IMPACT OF IRON DEFICIENCY ANEMIA ON THE MEASUREMENT OF HbA1c IN NON-DIABETIC POPULATION

ABSTRACT

Background: Iron deficiency anemia (IDA) is a prevalent condition with significant implications for public health. The impact of IDA on glycated hemoglobin (HbA1c) levels in non-diabetic individuals remains underexplored.

Aims: This study aimed to investigate the effect of IDA on HbA1c levels by comparing the HbA1c levels of non-diabetic patients havingIDA and age/sex matched healthy controls. The study also compared the mean HbA1c levels with the severity of anemia, and the correlation between HbA1c and hemoglobin levels.

Study design: Case-control study

Place and Duration of Study: The study was conducted in the Medicine Emergency, Outpatient Department, and Medicine wards of the University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi from September 2022 to February 2024.

Methodology: We included 100 participants aged 18 to 65 years which comprised 50 lab-confirmed IDA cases and 50 age/sex matched healthy controls, after excluding those with diabetes or on hematinics. Detailed clinical history, examination, and investigations were carried out. Diagnosis of IDA was based on specific hematological parameters and confirmed by using serum iron and ferritin levels. Anemia severity was categorized based on hemoglobin levels into mild, moderate, and severe groups.HbA1c levels were measured using the standard HPLC method. The data was analyzed using SPSS software. The continuous variables were presented as means and standard deviations and the strength of correlation between variables was determined by Spearman's correlation coefficient.

Results: The mean HbA1c level was higher in the case group at 5.07% (SD = 0.42) compared to the control group at 4.68% (SD = 0.49) with ($P< .001$). A trend of increasing mean HbA1c values with the increasing severity of anemia was observed with mild, moderate, and severe anemia having mean HbA1c of 4.92%, 5.15%, and 5.20% respectively. We found a significant negative correlation between HbA1c and hemoglobin ($P< .001$).

Conclusion: The effect of IDA should be considered while interpreting HbA1c values.

Keywords: Iron deficiency anemia; anemia; glycated hemoglobin; HbA1c; diabetes mellitus

1.INTRODUCTION

The high prevalence of IDA and type 2 diabetes mellitus (T2DM) globally burdens public health, reducing life quality and causing patient suffering. Anemia is a condition in which hemoglobin concentration and/or red blood cell numbers are lower than normal and insufficient to meet an individual's physiological needs.[1] Anemia affects one-third of the world's population, contributing to increased morbidity and mortality.[2] Iron which is crucial for hemoglobin, is primarily deficient in anemia, progressing through stages: storage iron depletion, iron-deficient erythropoiesis, and IDA. Various factors such as age, gender, and socioeconomic status, influence iron deficiency.Treatment involves addressing the underlying cause and iron supplementation.[1]

The International Expert Council of the American Diabetes Association (ADA) incorporated hemoglobin A1c as a diagnostic tool for diabetes mellitus (DM) in 2009.[3] Elevated HbA1c levels correlate with chronic hyperglycemic DM and indicate poorer glucose control. HbA1c levels, reflecting average blood glucose over 2-3 months, are influenced by factors such as blood glucose control and the effectiveness of diabetes management, including diet, exercise, and medication. Other factors affecting HbA1c include variations in hemoglobin, red blood cell lifespan, kidney function, pregnancy, ethnicity, medications, stress, illness, age, smoking, and miscellaneous conditions like iron deficiency and hemoglobinopathies. Thus, healthcare providers must scrutinize non-glycemic factors that could impact a patient's HbA1c levels.[4,5]

Despite the importance of accurate HbA1c interpretation, the relationship between IDA and HbA1c remains complex and requires further research. Particularly in developing nations like India, where both IDA and DM prevail, understanding this relationship is crucial. However, extensive research is lacking on this topic, with disparities in results and study populations, indicating a significant knowledge gap. This study aims to elucidate the impact of IDA on HbA1c levels in non-diabetic patients and investigate potential correlations with anemia severity and hemoglobin levels.

Aim and objectives of the study: This study aimed to examine the influence of IDA on HbA1c levels among individuals without diabetes. The primary objective was to compare HbA1c levels between non-diabetic patients diagnosed with IDA and age/sex matched healthy controls. Secondary objectives included investigating the association between mean HbA1c levels and the severity of anemia, as well as exploring potential correlation of HbA1c with hemoglobin levels.

