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A STUDY ON THE EFFECT OF MICROCYTIC HYPOCHROMIC ANEMIA ON HBA1C LEVELS IN NON-DIABETIC INDIVIDUALS

Pathology

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ABSTRACT

Introduction - Microcytic hypochromic anemia, predominantly caused by iron deficiency, is highly prevalent in developing countries and has been reported to influence glycated hemoglobin (HbA1c) independent of glycemic status. The present study aimed to evaluate the effect of microcytic hypochromic anemia on HbA1c levels in non-diabetic individuals and to determine the relationship between hematological parameters and HbA1c. **Methodology -** This case-control study was conducted in the Department of Pathology, Jhalawar Medical College, Rajasthan from July 2024 to April 2026 involving 776 non diabetic participants (388 cases of microcytic hypochromic anemia and 388 non-anemic controls). Complete blood count and fasting blood glucose were analyzed using standard automated methods. HbA1c was estimated using High Performance Liquid Chromatography (HPLC) method . Statistical analysis was performed using SPSS software, and p-value <0.05 was considered statistically significant. **Results -** The mean HbA1c level was significantly higher in cases than controls (5.48 ± 0.37% vs. 5.17 ± 0.36%; p<0.001), despite comparable fasting blood glucose levels, indicating a non-glycemic influence on HbA1c. HbA1c demonstrated a significant inverse correlation with hemoglobin concentration (r = −0.35, p<0.001). HbA1c also increased progressively with anemia severity (5.32 ± 0.30%, 5.47 ± 0.35%, and 5.62 ± 0.40% in mild, moderate, and severe anemia, respectively; p<0.001). **Conclusion -** Microcytic hypochromic anemia is associated with a significant non-glycemic elevation of HbA1c in non-diabetic individuals. Evaluation and correction of anemia before interpreting HbA1c may improve the diagnostic accuracy of diabetes and prediabetes, particularly in populations with a high prevalence of iron deficiency anemia.

KEYWORDS

INTRODUCTION

Microcytic hypochromic anemia is characterized by reduced hemoglobin concentration, decreased mean corpuscular volume, and diminished hemoglobin content within red blood cells. These hematological alterations lead to impaired oxygen delivery to tissues and are associated with a range of clinical manifestations, including fatigue, reduced physical capacity, cognitive impairment, and decreased quality of life.[1] Beyond these well-recognized consequences, anemia can also influence various biochemical parameters, thereby affecting the interpretation of laboratory investigations used in routine clinical practice.[2]

In recent years, HbA1c has gained further prominence with its inclusion as a diagnostic criterion for diabetes mellitus. This has led to an increasing reliance on HbA1c values for clinical decision-making. However, it is now well recognized that HbA1c levels can be influenced by factors other than blood glucose concentration. Conditions that affect red blood cell lifespan, hemoglobin structure, or erythropoiesis can alter HbA1c values independently of glycemia. This raises important concerns regarding the accuracy and reliability of HbA1c measurements in certain clinical settings.[3]

India bears a dual burden of communicable and non-communicable diseases, with diabetes mellitus emerging as a major health concern. The country is home to a large number of individuals with diabetes and an even larger population at risk. At the same time, anemia continues to affect a considerable segment of the population. The coexistence of these conditions creates a complex clinical scenario where interpretation of glycemic markers requires careful consideration. In such a context, understanding the interaction between microcytic hypochromic anemia and HbA1c levels becomes essential.[4]

In the Indian healthcare setting, where resource constraints often necessitate reliance on single laboratory parameters, HbA1c is frequently used as a standalone test for assessing glycemic status. The present study aimed to evaluate the effect of microcytic hypochromic anemia on HbA1c levels in non-diabetic individuals and to determine the relationship between hematological parameters and HbA1c.

MATERIALS AND METHODS

The study was carried out in the Department of Pathology, Jhalawar Medical College and associated hospitals, Jhalawar, Rajasthan. This is a case-control study conducted to evaluate the effect of microcytic hypochromic anemia on HbA1c levels in non-diabetic individuals during the period of July 2024 to April 2026.A total of 776 participants were enrolled including 388 cases and 388 controls.

Inclusion Criteria: Patients with Microcytic and hypochromic blood picture and indices. Non- diabetics and adults aged 18 years or older were included in the study.

Exclusion Criteria: Patients with anemia other than microcytic hypochromic, age less than 18 years, known diabetics, with hemoglobinopaties, H/o kidney disease, liver disease or any major systemic illness, pregnant females, H/o endocrinopathy with effects on glucose tolerance and H/o blood transfusion in the past two months were excluded from the study.

Non-Diabetic Patients diagnosed with microcytic hypochromic anemia were considered as cases. Non-anemic individuals were considered as controls.

