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Research Article

Effect of Iron Deficiency Anemia on HbA1c Levels in a cohort of patients with Type 2 diabetes mellitus in a tertiary care hospital

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Abstract

Introduction: Studies have reported that HbA1c levels can be changed by iron deficiency (ID) which is the commonest nutritional anaemia globally. This study was aimed at determining the effect of iron deficiency anaemia (IDA) on HbA1c levels in a cohort of type 2 diabetes mellitus (T2DM) attending a tertiary care hospital in Sri Lanka. Methods: A retrospective analytical study was performed for a period of six months from January 2021. Laboratory records of 281 adult T2DM patients both, anaemic (n=135) with Hb <12g/dL in females and <13g/dL males and transferrin saturation <16%, and non-anaemic (n=146) with Hb >12g/dL in females and >13g/dL in males were analyzed. Data were analyzed using (SPSS) version 20, descriptive statistical methods, Pearson's Correlation test, independent sample t-test, one-way analysis of variance (ANOVA) and Chi-Square test were used. P<0.05 was considered significant. Results: Out of the total 281 patients diagnosed with T2DM, 150 were female, and 131 were male, spanning an age range of 22 to 96 years. The mean ± standard deviation (SD) values for HbA1c in both anaemic and non-anemic groups were 7.4±2.2% g/dL and 7.3±2.36% g/dL, respectively. Importantly, no statistically significant relationship was observed between the two groups (p=0.889). Furthermore, there was no significant correlation between HbA1c levels with serum iron (p=0.617), total Iron Binding Capacity (TIBC) (p=0.340), and transferrin saturation (p=0.168). Conclusion: The study reveals, HbA1c in T2DM patients with and without IDA show no significant difference. Therefore, HbA1c could be reliably used in monitoring patients with T2DM and IDA.

Keywords: T2DM, HbA1c, iron deficiency anaemia, red blood cell indices.

Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disorder characterized by elevated blood glucose levels resulting from varying degrees of insulin resistance and deficiency. Chronic hyperglycemia can lead to multi-organ damage, affecting the renal, neurologic, and cardiovascular systems, as well as causing disruptions in carbohydrate, lipid, and protein metabolism [1,2].

Effective glycemic control is crucial for reducing the morbidity and mortality associated with diabetes. Assessing blood glucose levels in diabetic patients involves measuring Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS), and Post Prandial Blood Sugar (PPBS) [3].

The formation of HbA1c occurs through the glycation of the terminal valine of the β-chain of hemoglobin. HbA1c levels serve as the gold

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standard for diabetes assessment during follow-up. High-performance liquid chromatography is a rapid and convenient method for measuring HbA1c levels, minimally affected by physiological and pharmacological conditions [4].

Thalassemia, structural hemoglobinopathies, and alteration in the quaternary structure of Hb affect the levels of HbA1c [5]. Also, studies have reported that HbA1c levels can be changed by anemia. Anemia is the most prevalent form of nutritional deficiency and, 50% of the anemic burden is associated alone with Iron Deficiency (ID) globally [6].

According to the World Health Organization (WHO),[18] ID is a condition of reduced content of total body iron and the main manifestation of nutritional deficiency. Iron Deficiency Anaemia (IDA) occurs when the iron content is insufficient for erythropoiesis and causes low Hb levels. The most common type of anemia is IDA [5]. There are studies to support the idea that diabetes is influenced by changes in the iron level in the body [7].

Increased glycation of HbA1c is linked with lower levels of serum iron and serum ferritin [8,9]. Research done by Schindler C et al., [10] reported that there is a bidirectional relationship between iron metabolism and glucose homeostasis. This study showed that higher iron levels modulate both the action and secretion of insulin. Coban E et al., [11] showed, that lowering the serum iron levels, increases the glycation of HbA1c, causing its false-high values in both diabetic and non-diabetic groups.