2. MATERIAL AND METHODS

The study was carried out at the Department of Medicine, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, and employed a case-control study design spanning from September 2022 to February 2024. The sample size was determined based on the mean (SD) of HbA1c among anemics and non-anemics, which were 7.6 (0.5) and 5.5 (0.8) respectively, from a previously published study by Shanthi B et al. in Chennai.[6] With a type 1 error of 5% and power of 80%, 50 subjects were included in each group to obtain a normal distribution for robust significance testing.

Institutional ethical clearance was obtained. After obtaining written and informed consent, the study subjects were evaluated using clinical history, examination, and investigations.

The inclusion criteria for cases comprised individuals aged 18 to 65 years diagnosed with IDA who were not currently receiving hematinics. Those having hemoglobin <13 g/dl in males and <12 g/dl in females, hematocrit <40% in males and <36% in females, mean corpuscular volume (MCV) <80 fl, mean corpuscular hemoglobin (MCH) <26 pg/cell, mean corpuscular hemoglobin concentration (MCHC) <32 g/dl and peripheral smear showing microcytic hypochromic picture were considered to have IDA and confirmed by their serum iron (<60 µg/dl) and serum ferritin (<15 µg/l) levels.[7]Based on hemoglobin levels, anemic patients were categorized as mild anemia (11-12.9 g/dl for males and 11-11.9 g/dl for females), moderate anemia (8-10.9 g/dl for both genders), and severe anemia (<8 g/dl for both genders).[1]Medical history was recorded to exclude subjects having fasting plasma glucose >126 mg/dl or 2-hour postprandial plasma glucose >200 mg/dl or random plasma glucose >200 mg/dl with symptoms of hyperglycemia. Patients with a history of chronic alcohol ingestion, kidney diseases, liver diseases, hemoglobinopathies, hemolytic anemia, acute blood loss, repeated blood transfusions, and pregnant patients were also excluded. The control group comprised age and sex-matched healthy subjects. Diabetics were excluded using the ADAcriteria.[8]

Blood samples were withdrawn from the antecubital vein under aseptic precautions and collected into plain and EDTA vacutainers for the estimation of various biochemical and hematological parameters. Three ml of whole blood was taken in EDTA vials, and HbA1C was measured by the HPLC method (D-10, BioRad, USA).

Statistical analysis: The data was entered into a Microsoft Excel spreadsheet and cleaned. SPSS 20.0 software was used for analysis. Continuous variables such as HbA1c and hemoglobin were presented as means and standard deviations. Independent sample t-test was utilized to compare the HbA1c values between the anemic and non-anemic groups. All tests were two-tailed. Spearman's correlation coefficient was computed to determine the strength of correlation between the different variables. A p-value of less than 0.05 was considered statistically significant, and less than 0.001 was considered highly significant.

RESULTS

The case group exhibited a mean age of 32.08 ± 10.91 years while the control group had a mean age of 33.68 ± 10.84 years. Both groups exhibited an equal proportion of males and females, with 22 males constituting 44% and 28 females comprising 56% of each group. All the participants in our study were literate and had no smoking or drinking habits. The relevant difference in mean hemoglobin levels between the case group was 9.93 ± 1.87 g/dL and the control group 13.56 ± 1.20 g/dL, with significantly lower hemoglobin concentrations observed in the case group as shown in Table 1. The number of participants with mild, moderate, and severe anemia was 19, 24, and 7 respectively, in the case group.

The proportion of individuals in the case group adhering to a mixed diet (48%) was found to be lower than the control group (72%). The mean hemoglobin level for those with a mixed diet stood at 10.96 (SD = 1.17), significantly surpassing the mean hemoglobin level of 8.98 (SD = 1.90) observed in individuals adhering to a vegetarian diet. This distinction was further highlighted by the median hemoglobin levels, where the mixed diet group demonstrated a median of 11.2 (IQR: 10.45-11.8), in stark contrast to the median of 9.05 (IQR: 7.95-10.33) observed in the vegetarian diet group as shown in table 2.