Techniques Used

After obtaining informed consent,under strict aseptic precautions, venous blood samples were collected by peripheral venepuncture from all participants and appropriately distributed into different vacutainers depending on the required investigations. Blood collected in EDTA vacutainer was used for hematological analysis and HbA1c estimation. Sample for biochemical analysis, including fasting blood glucose, was collected in plain vial after an overnight fasting of 8–12 hours.

1. **Complete Blood Count -**
   - CBC was done using SYSMEX-XN 1000, a 5 part analyser which gives the following parameters including RBC count, Hemoglobin concentration, MCV, MCH, MCHC, RDW, WBC parameters and platelet indices.

2. **Peripheral Blood Film -**

Peripheral blood film (PBF) was prepared using blood from EDTA vial and stained with field stain. PBF was then examined under a light microscope for morphological evaluation. Detailed assessment of red blood cells was carried out, including evaluation of their size, shape, and color, hemoglobin distribution, presence of any inclusions, and detection of nucleated red blood cells, which aided in confirming microcytic hypochromic anemia.

3. **HPLC** - HbA1c was analyzed by High Performance Liquid Chromatography method using Bio-Rad D -10 analyser. This method utilizes the principles of ion- exchange high-performance

liquid chromatography(HPLC).

The samples were automatically mixed and diluted on the D-10 and injected to the analytical cartridge. The D-10 deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins were separated based on their ionic interaction with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer where the changes in absorbance at 415mm were measured.

4. **Fasting Blood Glucose** - Fasting Blood Glucose estimation was done by glucose oxidase - peroxidise method using Beckman Coulter DxC 700 auto analyser in the department of Biochemistry.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical package for social Sciences software or a similar statistical software package.A p-value <0.05 was considered statistically significant.

RESULTS

The demographic distribution revealed that the majority of participants were clustered in the 31–40 years age group, with a marked female predominance, findings that reflect established epidemiological patterns of anemia in developing countries where nutritional deficiencies, menstrual blood loss, and increased physiological demands during reproductive years contribute significantly to disease burden.

Table 1: Comparison of Mean Values of Studied Parameters of Cases and Control Groups

	Cases	Controls	P value
Mean Hb (g/dL) ± SD	7.11 ± 1.91	13.88 ± 1.07	<0.001, sig
Mean MCV (fL) ± SD	65.13 ± 6.33	87.49 ± 4.15	<0.001, sig
Mean MCH (pg) ± SD	18.91 ± 4.85	29.57 ± 1.95	<0.001, sig
Mean MCHC (g/dL) ± SD	27.46 ± 2.55	33.88 ± 2.22	<0.001, sig
Mean RDW (%) ± SD	22.41 ± 4.02	14.04 ± 1.16	<0.001, sig
Mean RBC ($\times 10^6/\mu L$) ± SD	3.77 ± 0.77	4.70 ± 0.44	<0.001, sig
Mean WBC ($\times 10^3/\mu L$) ± SD	7.53 ± 2.67	7.85 ± 2.64	0.10 (NS)
Mean Platelets ($\times 10^3/\mu L$) ± SD	324.82 ± 93.84	265.05 ± 63.27	<0.001, sig
Mean FBG (mg/dL) ± SD	84.26 ± 7.67	84.62 ± 7.96	0.52 (NS)
Mean HbA1c (%) ± SD	5.48 ± 0.37	5.17 ± 0.36	<0.001, sig

Table 2 : Correlation Between Hb and HbA1c:

Parameter	Correlation (r)	p-value
Hb vs HbA1c	–0.35	<0.001 (HS)

The Pearson correlation analysis demonstrated a moderate negative correlation between hemoglobin and HbA1c levels ($r = –0.35$), which was highly statistically significant ($p < 0.001$). This indicates that as hemoglobin levels decrease, HbA1c levels tend to increase

Table 3 : Association Between Severity of Anemia and HbA1c

Severity of Anemia	HbA1c (%) Mean ± SD	p-value
Mild	5.32 ± 0.30	<0.001 (HS)
Moderate	5.47 ± 0.35	
Severe	5.62 ± 0.40	

The findings indicate a clear dose-response relationship, where worsening anemia is associated with higher HbA1c levels.This suggests that the severity of anemia directly influences HbA1c elevation, further supporting the concept that HbA1c may be spuriously elevated in anemic individuals, independent of actual glycemic status.

DISCUSSION

The present study demonstrated a markedly reduced mean hemoglobin level ((7.11 ± 1.91 g/dL) in the case group compared to controls (13.88 ± 1.07 g/dL), confirming the presence of significant anemia among cases and validating the selection of subjects with microcytic hypochromic anemia. Hemoglobin is a primary indicator of oxygen-carrying capacity, and its reduction reflects impaired erythropoiesis or increased loss/destruction of red blood cells. The findings of the present study are in strong agreement with earlier work by Kim C et al.[5] (2019) reporting markedly reduced hemoglobin levels in anemic individuals and emphasizing the hematological alterations associated with iron deficiency. Additionally, an Indian study by Sinha N et al.[6] (2020) observed comparable reductions in hemoglobin levels among patients with microcytic anemia, reinforcing the consistency of these findings across different populations.

