Contents lists available at Science-Gate

International Journal of Advanced and Applied Sciences

Journal homepage: http://www.science-gate.com/IJAAS.html

Effect of iron deficiency anemia on glycosylated hemoglobin level (HbA1c) in non-diabetic patients in Riyadh region of Saudi Arabia

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ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form
18 December 2016
Accepted 20 December 2016

Keywords:
Anemia
Iron deficiency
HbA1c

ABSTRACT

Iron deficiency anemia (IDA) is associated with higher HbA1c and higher fructose-amine. We proposed to analyze the effects of iron deficiency anemia on HbA1c levels. The aim of this study is to determine the association of IDA and HbA1c levels in non-diabetic patients in Riyadh region of Saudi Arabia in order to understand if IDA is an important factor influencing HbA1c levels in non-diabetics. In this observational case-control study conducted from December 2014 at College of Applied Medical Sciences, KSU, a complete blood count and biochemical analysis of ferritin, vitamin B12, folic acid level and total iron binding capacity with iron levels in 100 diabetic patients with IDA compared to healthy controls will be performed. All subjects will be selected from Riyadh Region of Saudi Arabia. This study revealed that IDA and corresponding CBC parameters along with vitamin B12, folic acid and liver function test parameters cause significant variations in HbA1c levels. In addition, correlations were observed in percentages of hemoglobin, iron, ferritin and fasting glucose with HbA1c levels in male and female subjects without diabetes. This study indicates that closer monitoring of IDA and all these parameters are needed to improve the overall quality of life of subjects. The study results signify that additional thorough cohort and interventional studies are necessary to assess the role of HbA1c with IDA for effective treatment management.

1. Introduction

Iron deficiency is one of the most widespread nutritional complications affecting 4 – 5 billion people across the world according to the World Health Organization. In developing countries, anemia is prevalent at epidemic levels among nutritionally compromised individuals and presents a major obstacle to the proper growth increasing the risk for mortality and morbidity. Both anemia and iron deficiency anemia (IDA) are identified by a decrease in Hb concentrations and consequently a reduction in the oxygen-carrying ability of the blood. Iron deficiency (ID) entails as a reduction in total body iron to the extent that iron stores are fully exhausted and some degree of tissue iron deficiency anemia is present (Clark, 2008). Iron deficiency is generally viewed as a continuum: iron depletion, iron-deficient erythropoiesis (IDE), and iron deficiency anemia (IDA) (Goddard et al., 2011). The symptoms of IDA are usually non-specific and are typically triggered by blood loss due to hemolysis or increased demand for iron and is characterized by shortness of breath, dizziness, fatigue, frequent infections, loss of appetite etc., (Cook, 2005). Presently about 50% of all cases of anemia are caused by iron deficiency (McLean et al., 2009). Globally, iron deficiency accounts for 841,000 deaths and 35,057,000 DALYs lost. On average while most of the developed countries regions have less than 2% proportion of disability adjusted life years that are attributable to iron deficiency, countries like Africa, India and Saudi Arabia still are at a high risk of IDA (McLean et al., 2009).

IDA is a major public health problem of multi-factorial origin in Saudi Arabia. In Saudi Arabia the overall country prevalence of iron deficiency anemia was 30–56% (Verster and Van Der Pols, 1995). A cross-sectional study conducted in Riyadh City among schoolgirls showed that the prevalence was 40.5% among female adolescents (16–18) years old (Musaiger, 2002). More recently, IDA was assessed in 1210 school girls aged 7-14 year old in Riyadh, Saudi Arabia. Severe anemia was in 1.4% of all children while 8.5% had levels of 8 to < 10 g/dL. A