Brooks et al., [12] have reported that excessive glycation of the beta-globin chain occurs due to alteration of the quaternary structure of the Hb molecule in a condition of a relative absence of iron. El Agouza et al., [13] revealed that a constant glucose level and lower levels of Hb can lead to an increase in the glycated fraction of HbA because HbA1c is measured as a percentage of total HbA. Coban et al., [11] showed that patients with IDA had higher HbA1c levels and on treatment with iron treatment HbA1c levels significantly decreased. A case study also showed that HbA1c levels significantly decreased with the correction of IDA [14]. Furthermore, another study revealed that there was a significant difference in mild, moderate, and severe IDA patients with and without T2DM.

In contrast to the above findings, Heyningen et al., [15] and Hansen et al., [16] reported that there was no difference between HbA1c levels in patients with IDA and control. These findings were consistent with the study by Rai et al., [17] who reported no difference in HbA1c levels concerning IDA using different methods to assay HbA1c.

According to the WHO, non-pregnant women who have a blood hemoglobin concentration of 12 g/dL or above are categorized as non-anemic, and subjects who have less than 12 g/dL of blood hemoglobin concentration are categorized as anaemic. Men (15 years of age and above) who have a blood haemoglobin concentration of 13 g/dL or above are categorized as non-anemic and subjects who have less than 13 g/dL of blood haemoglobin concentration are categorized as anaemic [6,18].

Based on the WHO criteria, the anaemic female individuals were further classified as mildly anaemic (haemoglobin concentration between 11.0 and 11.9 g/dL), moderately anaemic (haemoglobin concentration between 8.0 and 10.9 g/dL), and severely anaemic (haemoglobin concentration below 8.0 g/dL). Likewise, anaemic male individuals are also classified as mildly anaemic (haemoglobin concentration between 11.0 and 12.9 g/dL), moderately anaemic (haemoglobin concentration between 8.0 and 10.9

g/dL), and severely anaemic (haemoglobin concentration below 8.0 g/dL) [6,18].

The contradictory results from previous studies have generated uncertainties for clinicians regarding the effectiveness of therapy for diabetic patients with IDA. Consequently, this study is designed to investigate the impact of IDA on HbA1c levels among individuals diagnosed with T2DM. It's important to note that there is limited data on this specific relationship in Sri Lanka, with only one study conducted at the University of Jaffna revealing that diabetic patients with IDA exhibit higher HbA1c levels compared to diabetic patients without IDA [22]. Notably, the mean HbA1c level demonstrated an upward trend corresponding to the severity of anemia in these individuals. These findings underscore the necessity of further research to enhance our understanding of the complex interplay between iron-deficiency anemia and HbA1c levels in the context of T2DM patients.

Methods

Study Design

This study employed a retrospective analytical design.

Study Center

The study was conducted at the Department of Hematology at Sri Jayewardenepura General Hospital in Sri Lanka.

Sample Population, Selection and Size

Inclusion Criteria

The study included adult patients, both males and females, diagnosed with T2DM in the age group of 20 years and above. Both male and female T2DM patients with confirmed iron deficiency anemia (IDA) [Hb < 12 g/dL in females and < 13 g/dL in males with transferrin saturation < 16% (transferrin saturation = iron/transferrin x 1.2),] and non-anemic [Hb>12 g/dL in females and > 13 g/dL in males] groups were incorporated into this research. The anaemic population was categorized into mild, moderate, and severe groups according to the WHO classification [6,18].

Exclusion Criteria

Patients diagnosed with Type 1 diabetes mellitus, hypothyroidism, Cushing's syndrome, chronic systemic illnesses, acute and chronic liver diseases (including acute hepatitis of any cause, viral hepatitis, autoimmune hepatitis, and any history of jaundice), renal disorders, heart failure, pregnancy, cancer, and individuals with a history of acute blood loss, hemolytic anemia, or hemoglobinopathies were not considered in this study.

