 93.0	7.5 – 93.0	7.3 – 93.0		
Median (IQR)	9.5 (8.8 – 10.1)	9 (8.6 – 9.6)	9.2 (8.8 – 10.1)	9.6 (8.7 –12.7)	8.7 (7.5 – 93)	8.7 (8.7 – 12.7)	9.2 (8.7 – 10.1)		
	p2 <0.001*			p3 0.124					
PDW								**U 7195.0**	**p1 0.249**
Mean ± SD	15.5 ± 2.63	14.5 ± 2.02	15.2 ± 2.50	17.8 ± 7.47	12.7 ± 2.69	16.4 ± 6.89	15.3 ± 3.19		
Min. – Max.	10.2 – 21.7	11.5 – 18.6	10.2 – 21.7	10.1 – 27.9	9 – 15.4	9.0 – 27.9	9.0 – 27.9		
Median (IQR)	15 (13.7 – 17.5)	13.9 (13.225 –15.8)	14.8 (13.3 –16.8)	14.3 (11.375 –27.9)	14.3 (9 – 15.4)	14.3 (10.95 –21.65)	14.6 (13.3 – 16.8)		
	p2 <0.001*			p3 0.165					
RDW-SD								**U 7360.5**	**p1 0.239**
Mean ± SD	41.2 ± 3.17	40.4 ± 2.86	40.9 ± 3.09	41.9 ± 1.97	40 ± 1.02	41.4 ± 1.95	40.9 ± 3.01		
Min. – Max.	31.9 – 46.2	31.9 – 45.4	31.9 – 46.2	39.6 – 44.5	38.6 – 41.2	38.6 – 44.5	31.9 – 46.2		
Median (IQR)	40.3 (38.6 –43.7)	40.3 (38.6 –42.8)	40.3 (38.6 –42.8)	41.2 (40.125 –44.5)	40 (38.6 –41.2)	41.2 (39.8 –42.85)	40.3 (39.5 – 42.8)		
	p2 0.185			p3 0.009*					
P-LCR								**U 7404.0**	**p1 0.294**
Mean ± SD	29.5 ± 8.49	26.3 ± 5.87	28.5 ± 7.90	33.1 ± 14.61	21.3 ± 7.66	29.9 ± 14.06	28.6 ± 8.64		
Min. – Max.	13.7 – 53.3	17.3 – 36.6	13.7 – 53.3	17.4 – 53.3	11.6 – 30.3	11.6 – 53.3	11.6 – 53.3		
Median (IQR)	27.3 (23.4 –34.2)	25.8 (21.6 –32.7)	26.3 (23.1 –33.1)	24.5 (21.3 –53.3)	24.5 (11.6 –30.3)	24.5 (21.3 –41.8)	26.25 (23.1 – 33.1)		
	p2 <0.001*			p3 0.070					
P-LCC								**U 6005.0**	**p1 0.316**
Mean ± SD	70.7 ± 21.53	68.7 ± 22.53	70.1 ± 21.78	74.5 ± 23.91	48.7 ± 9.24	66.2 ± 23.59	69.8 ± 21.92		
Min. – Max.	32 – 119	42 – 129	32.0 – 129.0	43 – 101	37 – 58	37.0 – 101.0	32.0 – 129.0		
Median (IQR)	76 (54 – 87)	60 (51.75 –82)	68.0 (54.0 –83.25)	55 (55 – 101)	55 (37 – 58)	55.0 (55.0 –101.0)	66.0 (54.0 – 84.0)		
	p2 0.227			p3 0.015*					
Fasting bloodglucose								**U 2003.5**	**p1 <0.001***
Mean ± SD	94.0 ± 17.43	99.1 ± 10.35	95.6 ± 15.68	133.5 ± 43.01	112.3 ± 57.53	127.8 ± 47.53	98.6 ± 22.66		
Min. – Max.	5.48 – 124	84 – 122	5.48 – 124.0	5.5 – 186	5.5 – 165	5.5 – 186.0	5.48 – 186.0		
Median (IQR)	96 (91 – 101)	98 (91 –106.5)	96.0 (91.0 –101.0)	124 (106 –186)	137 (106 –165)	124.0 (106.0 –175.5)	97.0 (91.0 – 105.0)		
	p2 0.007*			p3 0.513					
Ferritin								**U 3608.0**	**p1 0.039**
Mean ± SD	89.9 ± 45.65	33.3 ± 25.68	74.8 ± 48.17	95.4 ± 59.00	20.5 ± 33.68	71.3 ± 62.83	71.6 ± 61.59		
Min. – Max.	17.2 – 140	11 – 72	11.0 – 140.0	10.1 – 251.0	4.19 – 167.0	4.19 – 251.0	4.19 – 251.0		
Median (IQR)	99.5 (38.7 –130.25)	27.8 (11 – 72)	72.0 (38.7 –127.0)	85 (42.6 – 139)	11.8 (8.02 –17.7)	47.1 (17.7 –101.0)	47.1 (17.7 – 127.0)		
	p2 <0.001*			p3 <0.001*					

