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RESEARCH ARTICLE

Diagnostic Implications of Microcytic Hypochromic Anemia on HbA1c in Non-Diabetic Adults: A Case-Control Study

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Abstract: ***Introduction:*** HbA1c is extensively utilized as a marker for diagnosis and monitoring of diabetes mellitus. Even so, accuracy of it has potential to be under influence of non-glycemic factors such as anemia, particularly microcytic hypochromic anemia, which is highly prevalent in the Indian population. ***Methods:*** This case-control study included 500 non-diabetic individuals with microcytic hypochromic anemia and 500 controls after adequate matching for age and sex. Hematological parameters and HbA1c were determined followed by statistical analysis within groups. Pearson's correlation coefficient for relationship between hemoglobin and HbA1c alongwith subgroup analysis was performed based on HbA1c range to evaluate potential for diagnostic misclassification. ***Results:*** Case group revealed significantly elevated mean HbA1c level ($5.766 \pm 0.4368\%$) in comparison to the control group ($5.273 \pm 0.6468\%$, $p < 0.001$). A substantial portion of participants in the case group had HbA1c in prediabetic range (59.2%) compared to the control group showing significant difference with p-value <0.001 and relative risk of 1.717 and odds ratio 3.027. Also a significant correlation was found between hemoglobin and HbA1c in anemic individuals ($r = -0.5833$, $p < 0.001$), albeit in the control group, weak inverse relationship was identified ($r = -0.0550$, $p = 0.220$). ***Conclusion:*** Microcytic hypochromic anemia falsely elevates HbA1c in non-diabetic individuals, potentially promoting misclassification of individuals into the prediabetic range. Anemic individuals have 72% higher likelihood of misclassification. Prudence is advised while relying solely on HbA1c for diagnosis of diabetes in anemic patients and alternative methods for accurate assessment warrants attention.

Keywords: Microcytic hypochromic anemia; HbA1c; Non-diabetic individuals; Iron deficiency anemia; Glycated hemoglobin; Hemoglobin; Case-control study.

INTRODUCTION

Diabetes mellitus refers to a chronic, non-communicable, disorder of human metabolism which stems from relative or absolute deficiency of insulin level in blood because of various etiological factors and interfere with fat, carbohydrate and protein metabolism[1]. Over the past four decades, the worldwide prevalence of diabetes has grown nearly eightfold, increasing from 108 million to more than 828 million in 2022[2]. This surge stands out in countries with emerging economies, including India and China[3]. Type 2 diabetes mellitus dominates the global statistics of diabetes and is attributed for every 9 out of 10 cases of diabetes and has transitioned from a disease of affluent to a major global health concern, affecting younger populations across all socioeconomic strata[4].

India has witnessed a sustained increase in diabetes prevalence since the 1990s, with significant acceleration after 2000. According to the ICMR-INDIAB study, approximately 101 million Indian adults are currently living with diabetes, with a national prevalence of 11.4% among individuals aged 20 years and older[5]. As of 2024, India has surpassed China to become the global epicenter of diabetes with estimated cases at 212 million, accounting for 26% of the global diabetic population[2]. A significant proportion of these cases remain undiagnosed or untreated, particularly in rural and underserved areas, reflecting critical gaps in awareness and healthcare access.

In adults, hemoglobin primarily comprises HbA (~97%), along with smaller fractions of HbA_2 (~2.5%) and fetal hemoglobin (HbF, ~0.5%). HbA includes a major non-glycated fraction (HbA_0, 93–95%) and a minor glycated component (HbA_1, 5–7%), which further includes HbA_1c (~4–6%), HbA_1a, and HbA_1b[6]. Among these, HbA_1c is the most clinically relevant, while the physiological roles of other glycated fractions remain uncertain[6]. The various hemoglobin fractions and their glycated derivatives are illustrated in Figure 1.