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https://doi.org/10.21833/ijaas.2017.01.009

nation-wide study conducted in four provinces of Saudi Arabia by El-Hazmi and Warsy (1999) indicated that the diagnosis of IDA in Saudi Arabia becomes more complicated due to the presence of genetical hemoglobin disorders. In addition, in Saudi Arabia grand multiparity, and shorter birth intervals and lower daily dietary intake of iron were reported as important factors. Therefore, IDA is a major public health concern especially in women of Saudi Arabia. In Saudi Arabia, most of the studies on anemia were based on nutritional status and concentrated on preschool children who were under six years old and mostly not conducted in children and adolescents. Previous studies performed in different regions of the Kingdom showed that the overall prevalence of anemia reported among Saudi school students in older age groups ranged from 16.1% (Al-Othaimeen et al., 1999), 20.5% (Abalkhail et al., 2002), and 26.4% (El-Hazmi and Warsy, 1999). Similar supportive studies were conducted in children by Nicklas et al. (1998) and Gari et al. (2008) that indicated a higher prevalence of anemia in children 10-12 years. Using other blood parameters, iron deficiency anaemia among pregnant women was also high.

HbA1c levels are not only affected by blood glucose levels but also altered in hemolytic anemias, hemoglobinopathies, acute and chronic blood loss, pregnancy, and uremia. Glycated haemoglobin (HbA1c) reflects average plasma glucose over the previous eight to 12 weeks (Nathan et al., 2008). Conflicting reports exists as to the role of HbA1c and Iron deficiency anemia (Sinha et al., 2012). In Saudi Arabia maximum of the scientific studies in anemia are focussed on nutritional status and primarily conduced on young children <6 years. This indicates that there is a lack of information in the status of IDA in children above 6 years and in adolescents from the Kingdom (El-Hazmi and Warsy, 1999; Al-Othaimeen et al., 1999; Abalkhail et al., 2002; Al-Hifzi et al., 1996; Hawsawi et al., 2015; Babiker et al., 1989). In addition, the effects of IDA on HbA1c levels in non-diabetic patients indicated that HbA1 levels were considerably elevated with lower iron levels; these levels decreased after treatment with iron (Brooks et al., 1980). Further supportive evidence was obtained from the studies of El-Agouza et al. (2002), Coban et al. (2004) and Shanthi et al. (2013) that HbA1c levels were higher in patients with IDA and reduced substantially upon treatment with iron. Glycation of HbA does not affect oxygen transport, but, glycated Hb has augmented oxygen affinity, and higher levels of glycated Hb.

Therefore, in Saudi Arabia while the knowledge of anemia prevalence is evaluated in women and children, the prevalence in adolescents, men, and the elderly are remote. In addition, the levels of HbA1c and their role in modulating anemia has not been widely assessed. Therefore, the aim of this study was to determine the prevalence of IDA in the Saudi non-diabetic populations and assess the levels of HbA1c in these subjects and correlate with various blood parameters and other variables such as low iron, vitamin B12 and folic acid levels along with liver function test parameters.

2. Materials and methods

2.1. Study design

Identification and screening of eligible patients was carried out at Prince Mohamed Bin Abdulaziz Hospital (PMAH), Riyadh, Saudi Arabia. Data from clinical history (from both parents/guardians and available in the clinical notes) and physical exam were recorded. The patients in this observational case-control clinical study were selected based on plasma iron levels provided they are non-diabetic. Patients with low iron, B12, folic acid and low hemoglobin were chosen. A screening test was conducted to determine iron levels based on hemoglobin levels. Ethical approval (CAMS 16/3536) for the project was obtained College of Applied Medical Sciences, KSU as well as Prince Mohamed Bin Abdulaziz Hospital (PMAH), Riyadh, Saudi Arabia. Written informed consent was sought from participants. Any patients <16 and >50 years of age in both genders, pregnancy, patients with cardiac disease, transfusion history, oncology diagnosis, liver or kidney disease and diabetic patients were excluded.

2.2. Sample collection

5 ml blood samples from 100 patients with IDA (age range: 16-45 years [males: 16-42 years; females: 17-45 years] in Riyadh region were collected from 14/12/2014 for complete blood count (CBC) and biochemical analysis of ferritin, vitamin B12, folic acid level and HbA1c levels with iron levels. The detection of RBCs with characteristics of microcytic hypochromic anemia was evaluated in the microscopic slides. A total of 100 age (age range: 16-38 years) and gender-matched healthy volunteers were included in parallel as controls. Approximately 86% female subjects and 14% male subjects were planned to be included. Among the female subjects, 27% came for pre-employment test, 30% for pre-marriage testing, 18% for routine check-up and 11% for pre-school test. Among the male subjects, about 1% subjects came for pre-school test while the rest came for blood donation.