IQR: Inter quartile range, SD: standard deviation, *p ≤ 0.05 (Statistically significant), U: Mann Whitney U test, t: Independent t test
p1 (Between non-diabetic and diabetics), p2 (Between anemic & not anemic in non-diabetic group) p3 (Between anemic & not anemic in diabetic group)

Table (4): Correlation between HbA1C and other factors in the studied patients inlcuding including non-diabetic and diabetic patients

Factors showed significant variations among diabetic andnon-diabetic patients	Hb A1C level		Hb A1C level	
	Non-diabetic patients (n = 404)		Daibetic patients (n = 41)	
	r_s	p	r_s	p
Age	-0.035	**0.480**	-0.212	**0.183**
Hemoglobin	-0.433	**<0.001***	0.086	**0.593**
Hematocrit -PCV	-0.301	**<0.001***	0.058	**0.718**
MCV	-0.134	**0.007***	-0.338	**0.031***
MCHC	-0.153	**0.002***	0.118	**0.461**
MPV	-0.140	**0.005***	0.277	**0.080**
Fasting blood glucose level	0.182	**<0.001***	0.580	**<0.001***
Ferritin	-0.618	**<0.001***	-0.717	**<0.001***

r_s : Spearman correlation, *p ≤ 0.05 (Statistically significant)

Table (5). Linear logistic regression for significant variables as predictors for HbA1C in non-diabetic patients (n = 404)

Predictors		Regressioncoefficient	p	B	95.0% Confidence Interval for B	
					Lower Bound	Upper Bound
	Age	3.613	<0.001*	0.005	0.002	0.008
	Gender	4.642	<0.001*	0.213	0.123	0.304
	Hemoglobin	-2.416	0.016*	-0.033	-0.059	-0.006
	Hematocrit -PCV	0.326	0.745	0.012	-0.058	0.081
	RBCs Count	0.258	0.796	0.074	-0.492	0.641
	MCV	-2.484	0.013*	0.089	0.019	0.159
	MCH	-3.106	0.002*	-0.280	-0.458	-0.103
	MCHC	-3.560	<0.001*	0.282	0.126	0.438
	RDW-CV	0.735	0.463	0.005	-0.008	0.018
	MPV	-2.124	0.034*	-0.065	-0.125	-0.005
	PDW	-.0093	0.926	-0.001	-0.028	0.025
	P-LCR	-0.417	0.677	-0.002	-0.009	0.006
	Fasting blood glucose level	4.157	<0.001*	0.004	0.002	0.006
	Ferritin level	-6.630	<0.001*	-0.002	-0.002	-0.001

*p ≤ 0.05 (Statistically significant)

Table (6). Linear logistic regression for significant variables as predictors for HbA1C in diabetic patients (n = 41)

