



www.bioinformation.net
Volume 22(9)



Research Article

Received September 1, 2026; Revised September 30, 2026; Accepted September 30, 2026, Published September 30, 2026

DOI: 10.6026/973206300225879

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478

2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

Declaration on Publication Ethics:

The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at <https://publicationethics.org/>. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Declaration on official E-mail:

The corresponding author declares that lifetime official e-mail from their institution is not available for all authors

License statement:

This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License

Comments from readers:

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

Disclaimer:

Bioinformation provides a platform for scholarly communication of data and information to create knowledge in the Biological/Biomedical domain after adequate peer/editorial reviews and editing entertaining revisions where required. The views and opinions expressed are those of the author(s) and do not reflect the views or opinions of Bioinformation and (or) its publisher Biomedical Informatics. Biomedical Informatics remains neutral and allows authors to specify their address and affiliation details including territory where required.

Edited by Vini Mehta

E-mail: vmehta@statsense.in

Citation: Azees *et al.* Bioinformation 22(9): 5879-5883 (2026)

Comparative analysis of HbA1c level among patients with non-diabetic iron deficiency anemia

Surumi Azees¹, M. Dhanuthra ^{2,*} & Numan A. Mandaty¹

¹Department of General Medicine, Amrita Institute of Medical Sciences & Research Centre, Amrita Vishwa Vidyapeetha, Kochi, Kerala, India; ²Department of General Medicine, KIMS Avitis Hospital, Nenmara, Palakkad, Kerala, India; *Corresponding author

Affiliation URL:

<https://www.amritahospitals.org/kochi>

<https://www.avitishospital.com/>

Author contact:

Surumi Azees - E-mail: 1991surumiazees@gmail.com

M. Dhanuthra - E-mail: dhanuthra@gmail.com

Numan A. Mandaty - E-mail: numanam24@gmail.com

Abstract:

Iron deficiency anaemia (IDA) alters haemoglobin A1C (HbA1c) independently of glucose, distorting glycaemic assessments in non-diabetics. Therefore, it is of interest to compare the HbA1c levels and haematological parameters among 50 non-diabetic patients with IDA and 50 non-IDA subjects. The IDA group showed significantly lower haemoglobin, mean corpuscular volume (MCV), serum iron, and ferritin ($p < 0.001$). A substantial negative correlation linked HbA1c to these iron parameters, indicating that worsening IDA increases HbA1c. Clinicians must account for IDA during HbA1c interpretation to prevent the misdiagnosis of prediabetes or diabetes.

Keywords: Glycated haemoglobin, iron deficiency anaemia (IDA), iron profile, non-diabetic patients, serum ferritin

Background:

Iron deficiency anaemia (IDA), affecting 30% of the population worldwide, has been recognised by the WHO as the most widespread nutritional deficiency. Constitutes a major global health concern. The World Health Organization (WHO) defines anaemia as haemoglobin (Hb) levels below 12 g/dL and 13 g/dL in females and males, respectively [1]. While IDA is predominantly observed in women and children, adult males might also be susceptible depending on their economic circumstances and general health status. Gastrointestinal (GI) bleeding and menstruation are the primary factors of IDA, although other causes, including diminished dietary iron absorption and intake, also contribute [2, 3]. Mature Hb undergoes a gradual, irreversible non-enzymatic process to produce haemoglobin A1C (HbA1c), a glycated form of Hb [4]. This is a gold-standard method for evaluating glycaemic status in diabetic individuals. HbA1c offers a stable assessment of glycemia, less influenced by short-term fluctuations than blood glucose levels. It is endorsed by the American Diabetes Association and the WHO for diagnosing type 2 diabetes, with a normal range for healthy individuals being 4 to 6% [5, 6]. Three principal variables influence the value of HbA1c: the mean lifespan of circulating red blood cells (RBCs), the rate of Hb glycation and the Hb content of reticulocytes at the time of their release from the bone marrow [7]. Nevertheless, there are several clinical diseases other than hyperglycemia that might interact with HbA1c readings. A low HbA1c level is frequently found in conditions that limit RBC longevity, without regard for the assay technique utilised [8]. Red blood cell turnover has been associated with blood loss, haemolysis, hemoglobinopathies, red cell diseases (Thalassaemia, Sickle cell anaemia) and myelodysplastic disease. This, in turn, can result in increased glycation of Hb and elevated HbA1c readings, which is induced by IDA [9]. Several processes explain the rise in HbA1c in anaemic individuals, particularly in IDA, where changes in the quaternary framework of the Hb may enhance the glycation of β -globin chains [10]. Therefore, it is of interest to report the change in HbA1c according to the severity of anaemia and to compare HbA1c levels among non-diabetic individuals with IDA and those without IDA.