Data Collection

A random sampling technique was employed between June 2021 and December 2021 to access the laboratory records of 281 patients through the Laboratory Information System (LIS) and patients' records. Recent investigations, including HbA1c, Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) for non-anemic T2DM patients, were documented at the most recent or closest value relative to the date of Full Blood Count (FBC) or HbA1c testing. Additionally, values of serum iron, Transferrin Iron Binding Capacity (TIBC), and saturation were recorded for T2DM patients with IDA at the corresponding most recent or closest time to the date of FBC/HbA1c analysis.

Study Tool

A data extraction sheet was used to enter the demographic, medical, and laboratory details.

Ethical Considerations

Ethical approval was obtained from the Ethics Review Committee of Sri Jayewardenepura General Hospital and Post Graduate Training Centre, Thalapathpitiya, Nugegoda, Sri Lanka.

Statistical Analysis

All numerical and coded data derived from LIS and patients' records were introduced in a database using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistical methods were used to calculate the median, mean, and ± standard deviation of age, HbA1c, red cell indices, and iron profile parameters. Correlations of HbA1c level with age, Hb, serum iron, TIBC, and transferrin saturation were done with Pearson's Correlation method. The p-value, lower than 0.05 was considered statistically significant. The coefficient of determination (R Sq) was used as a statistical measure of how close the data are to the fitted regression line. Age, HbA1c, Hb, and red cell indices were compared by independent sample t-test between anaemic and non anaemic groups for detecting significant differences, and one-way analysis of variance (ANOVA) was used for comparing HbA1c value of anaemic and non anaemic groups according to their age categories. The Chi-Square test was applied for categorical variables such as gender.

Results

Among the 281 patients diagnosed with T2DM, 150 were female, and 131 were male, spanning an age range of 22 to 96 years, with a mean age ± Standard Deviation (SD) of 59.5±15.6 years. Descriptive data for Hb, MCV, MCH, MCHC, and HbA1c in the entire cohort were recorded with mean ± SD values of 11.7±2.4 g/dL, 86.0±10.3 fL, 28.5±3.1 pg, 32.9±1.4 g/dL, and 7.4±2.3%, respectively.

Table 1 and 2 illustrates the descriptive data specifically for anemic and non-anemic diabetes groups.

Mean HbA1c values were 7.1% for the mild anaemic group, 7.0% for the moderate anaemic group, and notably lower at 6.7% for the severe anaemic group. However, no significant difference in HbA1c levels was observed among these three anaemic groups (p=0.16).

The mean ± SD values for HbA1c in the anaemic and non-anaemic groups were 7.4±2.2% and 7.3 ± 2.36%, respectively. No statistically significant relationship (p=0.889) was found between anaemic and non-anaemic groups regarding HbA1c. However, a significant relationship with regard to the Hb and red cell indices were observed between anaemic and non-anaemic groups for Hb (p=0.01), MCV (p<0.001), MCH (p<0.001), and MCHC (p<0.001).

Age groups of both male and female were grouped into anaemic and non-anaemic categories based on the mean values of HbA1c and were compared using ANOVA. No statistically significant relationship was observed among the groups (Table 4).

The mean HbA1c value for anaemic males was lower than non-anaemic males, whereas, for anaemic females, it was higher than non-anaemic females. However, no significant relationship was observed. The mean HbA1c values for anaemic males and females were 7.3% and 7.5%, respectively. Although the mean HbA1c value for non-anaemic males was higher (7.7%) than non-anaemic females (7.0%), no significant relationship was noted among groups of male and female (Table 5).

Pearson's correlation analysis was conducted to assess the relationship between HbA1c and serum iron, total iron-binding capacity (TIBC), and transferrin saturation in the anaemic diabetes population. However, no significant correlation was found between HbA1C and serum iron (p= 0.617), HbA1C and TIBC (p=0.340), as well as transferrin saturation (p=0.168).