Predictors	Regressioncoefficient	p	B	95.0% Confidence Interval for B	
				Lower Bound	Upper Bound
Age	0.145	0.885	0.675	-8.791	10.141
Hemoglobin	-1.381	0.177	-5.336	-13.208	2.535
Hematocrit -PCV	0.297	0.768	3.535	-20.698	27.767
MCH	0.035	0.973	0.079	-4.604	4.762
MCHC	0.265	0.792	15.653	-104.516	135.822
RDW-SD	0.250	0.804	7.012	-50.135	64.160
P-LCC	-0.224	0.824	-1.113	-11.229	9.003
Ferritin	-0.230	0.819	-0.258	-2.539	2.023

Figure captions

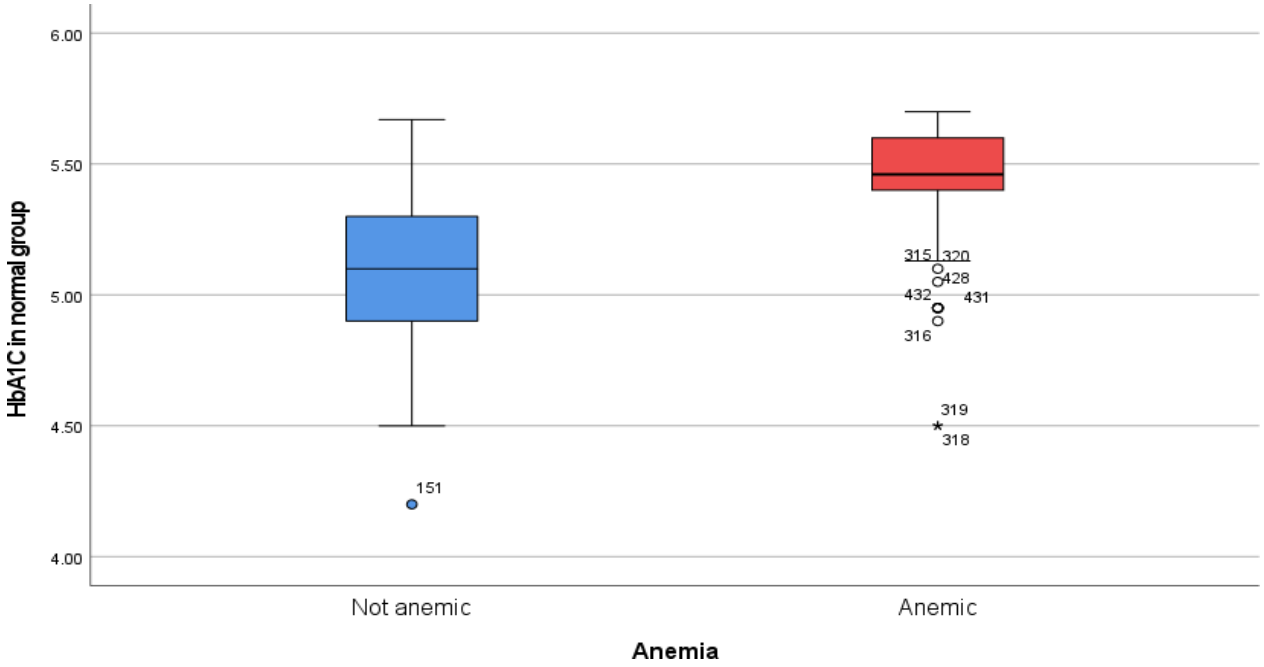

Figure (1) Box plot comparing HbA1C between anemic & not anemic in the non-diabetic patients