2.3. Investigations

Complete blood picture investigations were measured on full Automatic blood cell counter hematology analyzer (ADVIA 120 hematology analyser from Seimens) (Meintker et al., 2013). Ferritin levels was conducted based on a two-step test based on chemiliminescent microparticles immunoassay (CMIA) on the ARCHITECT i2000 Assay using system specific reagents. Iron levels were determined by measuring the quantity of iron bound to the protein transferrin on ABBOTT Architect Ci8200/C8000 System. Liver function tests were performed using the Siemens Dimension RxL Max System. Vitamin B12, Folic Acid (folate) and Blood glucose were measured based on Chemiluminescent Microparticle Immunoassay (CMIA) technology in the ARCHITECT Assay System. Hb1Ac levels were determined using the Bio-Rad D-10 Hemoglobin Testing System specific reagents.

3. Results

3.1. Clinical characteristics of the enrolled subjects

The study enrolled 100 subjects aged (16-45 years) in non-diabetic Saudi patients attending Prince Mohamed Bin Abdulaziz Hospital in Riyadh Region of Saudi Arabia. The mean age of patients was 31.8 years with more than 80% being females and rest of them were males. The controls enrolled were 100 healthy volunteers having age 16-38 years. The mean age of controls was 27 years with 64% females and 36% males. Results are shown in Table 1.

Table 1: Clinical characteristics of enrolled subjects

Parameter	**Controls**	**Patients**
N	100	100
Age (range)	16-45 years	16-38 years
Mean age	31.8 years	27.2 years
% females	86%	24%
% males	64%	36%

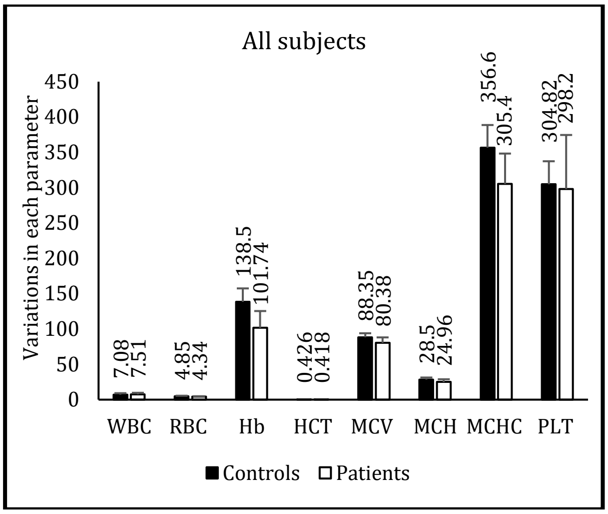
3.2. Comparison between results of all patients and controls in complete blood count

Overall, the total WBC count in controls and patients was 7.08x10⁹/L and 7.51x10⁹/L respectively, while RBC count varied from 4.34-4.85x10¹²/L (Table 2).

The levels of hemoglobin, hematocrit, MCV, MCH, MCHC and platelets, were decreased in all the patients. While there was a significant decrease in RBC count ($p<0.05$) and MCH ($p<0.05$) in patients, the values of Hb ($p<0.001$), HCT ($p<0.01$), MCV ($p<0.001$) and MCHC ($p<0.0001$) were very highly significantly altered. The levels of WBC and platelets were not significantly increased or decreased in patients (Fig. 1; Table 2).

3.3. Comparison between results of all patients and other test parameters

The levels of iron and ferritin in controls remained well elevated with mean levels of 18.18 Umol/L iron and 82.92 Ug/L ferritin. In patients however, the levels of both iron and ferritin were significantly decreased with 4.6 Umol/L iron and 28.5 Ug/L. ferritin. The levels of both iron ($p<0.001$) and ferritin ($p<0.001$) were highly significantly altered in patients (Table 2). Analyses between the levels of iron and hemoglobin in the current study indicated comparable variations.