The present study showed a significantly reduced mean corpuscular volume (MCV) (65.13 ± 6.33 fL) in the case group compared to controls (87.49 ± 4.15 fL)., indicating the presence of microcytosis, which is a hallmark feature of iron deficiency anemia. A decrease in MCV signifies the production of smaller-than-normal erythrocytes due to defective hemoglobin synthesis.The findings of this study are consistent with the observations of Camaschella C et al.[7] (2020), who emphasized that an MCV value below 80 fL is a key diagnostic indicator of iron deficiency anemia. Similarly, Urrechaga E et al.[8] (2018) reported significantly reduced MCV values in patients with iron deficiency, highlighting its utility as an essential parameter in differentiating microcytic anemias from other types.

We found a significant reduction in mean corpuscular hemoglobin (MCH) among cases (18.91 ± 4.85 pg) compared to controls (29.57 ± 1.95 pg), indicating hypochromia, which is characterized by decreased hemoglobin content within individual red blood cells. The findings of the present study are consistent with those reported by Urrechaga E et al.[8] (2018), who demonstrated significantly decreased MCH values in patients with iron deficiency anemia and emphasized its diagnostic importance in conjunction with MCV. Furthermore, a study by Sherwani SI et al.[9] (2016) also reported reduced MCH levels in individuals with microcytic hypochromic anemia, supporting its role as a key indicator of red cell hemoglobinization.

We found a significantly reduced mean corpuscular hemoglobin concentration (MCHC) in the case group (27.46 ± 2.55 g/dL) compared to controls (33.88 ± 2.22 g/dL), further confirming the presence of hypochromia in individuals with microcytic hypochromic anemia. MCHC reflects the concentration of hemoglobin within a given volume of packed red blood cells and is an important indicator of the degree of hemoglobinization of erythrocytes. The findings of the present study are in agreement with the work of El-Agouza I et al.[10], who reported significantly lower MCHC levels in patients with IDA and emphasized its role in identifying hypochromic states.

We found a significantly elevated red cell distribution width (RDW) in the case group (22.41 ± 4.02%) compared to controls (14.04 ± 1.16%), indicating marked anisocytosis, or variation in red blood cell size. In IDA, ineffective erythropoiesis and variable iron availability result in the production of both smaller (microcytic) and relatively normal-sized red blood cells, leading to increased variability in cell size and hence elevated RDW. The findings of this study are consistent with those reported by Patel A et al.[11] (2024), who demonstrated that RDW is significantly increased in individuals with iron deficiency and may serve as an early marker of anemia.

The present study demonstrated a significantly reduced RBC count in the case group ($3.77 \pm 0.77 \times 10^6/\mu L$) compared to controls$4.70 \pm 0.44$ $\times 10^6/\mu L$), reflecting decreased erythrocyte mass and impaired red cell production, which are hallmark features of anemia. In iron deficiency anemia, the lack of adequate iron limits hemoglobin synthesis and consequently affects erythropoiesis in the bone marrow, resulting in reduced production of red blood cells. Sinha N et al.[6] (2020) also reported a significant reduction in RBC count among patients with microcytic hypochromic anemia, further supporting the present findings.

We found, no statistically significant difference was observed in the mean WBC count between cases ($7.53 \pm 2.67 \times 10^3/\mu L$) and controls ($7.85 \pm 2.64 \times 10^3/\mu L$), indicating that leukocyte levels remain largely unaffected in iron deficiency anemia.The findings of this study are consistent with the observations of Camaschella C et al.[7] (2019), who noted that WBC counts typically remain within normal limits in patients with iron deficiency anemia. Similarly, a study by Sherwani SI et al.[9] reported no significant alteration in leukocyte counts among individuals with microcytic anemia, reinforcing the notion that IDA primarily affects red cell parameters without significantly impacting white blood cell indices.

We found a significantly higher mean platelet count in the case group ($324.82 \pm 93.84 \times 10^3/\mu L$) compared to controls ($265.05 \pm 63.27$ $\times 10^3/\mu L$), suggesting the presence of reactive thrombocytosis in individuals with iron deficiency anemia (IDA). Reactive (secondary) thrombocytosis is a well-recognized hematological response in IDA

and is believed to result from shared regulatory pathways between erythropoiesis and thrombopoiesis in the bone marrow. The findings of the present study are consistent with those reported by Gilstrap LG et al.[14] (2019), who observed elevated platelet counts in patients with iron deficiency anemia and described it as a compensatory physiological response. Similarly, Kawabata H et al.[15] also reported increased platelet counts in iron-deficient individuals, reinforcing the association between IDA and thrombocytosis.