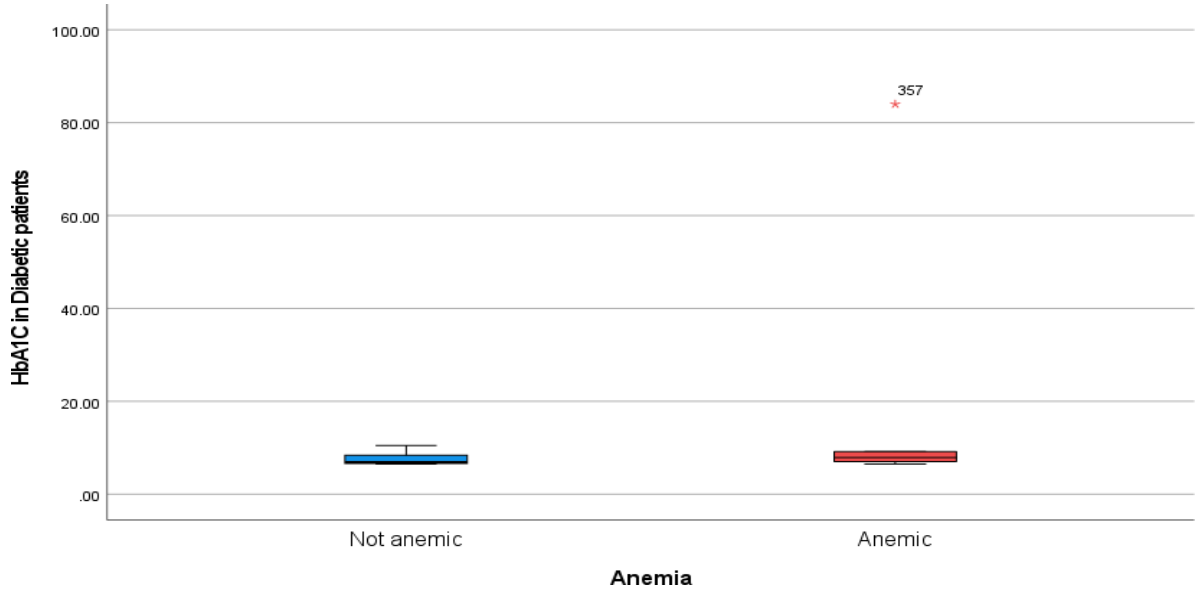

Figure (2) Box plot comparing HbA1C between anemic & not anemic in the diabetic patients