WBC: white blood cells (10^9/L); RBC: red blood cells (10^{12}/L); Hb: hemoglobin (gm/L); HCT: hematocrit (L/L); MCV: mean corpuscular volume (fl); MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (g/L); PLT: platelets (10^9/L)

Fig. 1: Complete blood picture of all subjects enrolled in the study

Subjects with high iron also had higher levels of hemoglobin and patients with significantly decreased iron levels showed significant decreases in hemoglobin. The levels of Hb in patients significantly decreased from 138.5 g/L to 101.7 g/L ($p<0.001$) whereas the levels of ferritin very highly significantly decreased from 82.92 Ug/L to 28.5 Ug/L ($p<0.0001$). These parameters indicate that iron and hemoglobin are inter-related and influence respective levels (Table 2). The levels of iron and HbA1c were found to be inter-related in the current study. In subjects having high iron levels, the HbA1c levels were considerably lower. In patients, however, there were significant decreases in iron ($p<0.001$) and there was a substantial increase in HbA1c levels from 5.05 in controls to 7.14 ($p<0.0001$) (Table 2). Statistically very high and significant variations were observed in the levels HbA1c ($p<0.0001$) and fasting glucose ($p<0.0001$) test parameters in patients compared to controls. The levels of HbA1c and fasting glucose in controls was 5.05% and 4.89 Mmol/L, the levels were 7.14% and 5.06 Mmol/L in patients, respectively (Table 2). Differences in the levels ALT and AST were observed in patients. While the levels of ALT decreased in patients (18.18 vs 16.94 U/L, $p<0.05$), the levels of AST were similar in both the groups (20.39 vs 20.54 U/L, $p>0.05$) (Table 2). The levels of vitamin B12 decreased significantly in patients compared to healthy controls (302.02 vs 294.84, $p<0.05$). However, the levels of folic acid was not much altered in both groups ($p>0.05$) (Table 2).

3.4. Comparison between results of male patients and controls in complete blood count

The total WBC count was 6.79x10⁹/L in male controls and significantly elevated in male patients to 7.51x10⁹/L ($p<0.001$) while RBC count was 5.24x10¹²/L in controls and significantly decreased ($p<0.01$) in patients to 4.44 x10¹²/L. The levels of hemoglobin were very highly significantly lower in patients (p<0.0001) as well as MCHC (p<0.01) compared to normal healthy controls (Table 3; Fig. 2).

3.5. Comparison between results of male patients and other test parameters

Iron and ferritin levels remained very highly significantly decreased in male patients compared to healthy controls in males. While the levels of iron decreased from 19.18 to 6.08 (p<0.0001) in patients, the levels of ferritin drastically dropped in males subjects from 101.7 to 71.33 (p<0.0001).

The levels of iron and ferritin were normal in healthy controls (Table 3). The levels of iron and hemoglobin in the male subjects of current study indicated comparable variations between both these parameters. Male subjects with high iron (19.28) also had higher levels of hemoglobin (149.7) and patients with highly significantly decreased iron levels (6.08, p<0.0001) showed significant decreases in hemoglobin (108 g/L, p<0.0001). These parameters indicate that iron and hemoglobin are inter-related and influence respective levels (Table 3). The levels of iron and HbA1c in the male subjects correlated with each other in the current study. In male healthy controls having high iron levels (19.28) the levels of HbA1c levels were considerably lower (5.14). In male patients, the iron levels were significantly decreased (6.08, p<0.0001) and also an increase in HbA1c levels to 7.08 (p<0.0001) were observed (Table 3).