Materials and Methods:

The Department of General Medicine at a tertiary hospital conducted a comparative observational study of adult males and

females who were non-diabetic and presented with IDA or without IDA. As per the research by Rajagopal *et al.* [11], to identify a difference of 1.72 (L) in HbA1c levels between two groups, a 95% confidence interval, 90% power and a pooled standard deviation of 2 were assumed. The expected sample size for the study is about 28.4, approximated to 28 in each category. Consequently, 56 participants who meet the eligibility criteria were to be recruited for the study.

$$n = [(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2 \times SD^2] / L^2$$

However, we enrolled 50 patients in each group to enhance the study's power and generalisability, resulting in a total sample size of 100. Data were gathered from August 2022 to August 2024. The study commenced after securing approval from the institutional ethics committee (AJEC/REV/127/2022). The inclusion criteria consisted of: all patients who volunteered to sign the informed consent form, nondiabetic adults over 18 years of age with IDA and those without IDA. The exclusion criteria encompassed patients unwilling to sign the informed consent document, individuals with hemoglobinopathy, chronic liver or kidney disease, pregnant and lactating women, individuals with malignancies of any aetiology, chronic alcoholics, those with a history of acute blood loss, individuals with Vitamin B12 and folic acid deficiencies, patients diagnosed with anaemia other than IDA and those exhibiting abnormal renal function. The study recorded patients' demographic information and conducted standardized clinical and laboratory assessments, including a complete blood count, liver and renal function tests, serum iron, ferritin and blood sugar levels (fasting, post-prandial and HbA1c). Diagnosis of IDA is based on specific criteria: Hb levels below 13 gm/dl for males and 12 gm./dl for females, haematocrit below 40% for males and 36% for females, low mean corpuscular volume (MCV less than 80 fl), mean corpuscular haemoglobin (MCH) less than 26 pg/cell, mean corpuscular haemoglobin concentration (MCHC) less than 32 gm./dl and a microcytic hypochromic peripheral smear, confirmed by serum iron levels below 60 μ g/dl and ferritin levels below 15 μ g/l. Fifty nondiabetic individuals without iron deficiency anaemia were enrolled as controls. Anaemic patients were classified according to haemoglobin levels as follows: mild anaemia (12-12.9 gm/dl for males and 11-11.9 gm/dl for females), moderate anaemia (9-11.9 gm/dl for males and 8-10.9 gm/dl for females) and severe anaemia (<9 gm/dl for males and <8 gm/dl for

females). The study population was categorised into group 1, which included 50 nondiabetic patients with IDA and group 2 comprising 50 nondiabetic patients without IDA. The variables recorded for the study comprised the following:

- [1] Demographic details like age, gender, body weight, height, body mass index (BMI), comorbidities
- [2] General physical examination (Presence or absence of pallor/icterus/clubbing/cyanosis/koilonychia/edema)
- [3] Detailed systemic examination.
- [4] Hb level
- [5] RBC indices: MCV, MCH, RDW
- [6] Serum iron and ferritin levels.
- [7] HbA1c levels
- [8] Fasting and post-prandial blood sugar levels.

Statistical analysis:

Data analysis utilized Graphpad InStat v3.0 software. Quantitative data were represented using Mean and Standard deviation, while qualitative data were expressed as frequency. An unpaired t-test compared group means and Pearson’s coefficient assessed correlation between two variables at a 5% significance level.