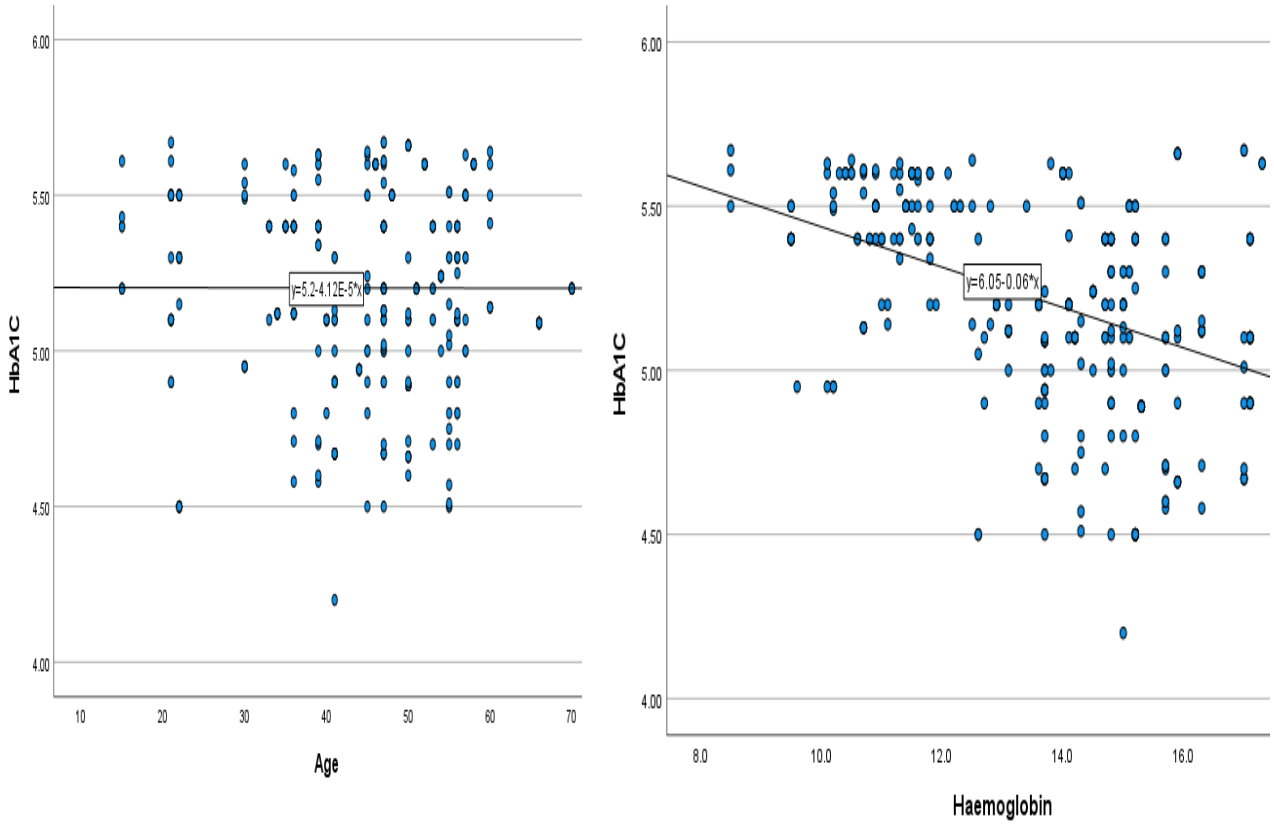

Figure (3) Correlation between HbA1C level from one side, age and hemoglobin level from the other side

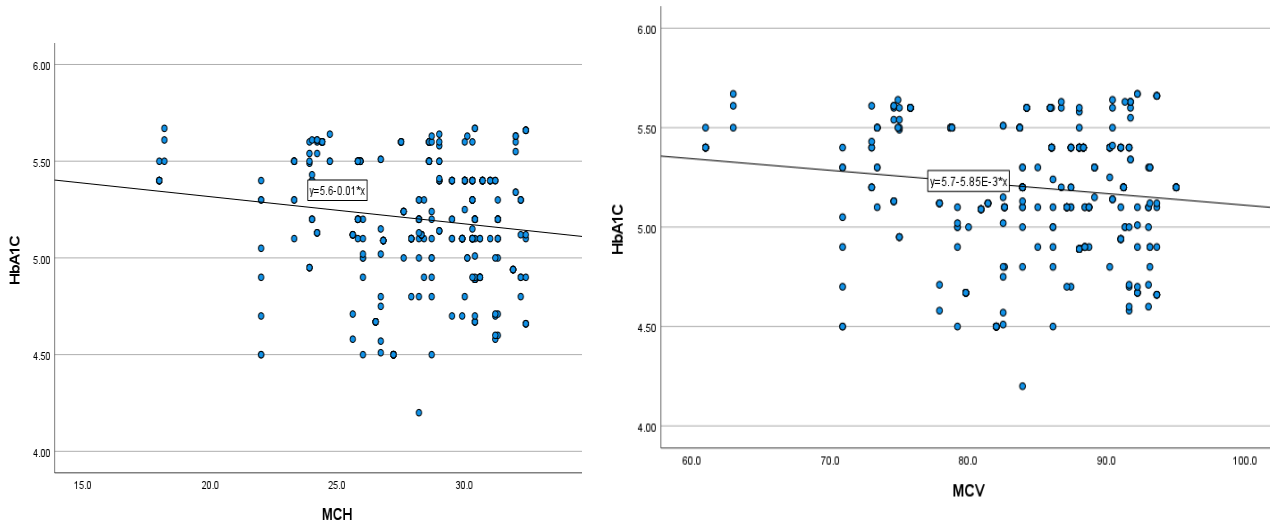

Figure (4) Correlation between HbA1C level from one side, MCH and MCV from the other side