Table 2: Summary of Clinical Characteristics of subjects enrolled in this study

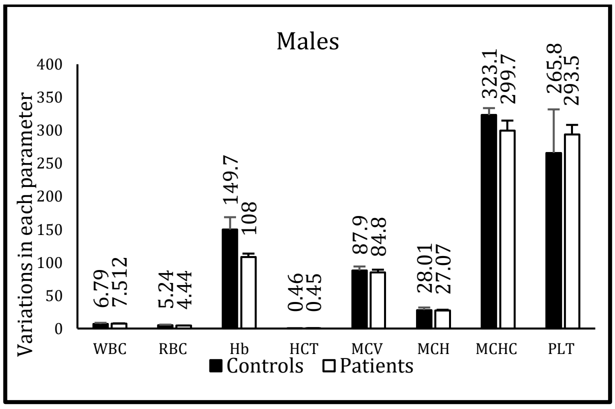
Parameters	Controls		Patients		Two- tailed P. value
	MEAN	SD	MEAN	SD	
COMPLETE BLOOD COUNT					
WBC (10^9/L)	7.08	2.07	7.51	2.19	**>0.05**
RBC (10^{12}/L)	4.85	0.687	4.34	0.53	**<0.05**
Hb (gm/L)	138.5	18.95	101.74	23.64	**<0.001**
HCT (L/L)	0.426	0.056	0.418	0.096	**<0.01**
MCV (fl)	88.35	5.59	80.38	7.73	**<0.001**
MCH (pg)	28.5	2.82	24.96	3.88	**<0.05**
MCHC (g/L)	356.6	32.1	305.4	42.9	**<0.0001**
PLT (10^9/L)	304.82	32.6	298.2	76.4	**>0.05**
LIVER FUNCTION TESTS					
AST (U/L)	18.16	1.60	16.94	1.89	**<0.05**
ALT (U/L)	20.39	1.09	20.54	2.62	**>0.05**
IRON & FERRITIN					
Iron (Umol/L)	18.18	4.63	4.6	2.08	**<0.0001**
Ferritin (Ug/L)	82.92	5.08	28.5	3.42	**<0.0001**
HbA1c & FASTING GLUCOSE					
HbA1c (%)	5.05	0.48	7.14	0.56	**<0.0001**
Fasting glucose (Mmol/L)	4.89	0.413	5.06	0.57	**<0.0001**
VITAMIN B12 & FOLIC ACID					
Vitamin B12 (pmol/L)	302.02	11.10	294.84	9.44	**<0.05**
Folic acid (nmol/L)	22.50	9.81	22.56	9.73	**>0.05**

WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; AST: Aspartate Aminotransferase; ALT: Alanine transaminase

Table 3: Summary of Clinical findings of male subjects enrolled in this study

Parameters	Controls (Males)		Patients (Males)		Two- tailed P. value
	MEAN	SD	MEAN	SD	
COMPLETE BLOOD COUNT					
WBC (10^9/L)	6.79	1.87	7.512	2.19	**<0.001**
RBC (10^{12}/L)	5.24	0.67	4.44	0.49	**<0.01**
Hb (gm/L)	149.7	18.8	108	31.9	**<0.0001**
HCT (L/L)	0.46	0.05	0.45	0.56	**>0.05**
MCV (fl)	87.9	6.01	84.8	8.36	**>0.05**
MCH (pg)	28.01	3.8	27.07	3.52	**>0.05**
MCHC	323.1	10.5	299.7	27.6	**<0.01**
PLT (10^9/L)	265.8	65.9	293.5	80.02	**<0.05**
LIVER FUNCTION TESTS					
AST (U/L)	23.35	1.65	17.85	3.73	**<0.001**
ALT (U/L)	22.45	2.06	18.14	8.52	**<0.01**
IRON & FERRITIN					
Iron (Umol/L)	19.28	4.4	6.08	2.32	**<0.0001**
Ferritin (Ug/L)	101.7	8.45	71.33	1.73	**<0.0001**
HbA1c & FASTING GLUCOSE					
HbA1c (%)	5.14	0.57	7.08	0.65	**<0.0001**
Fasting glucose (mol/L)	4.94	0.44	4.96	0.63	**>0.05**
VITAMIN B12 & FOLIC ACID					
VitaminB12 (pmol/L)	292.78	11.01	257.85	5.82	**<0.01**
Folate (nmol/L)	22.18	9.68	25.2	10.16	**<0.05**

WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; AST: Aspartate Aminotransferase; ALT: Alanine transaminase