The mean HbA1c level in the case group was notably higher at 5.07% (SD = 0.42) compared to the control group, which exhibited a lower mean HbA1c of 4.68% (SD = 0.49) ($P < .001$). The median HbA1c of the case group was 5.1% (IQR: 4.82-5.4), whereas the control group displayed a lower median of 4.6% (IQR: 4.3-5.1) as shown in Table 3. The IQR illustrates the spread of values, with the case group exhibiting a wider range compared to the control group, potentially indicating greater variability in glycemic control within the case group.Mean HbA1c levels increased with the severity of anemia. Kruskal Wallis Test was used to make group comparisons. The WNL (Within normal limits) category has the lowest mean HbA1c (4.68%), followed by mild anemia (4.92%), moderate anemia (5.15%), and severe anemia (5.20%). The median HbA1c values reflected a similar trend, with higher values observed as anemia severity increased. The *P* value (< .001) indicated a highly significant difference in mean HbA1c levels across different hemoglobin inference categories (table 4, figure. 1).

Our study found a moderate negative correlation between HbA1c and hemoglobin (Spearman correlation coefficient = -0.5), and this correlation was statistically significant (*P*<.001) (figure2).

Table 1: Comparison between cases and controls

Parameter	CASE (n=50)	CONTROL (n=50)	P value
Age (in years)	32.08 (10.91)	33.68 (10.84)	0.26
Male	22	22	1
Female	28	28	1
Mixed diet	24 (48.0%)	36 (72.0%)	0.014
Vegetarian diet	26 (52.0%)	14 (28.0%)	0.014
Weight (kg)	67.46 ± 12.44	70.62 ± 11.11	0.184
Height (m)	1.64 ± 0.09	1.65 ± 0.11	0.912
BMI (Kg/m²)	24.89 ± 3.31	26.02 ± 2.96	0.225

Hemoglobin (g/dL)	9.93 ± 1.87	13.56 ± 1.20	<0.001

Table 2: Association between hemoglobin and diet

Hemoglobin (g/dL)	Diet		
	Mixed	Vegetarian	p value
Mean (SD)	10.96 (1.17)	8.98 (1.90)	<0.001
Median (IQR)	11.2 (10.45-11.8)	9.05 (7.95-10.33)	
Min - Max	8.5 - 12.5	5.2 - 12.5	

Table 3: Comparison of HbA1c between study groups

HbA1c (%)	Group		
	Case	Control	p value
Mean (SD)	5.07 (0.42)	4.68 (0.49)	<0.001
Median (IQR)	5.1 (4.82-5.4)	4.6 (4.3-5.1)	
Min - Max	4.1 - 5.6	4 - 5.6	

Table 4: Association between HbA1c and severity of anemia

HbA1c (%)	Hemoglobin Inference				
	WNL	Mild Anemia	Moderate Anemia	Severe Anemia	p value
Mean (SD)	4.68 (0.49)	4.92 (0.40)	5.15 (0.44)	5.20 (0.26)	<0.001

HbA1c (%)	Hemoglobin Inference				
	WNL	Mild Anemia	Moderate Anemia	Severe Anemia	p value
Median (IQR)	4.6 (4.3-5.1)	5 (4.6-5.2)	5.25 (5.07-5.43)	5.1 (5-5.45)	
Min - Max	4 - 5.6	4.2 - 5.6	4.1 - 5.6	4.9 - 5.5	