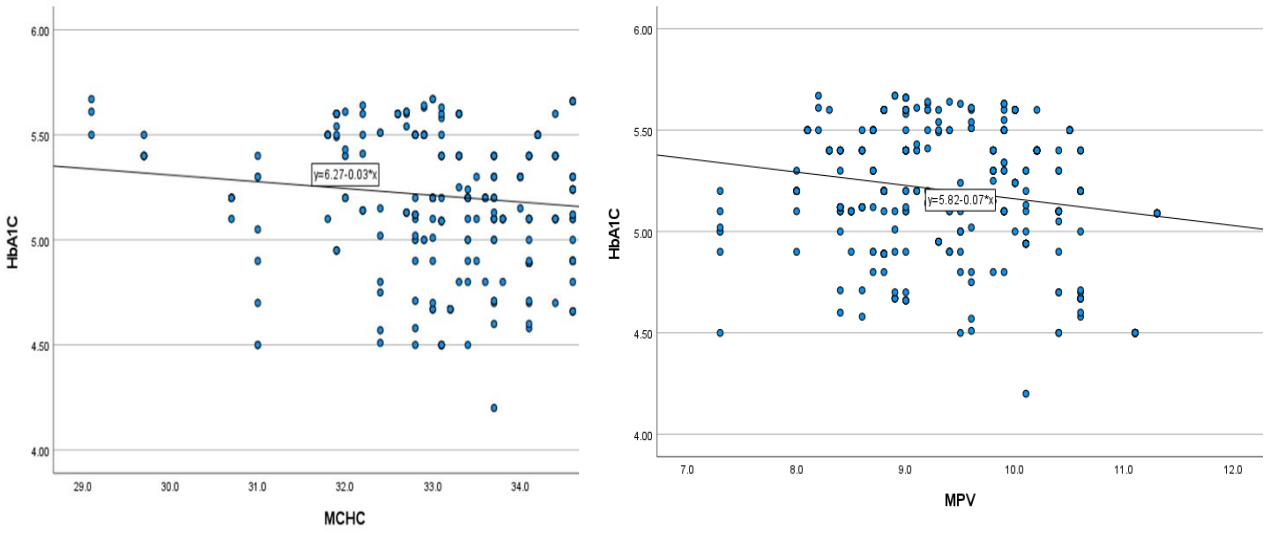

Figure (5) Correlation between HbA1C level from one side, MCHC and MPV from the other side