WBC: white blood cells (10^9/L); RBC: red blood cells (10^{12}/L); Hb: hemoglobin (gm/L); HCT: hematocrit (L/L); MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (g/L); PLT: platelets (10^9/L)

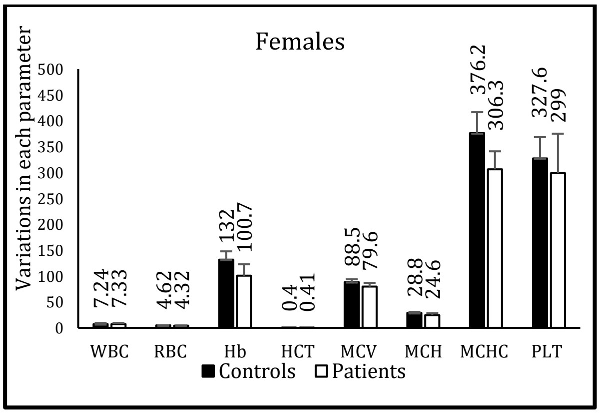
Fig. 2: Complete blood picture of male subjects enrolled in the study

In males, the levels of liver function test parameters were AST (23.35 U/L), ALT (22.45 U/L) in healthy controls while in patients AST (18.14 U/L, $p<0.001$) and ALT (17.85 U/L, $p<0.01$) were significantly decreased (Table 3). The levels of vitamin B12 and folic acid were significantly altered. Unlike the entire population, the levels of vitamin B12 significantly decreased from 292.78 to 257.85 ($p<0.01$) in male patients. However, the levels of folic acid was not significantly altered in male patients compared to healthy controls (Table 3).

3.6. Comparison between results of female patients and controls in complete blood count

The total RBC count decreased significantly in female patients ($4.32x10^{12}$/L, $p<0.05$). The levels of hemoglobin decreased very highly significantly from 132 g/L to 100 g/L ($p<0.0001$) (Table 4, Fig. 3).

There was a significant decrease of MCV ($p<0.05$), MCH ($p<0.01$), MCHC ($p<0.001$) and PLT ($p<0.01$) values in female patients compared to female controls.



WBC: white blood cells (10^9/L); RBC: red blood cells (10^{12}/L); Hb: hemoglobin (gm/L); HCT: hematocrit (L/L); MCV: mean corpuscular volume (fl); MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (g/L); PLT: platelets (10^9/L)

Fig. 3: Complete blood picture of female subjects enrolled in the study

3.7. Comparison between results of male patients and other test parameters

The levels of iron (4.35, $p<0.0001$) and ferritin (21.44, $p<0.0001$) drastically decreased and were significantly altered in female patients compared to healthy controls (Table 4). Healthy controls has substantially better levels of iron and ferritin. Analyses between the levels of iron and hemoglobin in the female subjects recruited in the current study indicated comparable variations. Subjects with high iron (17.5 Ug/L) also had higher levels of hemoglobin (132 g/L). In contrast, patients with significantly decreased iron levels (4.35, $p<0.0001$) showed noteworthy decreases in hemoglobin (100.7, $p<0.0001$) (Table 4). The levels of iron and HbA1c correlated in the female subjects enrolled in the current study. In subjects having high iron levels (17.5), the HbA1c levels were considerably lower (5). In patients, substantial decreases in iron (4.35, $p<0.0001$) were found in subjects who also showed elevated levels of HbA1c (7.15, $p<0.0001$) (Table 4). The levels of fasting glucose were lesser in female healthy controls (4.86) compared to patients (5.07, $p<0.05$), the levels of HbA1c were significantly elevated in patients (5 vs 7.15, $p<0.0001$) (Table 4). In females, the levels of liver function test parameters were AST (18.65 U/L), ALT (15.63 U/L) in healthy controls while the levels were elevated in patients (AST: 16.75 U/L [$p<0.05$], ALT: 20.98 U/L [$p<0.05$]) (Table 4). Unlike male subjects, the levels of both AST and ALT were significantly altered in female subjects compared to controls. While the levels of vitamin B12 decreased significantly ($p<0.01$) in female patients compared to healthy controls, the levels of folic acid were not significantly altered ($p>0.05$) in both groups (Table 4).