The present study found no statistically significant difference in Fasting Blood Glucose (FBG) levels between cases (84.26 ± 7.67 mg/dL) and controls (84.62 ± 7.96 mg/dL), indicating that both groups were metabolically comparable and largely non-diabetic. This finding is important as it establishes that any observed differences in HbA1c levels between the two groups are unlikely to be due to variations in actual glycemic status. The absence of significant differences in FBG suggests that both groups had similar baseline glucose homeostasis. This observation is consistent with the recommendations of the American Diabetes Association (2023), which emphasize that HbA1c values can be influenced by non-glycemic factors such as anemia, hemoglobinopathies, and alterations in red blood cell turnover. Therefore, in conditions like iron deficiency anemia, HbA1c may not accurately reflect true glycemic status, particularly when blood glucose levels are within normal limits.

The present study demonstrated that HbA1c levels were significantly higher in the case group (5.48 ± 0.37%) compared to controls (5.17 ± 0.36%), despite no significant difference in fasting blood glucose levels. This suggests that the observed elevation in HbA1c among anemic individuals is not due to true hyperglycemia but rather represents a spurious increase associated with iron deficiency anemia. The underlying mechanism is thought to involve prolonged red blood cell lifespan and reduced turnover in IDA, which allows more time for glycation of hemoglobin, thereby elevating HbA1c levels. Additionally, structural changes in hemoglobin and increased oxidative stress in iron deficiency may further contribute to enhanced glycation. These findings are strongly supported by the work of El-Agouza I et al.[10] (2002), who demonstrated that HbA1c levels are significantly elevated in patients with iron deficiency anemia and decrease following iron therapy. Similarly, Kim C et al.[5] (2019) reported higher HbA1c levels in anemic individuals independent of plasma glucose levels, emphasizing the confounding effect of anemia on glycemic markers. Earlier work by Brooks AP et al.[16] also supported the concept that alterations in erythrocyte lifespan can significantly influence HbA1c values. Therefore, the findings of the present study are in strong agreement with existing literature and underscore the limitation of relying solely on HbA1c for diagnosing or monitoring diabetes in patients with iron deficiency anemia, as it may lead to overestimation of glycemic status.

We found a statistically significant negative correlation between hemoglobin (Hb) levels and HbA1c [Pearson correlation(r) = –0.35], indicating that as hemoglobin decreases, HbA1c levels tend to increase. This inverse relationship is clinically important, as it suggests that lower hemoglobin levels—reflecting more severe anemia—are associated with spuriously elevated HbA1c values, independent of actual glycemic status. The underlying mechanism is thought to involve prolonged red blood cell (RBC) lifespan in iron deficiency anemia (IDA), which allows for increased exposure of hemoglobin to circulating glucose, thereby enhancing glycation and artificially elevating HbA1c levels.. These findings are consistent with studies by Ford ES et al[17]., who reported an inverse association between hemoglobin and HbA1c levels in population-based analyses, and Kim C et al. (2019)[5], who demonstrated a similar negative correlation in anemic individuals.

We found a clear dose-response relationship between the severity of anemia and HbA1c levels, with progressively higher HbA1c values observed as anemia worsened from mild to severe. These findings are in agreement with those reported by Sinha N et al. (2020)[6], who observed a progressive increase in HbA1c levels with increasing severity of anemia, and Kim C et al[5]., who similarly demonstrated that HbA1c values rise in proportion to the degree of anemia. Therefore, the present study highlights an important clinical implication: HbA1c may not only be falsely elevated in anemia but also varies according to its severity, potentially leading to misclassification of glycemic status in more severely anemic individuals.

CONCLUSION

Overall, the study emphasizes that HbA1c may not be a reliable marker of glycemic control in patients with microcytic hypochromic anemia especially iron deficiency anemia and may lead to overestimation of glycemic status if interpreted in isolation. Despite comparable fasting blood glucose levels between cases and controls, HbA1c values were found to be significantly elevated in the anemic group, indicating a spurious increase rather than true hyperglycemia. Furthermore, a significant inverse correlation between hemoglobin and HbA1c, along with a progressive rise in HbA1c with increasing severity of anemia, clearly establishes that the degree of anemia directly influences HbA1c levels.

Therefore, clinicians should exercise caution while interpreting HbA1c in anemic individuals and consider underlying hematological conditions before making diagnostic or therapeutic decisions related to diabetes. Alternative measures of glycemic assessment or correction strategies should be considered in such cases to ensure accurate diagnosis and appropriate management.

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