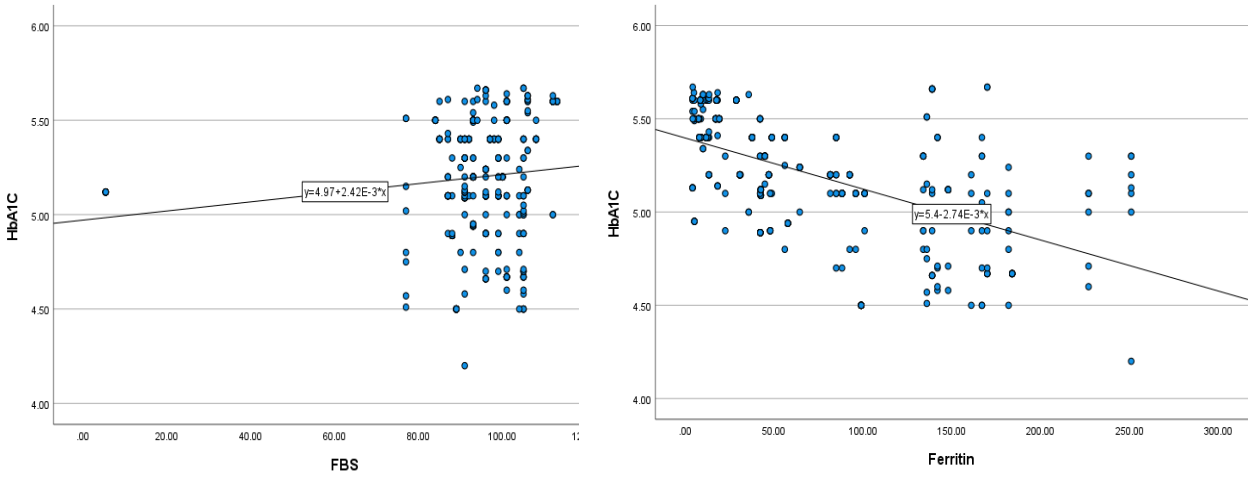

Figure (6) Correlation between HbA1C level from one side, Fasting blood sugar and ferritin level from the otherside

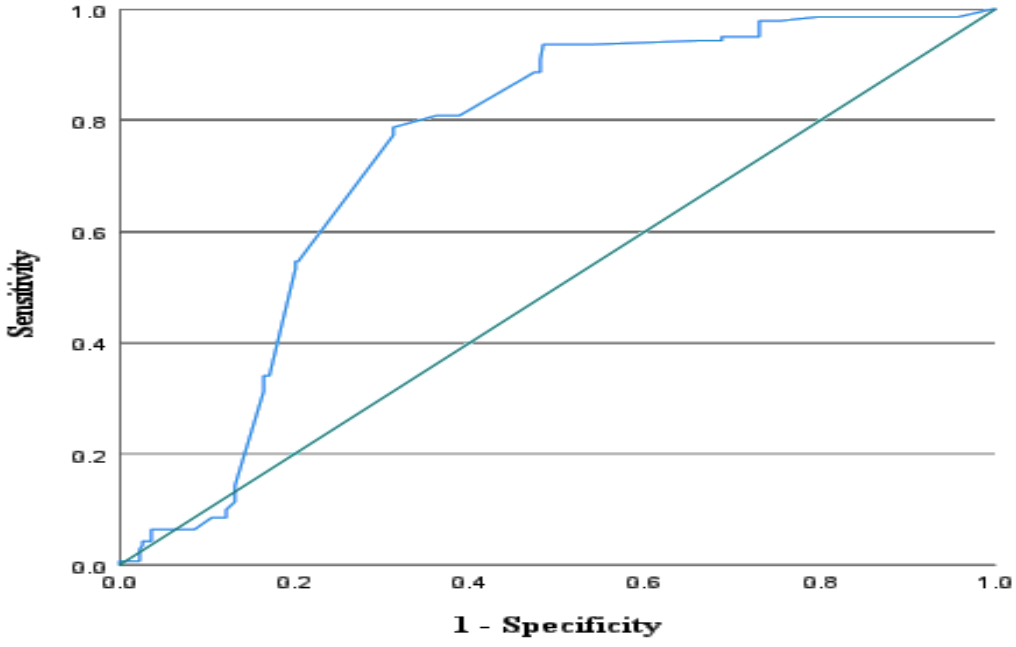

Figure (7) Reciver operting chartectic (ROC) curve illustrating that at cutoff higher than 5.32, HbA1C could significantly predict the anemic status with very good AUC (0.774), 731.9 % accuracy (sensitivity = 78.7% andspecificity = 68.8%), p < 0.001