Results:

The study consisted of 100 patients >18 years of age, nondiabetic, with or without IDA. The average age of study subjects was 40.2 ± 15.9 years, with a median age of 36 years (interquartile range: 28-48 years). The age range was broad, from 18 to 80 years, suggesting a fairly heterogeneous study population, with most of them being young to middle-aged adults. The age of the study participants is presented in the table. The age group of 21-30 years had the highest number of participants (n=32), followed by the age group of 31-40 years (n=28) and the age group of 41-50 years (n=16). The age group of 51-60 years had the least number of participants (n=5), followed by the 61-70 years and 71-80 years age groups (n=8 each) and only 3 subjects were younger than 20 years. Most of the study population were young and middle-aged adults. Upon evaluation of gender distribution, it was observed that there were 51% males and 49% females. The average age of female participants was 40.37 ± 18.2 years and that of male participants was 40.08 ± 13.65 years. The difference in the average age between males and females was statistically insignificant (p = 0.92), indicating that the age distribution was similar between the males and females. On assessment of past medical history, none of the study participants had a significant medical history. Moreover, only 2 participants had a positive history of cigarette smoking. Others denied a history of addiction. The most common symptom was generalised weakness, reported by 18 subjects, followed by breathlessness (n=16) and fever (n=13). Other presenting complaints were pedal oedema (n=5), headache (n=4), giddiness (n=3), neck swelling (n=3), cough (n=2), weight loss (n=2), abdominal pain, blood in stool, tremors and chest pain (n=1 each). These results indicate that generalised weakness and dyspnoea were the main clinical presentations in the study population. The mean systolic and diastolic blood

pressure and pulse rate of the study participants were 110.7±11.15 mmHg, 70.1±7.8 mmHg and 79.04±7.12 beats/minute, respectively. Mean BMI was 20.48 ± 2.3 kg/m² with a median BMI of 20 kg/m² and a range of 17 to 28 kg/m². According to BMI classification, the most prevalent participants were normal weight (n=56), followed by underweight (n=25), overweight (n=15) and obesity class I (n=4), while no participant was classified as obesity class II. The results indicate that most patients had a normal BMI, with a significant proportion of patients being underweight, which reflects the nutritional challenges that are commonly observed in patients with chronic kidney disease (**Table 1**). The IDA group had significantly lower Hb (10.5 ± 1.01 vs. 14.008 ± 1.6 g/dL, p = 0.0001), MCV (71.1 ± 18.4 vs. 87.1 ± 4.6 fL, p = 0.0001), MCHC (29.2 ± 4.1 vs. 31.2 ± 1.3 g/dL, p = 0.001), serum iron (44.5 ± 10.14 vs. 87.3 ± 14.4 µg/dL, p = 0.0001) and serum ferritin (78.9 ± 16.2 vs. 131.9 ± 41.4 ng/mL, p = 0.0001) than those without IDA. The IDA group had lower MCH (26.6 ± 16.2 pg) than their counterparts (28.6 ± 1.4 pg), but this difference was statistically insignificant (p = 0.19) (**Table 2**). These results demonstrate markedly impaired haematological and iron status in the IDA group than those without IDA. There was no significant difference in neutrophil percentage (60.03 ± 15.5% vs 64.6 ± 10.4%, p = 0.08), lymphocyte percentage (28.4 ± 13.8% vs 32.5 ± 7.4%, p = 0.06) and monocyte percentage (9.3% vs 9%, p = 0.3) between the two groups. However, eosinophil percentage was significantly higher in the IDA group than in those without IDA (3% vs. 1.5%, p = 0.0001). Basophil percentage was also significantly elevated in the IDA group (0.4% vs. 0.2%, p = 0.0001) (**Table 3**). The results indicate important differences in eosinophil and basophil counts between the two groups; while other parameters of leukocytes were similar Blood sugar profile of the two groups is shown in (**Table 4**). Fasting blood sugar (98.3±7.9 vs. 91.3±7.9 mg/dL, p=0.0001), post-prandial blood sugar (135.8±16.18 vs. 110.32±11.27 mg/dL, p=0.0001) and HbA1c levels (6.2±0.4% vs. 5.006±0.2%, p=0.0001) were significantly higher in IDA subjects compared to non-IDA subjects. These results show significantly worse glycaemic control between the groups. Haemoglobin (r = -0.70, p < 0.00001), mean corpuscular volume (MCV) (r = -0.40, p = 0.00003), serum iron (r = -0.776, p < 0.00001) and serum ferritin (r = -0.60, p < 0.00001) were significantly negatively correlated indicating that these parameters decreased as the study variable increased. By contrast, MCH (r = -0.0863, p = 0.30) and MCHC (r = -0.1006, p = 0.30) had weak negative correlations which were statistically insignificant (**Table 5**). Overall, haemoglobin, MCV, serum iron and ferritin were found to have significant inverse relationships with the study variable.