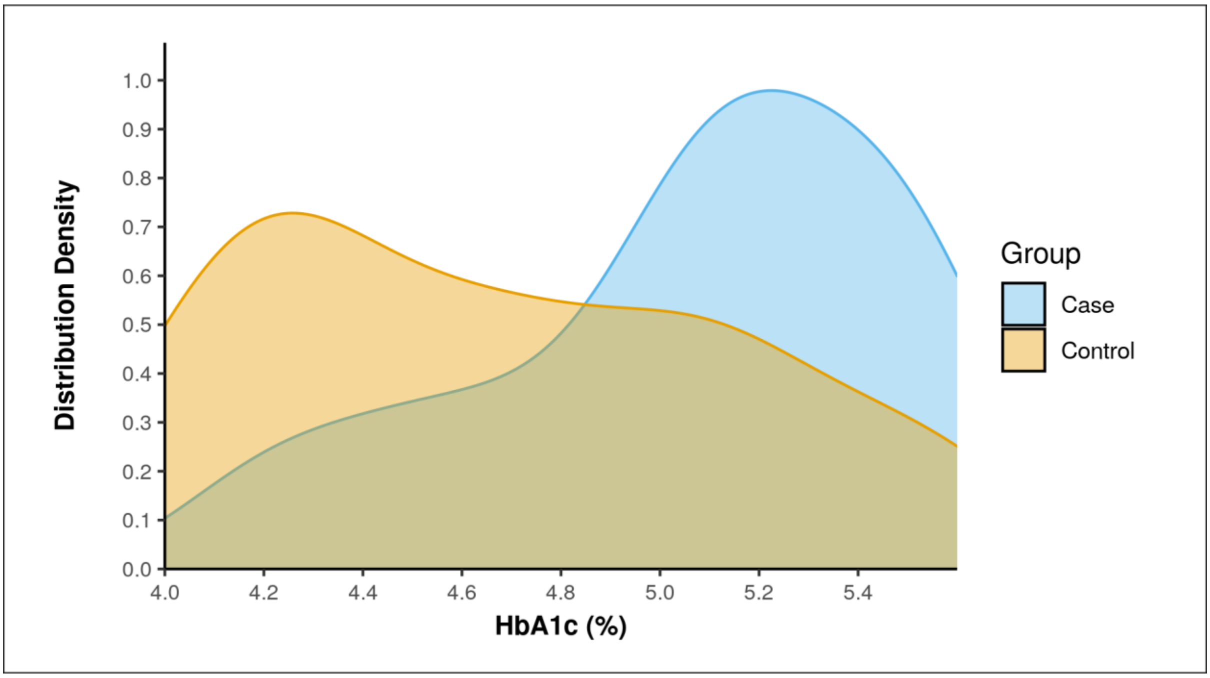

Figure 1: Distribution density of HbA1c across study groups

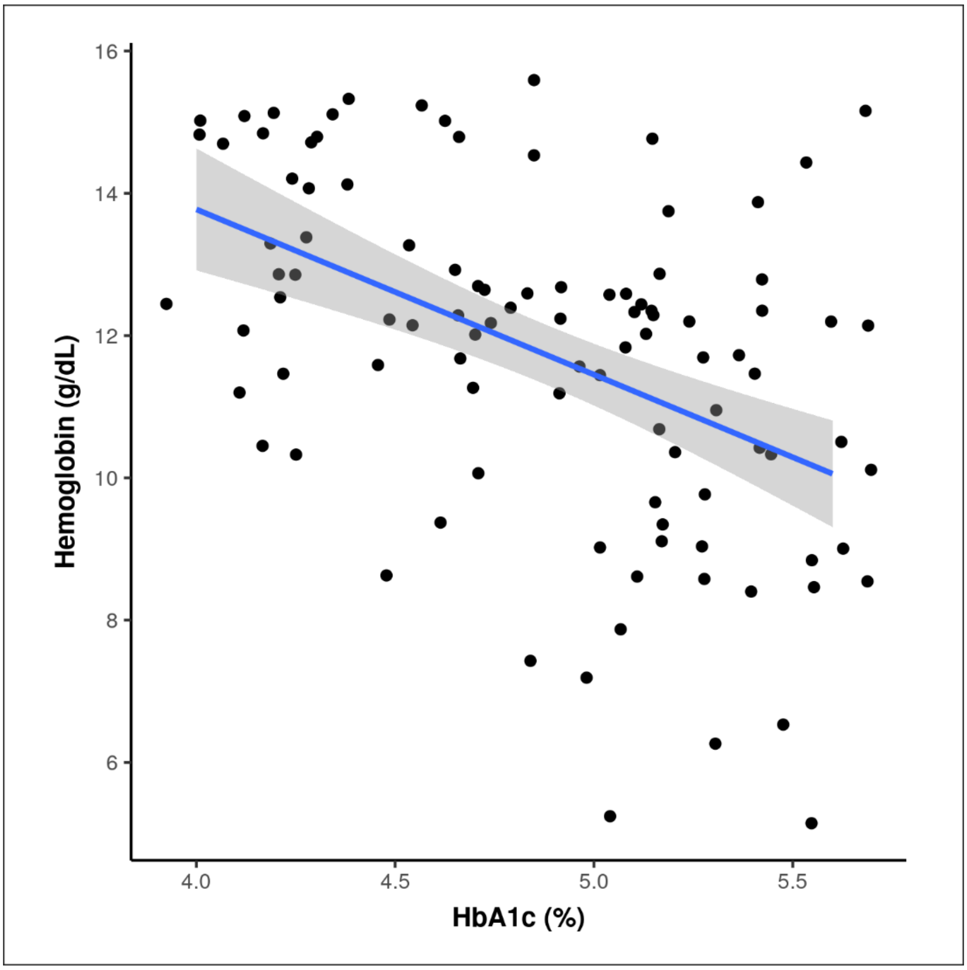

Figure 2: Correlation between hemoglobin and HbA1c.