Discussion

The HbA1c test is widely used in clinical settings due to its convenience and high level of repeatability. This test provides valuable information about the average blood glucose level of individuals over a period of three months. HbA1c measurements can be influenced by a variety of factors, including but not limited to age, ethnicity, genetics, and comorbidities. [19-21]. Anemia is a prevalent medical condition that frequently influences HbA1c levels, prompting numerous research investigations into their interrelationship [22-27]. In order to ensure the correctness of the HbA1c data, the present investigation was conducted on both nondiabetic and diabetic subjects, taking into consideration the impact of hyperglycemia on HbA1c [28]. There is a potential for patients with iron deficiency to be susceptible to an inaccurate categorization of diabetes when relying on HbA1c readings [29]. Hence, it is essential to thoroughly evaluate the pertinent hematologic parameters and risk factors associated with iron deficiency anemia (IDA) in order to prevent incorrect diagnoses and subsequent detrimental impacts on patient well-being. The results of our investigation suggest that individuals diagnosed with both diabetes and iron deficiency anemia (IDA), as well as non-diabetic individuals with IDA, had significantly higher levels of HbA1c in comparison to participants without anemia. The presented statistics align with findings from several investigations, indicating that iron deficiency anemia (IDA) leads to falsely raised readings of HbA1c [30-33]. Conversely, Sinha et al. (2012) [34] found that the average HbA1c levels were considerably lower in the no anemic population compared to the anemic persons from lower socioeconomic backgrounds.

In the current investigation, we have observed negative correlations between HbA1c and Hb, ferritin, and MCHC. There exists a correlation between decreased serum ferritin levels and the prolongation of red blood cell turnover, leading to an enhanced vulnerability of hemoglobin molecules to glycation. Consequently, this process contributes to an elevation in the levels of HbA1c. [28]. The observed association in the current study aligns with findings from prior epidemiological and clinical investigations, wherein elevated levels of HbA1c were detected in individuals diagnosed with IDA [32, 35,36]. Nevertheless, some prior investigations have yielded contradictory findings [37]. The observed disparities in the magnitude and direction of the findings could potentially be attributed to methodological variations, participant heterogeneity, and discrepancies in study design.

The HB A1C measurements of non-diabetic anemic patients were significantly greater than those of non-anemic individuals. HBA1C median was 5.46 in non-diabetic anemic patients, compared to 5.1 in non-diabetic not anemic patients ($p < 0.001$). The same was found in diabetics (median HB A1C = 7.9% in anemic patients vs. 6.91%). However, this change was not statistically significant ($p = 0.249$). Purbey et al (5.92% vs 5.11%) [33], Bharadwaj et al (6.6% vs 5.48%) [38], and Aggarwal et al (6.1% vs 4.1%) [39] reported that mean HbA1c levels among IDA patients were significantly higher.

Iron deficiency changes hemoglobin structure, promoting terminal proline glycation [40] and reducing erythrocyte turnover [41]. Iron deficiency may enhance peroxidation-induced glycation in diabetes. Reactive oxygen species damage during peroxidation. Unstable oxygen radicals react with cell molecules. These active free radicals destroy infections and affect immunological reactions, including immune complexes. However, extra oxygen radicals react with other substances [42]. Iron shortage reduces iron-containing enzyme activity and antioxidant capability [43]. Much recent research [44-45] found that peroxidation promotes glycation, and iron deficiency affects HbA1c levels through oxidative stress [28].

One of the important strengths of our research is the good sample size of both genders and different ages including both diabetic and non-diabetic patients to insure greater accuracy of its results. However, the study included a single health center, so its results cannot be generalized. Lack of long-term follow-up data further posed limitations in studying the relationship between IDA on HbA1c levels.

Conclusion

The study concentrated on the effect of iron deficiency anemia on HbA1c in non-diabetic anaemic patientsand on comparing the hematological parameters between non-diabetic and diabetic patients .There have been different research articles on this topic, but none of them compared the

hematological parameters of diabetic and non-diabetic subjects. Our study concludes that iron deficiency was associated with higher proportions of HbA1c Hence, the iron status must be considered during the interpretation of the HbA1c in diabetics and also non-diabetic subjects. Our data also imply that clinicians should be careful when identifying prediabetes and diabetes in anemic patients. IDA characteristics are strongly correlated with HbA1c in non-diabetics. Therefore, IDA-related variables should be assessed before diagnosing diabetes.

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