Table 1: Vital parameters and BMI distribution of the study sample

Vital parameters	Results
Mean SBP (in mmHg)	110.7 ± 11.15
Mean DBP (in mmHg)	70.1 ± 7.8
Mean Pulse rate (in beats/minute)	79.04 ± 7.12
BMI distribution	Results
BMI (Mean±SD in kg/m²)	20.48±2.3
Median [BMI Interquartile range] (in kg/m²)	20 [18.5-22]
Minimum BMI (in kg/m²)	17
Maximum BMI (in kg/m²)	28

Underweight (< 18.5 kg/m ²)	25
Normal weight (18.5-22.9 kg/m ²)	56
Overweight (23-24.9 kg/m ²)	15
Class I obesity (25-29.9 kg/m ²)	4
Class II obesity (≥ 30 kg/m ²)	0

Table 2: Comparison of Iron Study among those with and without IDA

Iron study	With IDA (n=50)	Without IDA (n=50)	p- value
Hb (g/dl)	10.5 ± 1.01	14.008 ± 1.6	0.0001*
MCV (fl)	71.1 ± 18.4	87.1 ± 4.6	0.0001*
MCH (pg)	26.6 ± 16.2	28.6 ± 1.4	0.19
MCHC (g/dL)	29.2 ± 4.1	31.2 ± 1.3	0.001*
Serum iron (mcg/dL)	44.5 ± 10.14	87.3 ± 14.4	0.0001*
Serum ferritin (ng/mL)	78.9 ± 16.2	131.9 ± 41.4	0.0001*

*significant (Unpaired t-test)

Table 3: Comparison of Hematocrit values between the two groups

Hematocrit values	With IDA (n=50)	Without IDA (n=50)	P-value[Unpaired t-test]
Neutrophil %	60.03 ± 15.5	64.6 ± 10.4	0.08
Lymphocyte %	28.4 ± 13.8	32.5 ± 7.4	0.06
Eosinophil %	3 [1.2-5]	1.5 [0-3]	0.0001*
Monocyte %	9.3 [6.6-10]	9 [8-10]	0.3
Basophil %	0.4 [0.2-0.6]	0.2 [0.1-0.2]	0.0001*

*significant

Table 4: Comparative analysis of Blood sugar variables in cases and controls:

Blood sugar profile	With IDA (n=50)	Without IDA (n=50)	P-value
Fasting blood sugar (mg/dL)	98.3 ± 7.9	91.3 ± 7.9	0.0001*
Post-prandial blood sugar (mg/dL)	135.8 ± 16.18	110.32 ± 11.27	0.0001*
HbA1c (%)	6.2 ± 0.4	5.006 ± 0.2	0.0001*

*significant (Unpaired t-test)

Table 5: Pearson's correlation analysis of HbA1c values with variables of Iron study

Variables of Iron study	r- score	P- value
Hb (g/dL)	-0.7	<0.00001*
MCV (fl)	-0.4	0.00003*
MCH (pg)	-0.0863	0.3
MCHC (g/dL)	-0.1006	0.3
Serum iron (mcg/dL)	-0.776	<0.00001*
Serum ferritin (ng/mL)	-0.6	<0.00001*