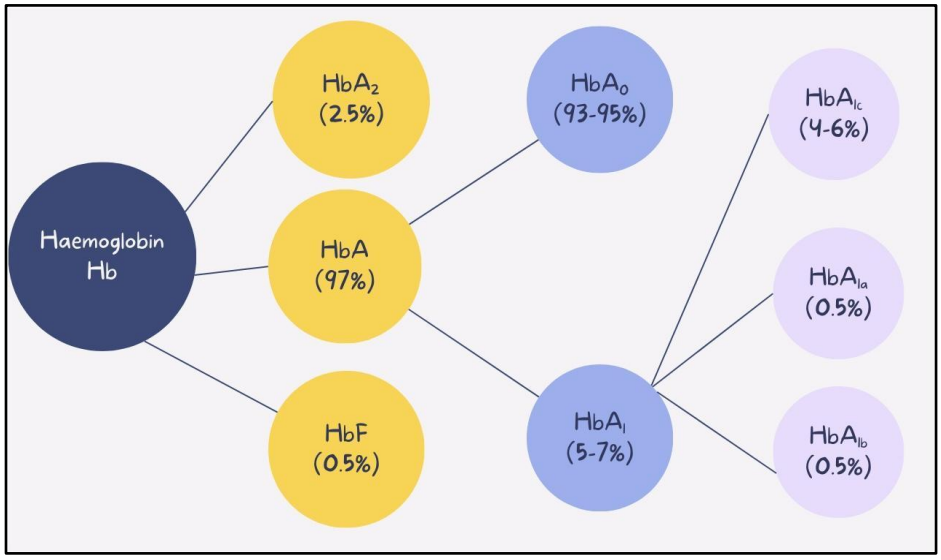

Figure 1: Schematic Representation of Hemoglobin Fractions and Glycated Forms.

HbA (97%) comprises non-glycated HbA_0 (93–95%) and glycated HbA_1 (5–7%), of which HbA_{1c} (4–6%) is the principal form used to assess long-term glycemic control.

Glycated hemoglobin (HbA1c) is extensively utilized as a marker for diagnosis and monitoring of diabetes mellitus, illustrating average glucose level of blood spanning over the last 8-12 weeks. Both the World Health Organization (WHO) and the American Diabetes Association (ADA) recommend HbA1c for the diagnosis of diabetes, with a threshold of ≥6.5% indicating diabetes and 5.7-6.4% for prediabetic[7,8]. However, factors such as anemia can possibly cause erroneous rise in HbA1c value, potentially resulting in misdiagnosis. Among the non-glycemic factors influencing HbA1c, microcytic hypochromic anemia has been shown to cause spuriously high HbA1c levels due to altered red blood cell turnover and changes in hemoglobin glycation rates[9,10]. Owing to the notable commonality of depleted iron stores in the Indian population, a significant challenge in accurately interpreting HbA1c results in non-diabetic individuals arises[11].

Thus, it becomes essential to evaluate the reliability of HbA1c in the presence of anemia. Clinicians must consider hematological status when interpreting HbA1c values to avoid misdiagnosis or inappropriate treatment. Current study was undertaken to investigate the impact of microcytic hypochromic anemia on HbA1c levels in non-diabetic individuals, with the aim of determining whether anemia leads to spurious HbA1c elevation and whether correction of anemia should be prioritized before relying on HbA1c for diagnosis or management.

MATERIAL AND METHODS

This case-control study was conducted in the Department of General Medicine at Maharishi Markandeshwar Medical College and Hospital, Kumarhatti, Solan, in collaboration with the Departments of Biochemistry and Pathology. Participants were recruited from both outpatient and inpatient services. The study was carried out over an appropriate time frame following approval by the Institutional Ethical Committee. A cohort of 1000 non-diabetic individuals with age bracket of 18 to 60 years were incorporated and divided equally into 2 study arms: 500 patients with microcytic hypochromic anemia were assigned as case group, 500 healthy individuals without anemia and diabetes who were matched for age and sex made up the control group.