4. Discussion

Of the different types of Hb present within the various phases of human life, HbA1c or the glycated haemoglobin has been found to play a very important role in understanding the blood glucose profiles, especially in diabetic patients. Apart from this, it has been reported that HbA1c levels are not only affected by blood glucose levels but also significantly altered in Vitamin B12, folate, and iron deficiency anemias. Early studies by Brooks et al. (1980) and Sluiter et al. (1980) showed a connection between IDA and HbA1c levels. Further studies of Heyningen et al. (1985) and Gram-Hansen et al. (1990) showed no differences between the HbA1c levels in patients with anemia and healthy controls. However, studies also showed that HbA1c levels were higher in patients with IDA and decreased significantly upon treatment with iron (El-Agouza et al., 2002; Coban et al., 2004). These contradicting results and lack of studies from the Saudi-Arabian population fuelled an interest to investigate the effects of IDA on HbA1c levels in non-diabetic patients. Therefore, the objective of the present study was to determine if the HbA1c levels are increased in non-diabetic anemic patients especially in association with IDA. Secondly, the correlation with low levels of vitamin B12 and folic acid have been specifically investigated to understand their role in IDA and levels of HbA1c.

Table 4: Summary of Clinical findings of female subjects enrolled in this study

Parameters	Controls (Females)		Patients (Females)		Two- tailed P. value
	MEAN	SD	MEAN	SD	
COMPLETE BLOOD COUNT					
WBC (10^9/L)	7.24	2.18	7.33	2.16	**>0.05**
RBC (10^{12}/L)	4.62	0.58	4.32	0.54	**<0.05**
Hb (gm/L)	132	15.8	100.7	22.06	**<0.0001**
HCT (L/L)	0.4	0.04	0.41	0.48	**>0.05**
MCV (fl)	88.5	5.37	79.6	7.42	**<0.05**
MCH (pg)	28.8	2.03	24.6	3.85	**<0.01**
MCHC	376.2	40.5	306.3	34.81	**<0.001**
PLT (10^9/L)	327.6	40.8	299	76.2	**<0.01**
LIVER FUNCTION TESTS					
AST (U/L)	18.65	4.88	20.98	2.82	**<0.05**
ALT (U/L)	15.63	9.16	16.75	9.06	**<0.05**
IRON & FERRITIN					
Iron (Umol/L)	17.52	4.65	4.35	1.94	**<0.0001**
Ferritin (Ug/L)	71.87	9.97	21.44	3.56	**<0.0001**
HbA1c & FASTING GLUCOSE					
HbA1c (%)	5.00	0.42	7.15	0.54	**<0.0001**
Fasting glucose (Mmol/L)	4.86	0.39	5.07	0.56	**<0.05**
VITAMIN B12 & FOLIC ACID					
Vitamin B12 (pmol/L)	307.44	11.17	300.94	9.80	**<0.01**
Folic acid (nmol/L)	22.69	9.97	22.13	9.65	**>0.05**

WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; AST: Aspartate Aminotransferase; ALT: Alanine transaminase