*significant

Discussion:

IDA is the most prevalent nutritional deficiency and poses a significant public health challenge, especially in developing nations. Recent findings indicate that IDA can influence HbA1c levels regardless of glycaemic status, potentially resulting in the misdiagnosis of diabetes and pre-diabetes. This study assessed HbA1c levels in non-diabetic individuals with IDA, comparing haematological, iron profile, inflammatory and glycaemic parameters with those of healthy controls. The average age of the study population was 40.2 ± 15.9 years, ranging from 18 to 80 years. Most participants were aged 21–30 and 31–40, with no significant gender difference in age distribution (p = 0.92). Such a similar demographic profile minimises confounding effects of age and sex on the interpretation of haematological and glycaemic parameters. Recent studies examining the effect of IDA on HbA1c have reported similar age distributions, with the majority of participants being young and middle-aged adults [12, 13]. Generalised weakness was the prevalent complaint,

with breathlessness and fever also reported. These findings are expected since the decreased oxygen-carrying capacity from iron deficiency often is associated with fatigue, generalised weakness and exertional dyspnoea. Clinical reviews of IDA have described similar clinical presentations with constitutional symptoms often preceding severe haematological abnormalities [14, 15]. The present study proved significantly lower Hb, MCV, MCHC, serum iron and serum ferritin levels in the IDA subjects compared to the non-IDA subjects and thus confirmed the diagnosis of IDA. Serum ferritin is the key sensitive biochemical indicator of body iron stores. Decreased MCV and MCHC are indicative of the typical microcytic hypochromic anaemia of iron deficiency [14, 16]. IDA group had lower MCH, but the difference was statistically insignificant and suggests that MCH alone might be less sensitive than other erythrocyte indices for iron deficiency. Correlation analysis also showed significant negative correlations of HbA1c with Hb, MCV and serum iron and serum ferritin. These findings suggest a progressive rise in HbA1c levels with increasing iron deficiency; in the absence of diabetes. This finding is concurrent with previous reports indicating that increased erythrocyte lifespan and glycation of circulating Hb in IDA lead to falsely elevated HbA1c values [17, 18]. Iron repletion has been shown to decrease HbA1c without significant changes in plasma glucose concentrations, supporting the influence of iron status on the interpretation of HbA1c [18, 19]. The critical finding of the present study was that the blood glucose levels (fasting, postprandial and HbA1c) were significantly higher in IDA subjects than non-IDA subjects. The IDA subjects showed significantly higher mean HbA1c, even though fasting and postprandial glucose levels were within the non-diabetic range. These findings support the hypothesis that iron deficiency affects HbA1c measurement independently and may lead to overestimation of long-term glycaemic status. HbA1c needs to be interpreted cautiously in IDA, one of the conditions with abnormal erythrocyte turnover according to recent guidelines from the American Diabetes Association and other expert panels [20]. The differential leukocyte count revealed significantly elevated eosinophil and basophil percentages in cases, whereas neutrophil, lymphocyte and monocyte counts were similar in both groups. Eosinophilia and basophilia are not classic findings in IDA, but mild alterations in leukocyte subsets may occur secondary to chronic inflammatory states, allergic disorders, parasitic infestations or nutritional deficiencies. Therefore, it is important to exercise caution when interpreting these results, as they might not be directly correlated with iron deficiency [21]. The present findings have clinical implications. HbA1c may be falsely elevated in patients with IDA and relying on HbA1c alone to diagnose diabetes or pre-diabetes may result in misclassification and unnecessary treatment. Therefore, evaluation of haemoglobin and iron status should be considered before interpreting elevated HbA1c values in individuals without established diabetes, especially in populations where iron deficiency is highly prevalent [20, 22]. Addressing iron deficiency before evaluating long-term glycaemic status might enhance diagnostic accuracy and avoid inappropriate clinical decisions. The present study had the

strength of including a non-IDA group and a comprehensive evaluation of haematological, biochemical and glycaemic parameters. However, the relatively small sample size and cross-sectional design impede causal inferences and post-treatment HbA1c measurements were not available to assess changes following iron therapy. Further prospective multicentre investigations are warranted to elucidate the magnitude of variations in HbA1c after correction of iron deficiency and also to identify appropriate thresholds for interpretation of HbA1c in populations with iron deficiency. Overall, the current study findings underscore the importance of evaluating iron status before making a diagnosis of diabetes or prediabetes based on HbA1c values alone and emphasise that iron deficiency should be considered as an important confounder in the interpretation of HbA1c. Long-term and large-scale studies are needed to provide accurate information regarding the effects of IDA on HbA1c.