DISCUSSION

The proximity in mean ages suggests that, on average, the participants in both groups were relatively close in age. Moreover, the examination of gender distribution revealed a balanced representation within the case and control groups. This equitable distribution of gender contributes to the overall demographic comparability between the two groups, thus enhancing the internal validity of the study and minimizing potential confounding factors related to age and gender. Body mass index (BMI) was comparable between the case and control groups, indicating that BMI alone may not be a differentiating factor between these groups in the context of the study.

Individuals with a mixed diet were found to exhibit markedly higher mean and median hemoglobin levels in comparison to their counterparts following a vegetarian diet. The observed differences in hemoglobin levels based on dietary habits suggest a potential correlation between nutritional choices and hematological parameters which can be attributed to a diverse intake of nutrients, including those essential for red blood cell production. Conversely, individuals adhering to a vegetarian diet may experience lower hemoglobin levels due to potential variations in iron and vitamin B12 intake, nutrients crucial for maintaining healthy hemoglobin levels. A systematic review and meta-analysis was conducted by Haider et al. in 2018 which showed that adult vegetarians had significantly lower iron stores in the form of serum ferritin levels than their non-vegetarian controls.[9] This makes those with a vegetarian diet more predisposed to develop IDA.

We found a significantly higher mean HbA1c level in the case group as compared to the control group.Similar results were obtained in a case-control study done by Shanthi et al. in non-diabetic participants in which the mean HbA1c (7.6 ± 0.5%) level in the patients with IDA was significantly higher than that in the control group (5.5% ± 0.8) ($p < 0.001$).[6] Our study results are also consistent with the findings of different studies conducted by Coban et al. and Pilla et al. who found that IDA patients had increased mean HbA1c levels which decreased significantly after iron treatment.[10,11] Our study contradicts the previous study by Sinha et al. which observed a significantly lower mean baseline HbA1c level in anemic patients (4.6% ± 0.6) as compared to the control group (5.5% ± 0.6; $p < 0.05$).[12] This difference in study results can be attributed to different age and gender distribution, the distribution of participants according to severity of anemia, and the inclusion of pre-diabetic levels of blood glucose.

Our research indicates a direct relationship between mean HbA1c levels and the degree of severity of anemia. Similar results were found in studies conducted by Rajagopal et al. and Silva et al. where they found an increase in HbA1c levels with the severity of anemia.[13,14] The studies concluded that IDA has an effect on HbA1c results, and the extent of this effect depends on the degree of anemia. Dissimilar results were found in a study by Altuntas et al. in which the mean HbA1c level was notably lower in the IDA group (5.4%) compared to the healthy control group (5.9%; $p < 0.05$).[15] This could be due to the differences in geographic areas, the exclusion of those with impaired fasting glucose levels, different age and gender distribution, and the number of participants in anemia severity categories.

CONCLUSION

We concluded that there is a significant positive association between IDA and HbA1c levels. The HbA1c level increased with the increasing severity of anemia. We found a negative correlation between hemoglobin and HbA1c levels.Thus, the effect of IDA should be kept in mind while interpreting HbA1c results. We would like to recommend larger population-based studies with a different demographic profile as compared to our study to further explore the influence of iron deficiency anemia on HbA1c levels.

CONSENT AND ETHICAL APPROVAL

Writtenconsent was obtained by all participants in this study. Institutional Ethics Committee - Human Research, University College of Medical Sciences, New Delhi, India issued approval IECHR-2022-55-20.

AUTHORS' CONTRIBUTIONS

Dr. Kuldeep Kumar and Dr. Aditi Singh contributed equally to the work and should be considered as co-first authors.All authors read and approved the final manuscript.