Discussion

HbA1c measures average blood glucose over 2-3 months by assessing glucose attachment to

Table 1: Descriptive data of anaemic and non-anaemic population

Variable	Anaemic group (N= 135)				Non-anaemic group (N= 146)			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
Age (yrs)	22	96	64.8	15.1	23	89	54.53	14.3
HbA1C (%)	5.0	13.8	7.4	2.2	4.4	15.8	7.3	2.36
Red cell parameters								
Hb(g/dL)	6.1	12.7	9.6	1.5	12.0	17.9	13.7	1.28
MCV (fL)	74	109.1	83.9	13.5	80.4	102	87.9	5.5
MCH (pg)	26.3	36.9	27.4	3.77	27.7	35.3	29.4	2.0
MCHC(g/dL)	27.2	37.4	32.2	1.5	29.8	35.6	33.5	1.02

Hb = Haemoglobin, HbA1c = Glycated Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration

hemoglobin in red blood cells, which lives for about 120 days. Testing every three months helps manage conditions like diabetes, allowing monitoring, treatment adjustments, and assessment of lifestyle changes. While red blood

Table 2: Descriptive data of mild, moderate, and severe anaemic groups

	Mild anaemic (N = 32)				Moderate anaemic (N = 84)				Severe anaemic (N = 19)			
	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD
Age (yrs)	24.0	96.0	63.4	17.4	22.0	90.0	65.5	14.6	38.0	89.0	64.4	14.15
Hb (g/dL)	11.0	12.7	11.5	0.4	7.6	10.9	9.4	0.86	6.1	7.9	7.1	0.57
MCV (fL)	11.6	98.9	82.6	15.5	57.8	109.1	85.7	10.2	8.4	99.9	78.1	20.2
MCH (pg)	19.2	32.3	27.9	3.2	19.2	36.9	27.6	3.70	15.5	31.4	25.0	4.40
MCHC (g/dL)	29.1	35.2	32.7	1.2	29.0	37.4	32.4	1.4	27.2	34.3	31.0	1.74
HbA1c%	5.0	12.2	7.1	1.8	4.1	13.8	7.0	2.3	3.9	12.2	6.7	2.29
Iron (µmol/L)	5.0	37.0	9.8	6.3	1.0	28.0	9.0	5.4	2.0	13.0	6.75	2.00
TIBC (µmol/L)	29.00	75.00	47.9	12.1	15.00	106.00	45.8	15.26	14.00	93.80	50.4	21.0
Saturation%	7.94	86.05	21.1	14.0	.95	85.19	22.0	15.28	2.38	50.00	16.6	10.0
Gender												
Male	17				29				06			
Female	15				55				13			

Hb = Haemoglobin, HbA1c = Glycated Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin MCHC = Mean Corpuscular Hemoglobin Concentration, TIBC = Transferrin Iron Binding Capacity

Table 3: Comparison of anaemic and non-anaemic groups by independent sample t-test and Chi-Square test

Variables	Anaemia T2DM	Non–anaemia T2DM	p-value
Number	**135**	**146**	
Male	52	79	**0.01**
Female	83	67	
Age (yrs)	64.8±15.1	54.53±14.3	0.586
HbA1C %	7.4±2.2	7.3±2.36	0.889
Hb (g/dL)	9.6±1.5	13.7±1.28	**0.01**
MCV (fL)	83.9±13.5	87.9±5.5	**<0.001**
MCH (pg)	27.4±3.77	29.4±2.0	**<0.001**
MCHC (g/dL)	32.2±1.5	33.5±1.02	**<0.001**

Hb = Haemoglobin, HbA1c = Glycated Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin MCHC = Mean Corpuscular Hemoglobin Concentration

cell turnover varies, HbA1c considers the overall lifespan, providing a stable indicator of long-term glycemic control. In conditions affecting turnover, like hemoglobinopathies, HbA1c interpretation may need adjustments. Overall, HbA1c is valuable for diabetes management in the general population.