This study is novel because these aspects have not been so far investigated in non-diabetic patient population from Prince Mohamed Bin Abdulaziz Hospital, Riyadh Region of Saudi Arabia. A step by step approach was taken up to understand the objectives of the current study. To determine if there is an association between reticulocyte cell counts and HbA1c levels, comparative studies were performed to determine the variations in various blood parameters. The levels of RBC, Hb, HCT, MCV, MCH, and MCHC, were significantly decreased in all the patients. Current evidence suggests that iron deficiency, demonstrated by low Hb, low MCV and low MCH, is associated with increased HbA1c levels. Studies have demonstrated that the survival rate of RBCs are altered in subjects with varying levels of HbA1c which may lead to RBC hemolysis and detection of mild hematologic disorders (Panzer et al., 1983; Coban et al., 2004). This study also showed that the levels of iron and ferritin in patients were significantly decreased with levels as low as 4.6 Umol/L for iron and 28.5 Ug/L for ferritin. The levels of Hb and ferritin very also significantly decreased in patients compared to controls. Our study results provide evidence that the levels of iron and HbA1c were found to be inter-related with subjects having high iron levels showing lower HbA1c levels and vice versa. A study by Sinha et al. (2012) also reported that low values of HbA1c in IDA were improved with increased iron replacement therapy. A study conducted by Ford et al. (2011) showed that in patients without diabetes but with low Hb and normal iron levels, the levels of HbA1c were significantly lower. This suggests that HbA1c may be spuriously elevated in IDA. Interestingly, this study also showed statistically significant variations observed in the levels HbA1c and fasting glucose in non-diabetic patients compared to controls and the levels of ALT decreased in patients. In addition, the levels of vitamin B12 but not folic acid decreased significantly in patients compared to healthy controls. A study by Hardikar et al. (2012) showed that in anaemia 30.8% had vitamin B12 deficiency, 15% had folate deficiency and 30% had multiple nutrient deficiencies. In their study, however, B12 and folate were not significantly related to HbA1c levels; but in the current study while folate is not significantly associated, vitamin B12 levels were significantly altered. This could be because, the study by Hardikar et al. (2012) was conducted in a small patient population while the current study had better sample size.

The current study also performed a sub-group analyses by males or females within the patient and control subjects. This was done to understand the relative role of individually assessed parameters and their probable variations in each sub-group since males and females respond differently in anemia. The total WBC count were significantly elevated while RBC count was significantly decreased in patients. The levels of haemoglobin, MCHC, iron and ferritin very highly significantly decreased in male patients with consequent increase in HbA1c. Unlike females, the levels of fasting glucose were not very altered in male patients; the levels of HbA1c was substantially elevated and significantly decreased levels of vitamin B12 were found. In the female and the majority sub-group also similar results were obtain barring a few alterations. The total RBC count, levels of haemoglobin, MCV, MCH, MCHC and PLT values in female patients was significantly decreased compared to female controls. Similarly, the levels of iron and ferritin drastically decreased and in subjects having high iron levels, the HbA1c levels were considerably lower. The levels of of HbA1c were significantly elevated in patients and the levels of both AST and ALT were significantly altered in female subjects compared to controls. The levels of vitamin B12 decreased significantly but, the levels of folic acid were not significantly altered. We report that IDA was more common among women and HbA1c levels along with vitamin B12, folic acid and various blood parameters and fasting glucose play a major role in its etiology.

In summary, this study revealed that IDA and corresponding CBC parameters along with vitamin B12, folic acid and liver function test parameters cause significant variations in HbA1c levels. In addition, correlations were observed in percentages of hemoglobin, iron, ferritin and fasting glucose with HbA1c levels in male and female subjects without diabetes. This study indicates that closer monitoring of IDA and all these parameters are needed to improve the overall quality of life of subjects. The study results signify that additional thorough cohort and interventional studies are necessary to assess the role of HbA1c with IDA for effective treatment management. Even though iron deficiency is the most common nutritional deficiency, the clinical relevance of iron deficiency on HbA1c has not been thoroughly investigated especially in reproductive-age women. Since women are more vulnerable to IDA, it is important to perform many such studies in this population. Although, a strong evidence supports the use of iron supplementation in adults, very long term iron treatment may cause unnecessary complications related to iron overload. Therefore, long-term follow-up is obligatory for patients with IDA with altered HbA1c levels. This is more important in regions like Saudi Arabia because such studies are less in number indicating a need for long-term comprehensive studies with special attention and timely screening of all subjects for IDA and HbA1c levels in each region. In conclusion, long-term studies are needed to study the effect of treatment options in order to identify more subjects with IDA and thus prevent disease complications.

Acknowledgements

We thank all the volunteers who participated in this study. The Authors would like to extend their sincere appreciation to the College of Applied Medical Sciences Research Centre and the deanship of scientific research at King Saud University for its funding of this research.

Conflict of interest

The authors do not have any conflicting interests with respect to the current study.

Patient consent

Written and verbal consent of patients was obtained before participating the study.

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