Conclusion:

Individuals with iron deficiency anaemia (IDA) exhibited substantially higher haemoglobin A1C (HbA1c) values and an inverse correlation with iron parameters. Clinicians should evaluate and rule out IDA before diagnosing diabetes or altering treatment courses based on glycemic status. Iron deficiency must be considered a crucial confounder to prevent misdiagnosis when interpreting HbA1c levels.

Advancement to Knowledge:

Before diagnosing diabetes or prediabetes based on HbA1c alone, iron status should be checked. Iron deficiency anemia can confound HbA1c interpretation, with lower Hb, MCV, serum iron and ferritin often linked to higher HbA1c levels. So, IDA should be ruled out before changing treatment.

References:

- [1] Kumar A *et al.* *BMJ Open Gastroenterol.* 2022 **9**:e000759. [PMID: 34996762]
- [2] Cotter J *et al.* *World J Gastroenterol.* 2020 **26**:7242. [PMID: 33362380]
- [3] Kolarš B *et al.* *Pharmaceuticals.* 2025 **18**:1104. [PMID: 40872496]
- [4] Marin MJ *et al.* *J Hematol.* 2024 **13**:99. [DOI: 10.14740/jh1268]
- [5] Chen J *et al.* *Cardiovasc Diabetol.* 2023 **22**:146. [PMID: 37349787]
- [6] Sakamoto N *et al.* *J Diabetes Investig.* 2020 **11**:719. [PMID: 31605656]
- [7] Gulavani GA *et al.* *Int J Clin Biochem Res.* 2023 **10**:123. [DOI: 10.18231/j.ijcbr.2023.020]
- [8] Abass AE *et al.* *Clin Pract.* 2017 **7**:999. [PMID: 29071062]
- [9] Alzahrani BA *et al.* *BMC Endocr Disord.* 2023 **23**:24. [PMID: 36709277]
- [10] Williams A *et al.* *Pathophysiology.* 2023 **30**:327. [PMID: 37606388]
- [11] Rajagopal L *et al.* *J Clin Diagn Res.* 2017 **11**:EC13. [PMID: 28384869]
- [12] <https://pubmed.ncbi.nlm.nih.gov/39651986/>
- [13] Gülsen S *et al.* *J Med Biochem.* 2023 **42**:714. [PMID: 38084249]
- [14] Camaschella C. *Blood.* 2019 **133**:30. [PMID: 30401704]
- [15] Rubbani A *et al.* *Cureus.* 2025 **17**:e84386. [PMID: 40535375]
- [16] Cappellini MD *et al.* *J Intern Med.* 2020 **287**:153. [PMID: 31665543]
- [17] Kumar D *et al.* *Pak J Med Sci.* 2023 **39**:956. [PMID: 37492295]
- [18] AlQarni AM *et al.* *J Clin Med.* 2023 **12**:7287. [PMID: 38068338]
- [19] Mukasa RJ *et al.* *PLoS One.* 2026 **21**:e0323034. [PMID: 41628151]
- [20] <https://pubmed.ncbi.nlm.nih.gov/39651989/>
- [21] Weiss G *et al.* *Blood.* 2019 **133**:40. [PMID: 30401705]
- [22] <https://journalahrj.com/index.php/AHRJ/article/view/170>

Caveat Emptor is applicable among the literate community where required and possible. The publisher, its journal, editors and the internal/external reviewers take adequate steps to check, evaluate, correct, edit, revise and improve content where possible and required.