The utilization of HbA1c for diabetes screening and diagnosis has increased, prompting clinicians to consider non-glycemic factors influencing HbA1c levels. Investigations reveal that IDA can elevate HbA1c levels independently of fasting glucose levels [15,16,17,20,21]. Different results have been obtained from the research on IDA and HbA1c but there is no clear explanation on the mechanism of how iron deficiency affects HbA1c. The initial study investigating the impact of IDA on HbA1c levels was conducted in the United States. It reported a mean HbA1c concentration of 4.9% for four patients with IDA, in contrast to a mean HbA1c concentration of 5.3% among 14 healthy adults [19]. Hong J W et al., [5] found that the presence of IDA led to an increase in HbA1c levels only within the normoglycemic and prediabetic ranges, not in the diabetic range.

Table 4: Comparison of anaemic and non-anaemic groups according to age category by ANOVA

Age group	Anemia		Non - anaemia		p-value
	Frequency	HbA1c % (Mean)	Frequency	HbA1c % (Mean)	
Female < 60 yrs	30	8.07	45	7.1	0.101
Female >= 60 yrs	54	7.29	22	6.7	0.302
Male < 60 yrs	18	6.8	45	7.4	0.474
Male > =60 yrs	33	7.5	34	8.0	0.222

Table 5: Comparison of male and female anaemic and non-anaemic groups by independence sample t test

Age group	Anemia		Non-anaemia		p-value (Anaemia vs Non-anaemia)
	Frequency	HbA1c %(Mean)	Frequency	HbA1c %(Mean)	
Male	52	7.3	79	7.7	0.268
Female	83	7.5	67	7.0	0.158
p-value (Male vs. female)		0.732		0.067	

Therefore, considering IDA is crucial before using HbA1c as a screening test for prediabetes.

In contrast to the findings of Coban E et al., [11] Brooks et al, and El Agouza et al., [13] a study conducted in Ethiopia discovered that HbA1c is significantly lower in diabetic patients with IDA compared to non-IDA diabetic patients [20]. The researchers emphasized that monitoring diabetic patients based solely on HbA1c levels should be approached with caution. This observation aligns with a study involving 120 subjects by Kalairajan et al., in 2019 [21], demonstrating that IDA leads to a decrease in HbA1c levels, followed by an increase upon correction. The study revealed a positive correlation between Hb and HbA1c in IDA before iron treatment, while no correlation was observed after correcting anemia through oral iron supplementation for three months.

In our study, the association between Hb, red cell indices, and HbA1c in the IDA group did not yield statistically significant differences. Similarly, a study conducted in India [8], no significant correlation was found between HbA1c and MCV (r= -0.23, p=0.06), and borderline significant association was found between HbA1c and MCH (r= -0.58, p=0.05). Concurrently, among the hematological parameters, Hb, MCV, MCH, and MCHC demonstrated a statistically significant mean difference between the two diabetic groups. This observation aligns with a study conducted in India in 2016 [20].

According to the WHO classification, 32 (23.7%) of the patients had mild, 84 (62.2%) had moderate, and 19 (14.1%) had severe anemia. A comparable study conducted in India reported severe anemia in 38 (76%) patients and moderate anemia in 12 (24%) patients [20]. Our study found no significant association between sex, age, and HbA1c in IDA diabetic patients, which was consistent with a similar study conducted in India [8].

Conclusion

The study found no significant difference in HbA1c levels between individuals with T2DM who had IDA and those who did not. Furthermore, the study observed no significant correlation between HbA1c levels and crucial iron parameters such as serum iron, TIBC, and transferrin saturation. This absence of associations suggests that variations in these pivotal iron indicators did not correspond with changes in HbA1c levels within the scrutinized cohort of individuals with T2DM.

Limitations

A limitation in our study was the absence of serum ferritin data for every patient, particularly in the non-anemic group, as this subgroup did not undergo testing for this test. Another limitation of this study is the exclusive focus on HbA1c levels as the primary glycemic marker, without concurrent consideration of FBS and PPBS values. By omitting these parameters, the study may not

capture the full spectrum of glycemic variability in individuals with T2DM. Expanding sample size and integrating FBS and PPBS values into the analysis could enhance the comprehensiveness of the study and offer a more nuanced understanding of the interplay between iron deficiency anemia and various glycemic markers in this population.

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