REFERENCES

1. World Health Organization. 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Accessed December 4, 2023. http://www.who.int/vmnis/indicators/haemoglobin.pdf.
2. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019 Aug;1450(1):15-31. doi: 10.1111/nyas.14092. Epub 2019 Apr 22. PMID: 31008520; PMCID: PMC6697587.
3. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care. 2009 Jul;32(7):1327-34. doi: 10.2337/dc09-9033. Epub 2009 Jun 5. PMID: 19502545; PMCID: PMC2699715.

4. Mayer TK, Freedman ZR. Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their clinical utility. Clin Chim Acta. 1983 Jan 24;127(2):147-84. doi: 10.1016/s0009-8981(83)80002-3. PMID: 6337751.
5. Gould BJ, Davie SJ, Yudkin JS. Investigation of the mechanism underlying the variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Clin Chim Acta. 1997 Apr 4;260(1):49-64. doi: 10.1016/s0009-8981(96)06508-4. PMID: 9101100.
6. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree. Effect of iron deficiency on glycation of haemoglobin in nondiabetics. J Clin Diagn Res. 2013 Jan;7(1):15-7. doi: 10.7860/JCDR/2012/4881.2659. Epub 2012 Sep 18. PMID: 23449741; PMCID: PMC3576740.
7. Bain BJ, Lewis SM. Dacie and Lewis Practical Haematology. 11th ed. Edinburgh: Elsevier Churchill Livingstone, 2012.
8. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003 Jan;26 Suppl 1:S5-20. doi: 10.2337/diacare.26.2007.s5. PMID: 12502614.
9. Haider LM, Schwingshackl L, Hoffmann G, Ekmekcioglu C. The effect of vegetarian diets on iron status in adults: A systematic review and meta-analysis. Crit Rev Food Sci Nutr. 2018 May 24;58(8):1359-1374. doi: 10.1080/10408398.2016.1259210. Epub 2017 Jul 5. PMID: 27880062.
10. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol. 2004;112(3):126-8. doi: 10.1159/000079722. PMID: 15345893.
11. Pilla R, Palleti SK, Rayana R, Skss SR, Abdul Razzack A, Kalla S. Glycated Haemoglobin (HbA1c) Variations in Nondiabetics With Nutritional Anemia. Cureus. 2020 Nov 13;12(11):e11479. doi: 10.7759/cureus.11479. PMID: 33329975; PMCID: PMC7735166.
12. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med. 2012 Jan;32(1):17-22. doi: 10.3343/alm.2012.32.1.17. Epub 2011 Dec 20. PMID: 22259774; PMCID: PMC3255499.
13. Rajagopal L, Ganapathy S, Arunachalam S, Raja V, Ramraj B. Does Iron Deficiency Anaemia and its Severity Influence HbA1C Level in Non Diabetics? An Analysis of 150 Cases. J Clin Diagn Res. 2017 Feb;11(2):EC13-EC15. doi: 10.7860/JCDR/2017/25183.9464. Epub 2017 Feb 1. PMID: 28384869; PMCID: PMC5376870.
14. Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Clin Biochem. 2016 Jan;49(1-2):117-20. doi: 10.1016/j.clinbiochem.2015.09.004. Epub 2015 Sep 10. PMID: 26365695.
15. Çetinkaya Altuntaş S, Evran M, Gürkan E, Sert M, Tetiker T. HbA1c level decreases in iron deficiency anemia. Wien Klin Wochenschr. 2021 Feb;133(3-4):102-106. doi: 10.1007/s00508-020-01661-6. Epub 2020 May 6. PMID: 32377869.

LIST OF ABBREVIATIONS

Serial no.	**ABBREVIATIONS**	**Full form**
1.	IDA	Iron deficiency anemia
2.	T2DM	Type 2 diabetes mellitus
3.	DM	Diabetes mellitus
4.	ADA	American Diabetes Association
5.	HbA1c	Hemoglobin A1c/glycated hemoglobin
6.	BMI	Body mass index
7.	SD	Standard deviation
8.	HPLC	High performance liquid chromatography
9.	MCV	Mean corpuscular volume
10.	MCH	Mean corpuscular hemoglobin
11.	MCHC	Mean corpuscular hemoglobin concentration
12.	IQR	Interquartile range
13.	WNL	Within normal limits