Each enrolled participant was subjected to thorough medical history evaluation and physical examination. Anemia was defined as hemoglobin <13 g/dL in males and <12 g/dL in females, with red cell indices showing MCV <80 fL and MCH <27 pg. Controls had normal hemoglobin levels and RBC indices confirmed on peripheral smear. Exclusion criteria included known cases of diabetes mellitus, RBS>140 mg/dL with symptoms of hyperglycemia, or HbA1c >6.5%. Subjects were also excluded if they had undergone recent surgery, had hemoglobinopathies, hemolytic anemia, acute blood loss, systemic illnesses such

as renal or hepatic disease, endocrinopathies, were pregnant, had received blood transfusion within the past 3 months, or were outside the 18–60 year age range. Venous blood samples were taken using aseptic procedure following the acquisition of signed informed consent. Complete blood count was performed using the Sysmex XN-550 automated hematology analyzer. HbA1c was estimated using the Roche Cobas 311 platform by a colorimetric enzymatic method. Peripheral blood smears were stained with Leishman stain and examined microscopically to confirm anemia type.

Data processing and statistical evaluation were carried out using Jamovi software (version 2.6.26.0) and Microsoft Excel (version 16.78.3). Quantitative data were summarized using mean and standard deviation, while categorical variables were expressed as percentages. To assess associations between cohorts, the Chi-square test was applied, with a p-value of <0.05 considered statistically significant. Pearson correlation analysis was used to assess the relationship between hemoglobin and HbA1c levels. The research protocol was reviewed and approved by the Institutional Ethical Committee. All enrolled participants provided informed consent in accordance with ethical guidelines, and confidentiality was maintained throughout the study.

RESULTS AND OBSERVATIONS:

The study included 1000 participants, 500 of which were entrusted with the case group and 500 to the control groups. The case group comprised non-diabetic individuals diagnosed with microcytic hypochromic anemia, while control group included healthy non-anemic, non-diabetic individuals. The average age in the case group was 37.63 ± 13.47 years, and in the control group, it was 36.38 ± 12.21 years; this difference was not statistically significant (p = 0.124). The participants in both groups were aged between 18 and 60 years. Gender distribution was also comparable between groups: 28.2% of the case group and 33.4% of the control group were male, while 71.8% and 66.6% were female, respectively; this difference was not significant statistically(p = 0.075). Obtained results indicate that both groups were demographically well matched, minimizing selection bias. All participants were screened for major systemic comorbidities such as chronic kidney disease, liver dysfunction, hemoglobinopathies, endocrinopathies, or recent major surgeries, and individuals with such conditions were excluded, ensuring homogeneity of the study population.

The comparison of hemoglobin (Hb) levels between both the groups revealed a statistically significant difference. The average Hb level in the case group was 8.92 ± 2.241 g/dL, with values stretching from 2.50 g/dL to 11.80 g/dL. In contrary, the control group had a significantly higher mean Hb of 15.36 ± 1.378 g/dL, ranging from 13.00 g/dL to 17.80 g/dL. This difference was *highly significant statistically* (p<0.001). Packed cell volume (PCV) followed a similar trend, with the case group showing a mean PCV of 26.78 ± 6.9234%, which was lower than the control group's average of 46.30 ± 6.064% (p<0.001). Median PCV values were 27.3% and 45.9%, respectively.

The red blood cell (RBC) count also showed a marked difference among both the groups. Case group had a significantly lower mean RBC count of 4.715 ± 0.3710 × 10^6/µL (range: 3.69 to 5.50), whereas the control group had an average RBC count of 5.841 ± 0.2425 × 10^6/µL (range: 4.79 to 6.00), with this difference being *statistically significant* (p<0.001). Mean corpuscular volume (MCV) was notably reduced in the case group (77.86 ± 2.669 fL) compared to the control group (98.53 ± 1.945 fL), again demonstrating a *highly significant difference*(p<0.001). Similarly, mean corpuscular hemoglobin (MCH) values were significantly lower in the case group, averaging 23.88 ± 1.214 pg versus 30.02 ± 1.517 pg in controls(p<0.001). Mean corpuscular hemoglobin concentration (MCHC) also showed a *statistically significant* reduction in the anemic group, with a mean of 30.87 ± 1.046 g/dL compared to 33.46 ±1.216 g/dL in the control group (p<0.001). This result affirm classical hematological profile of microcytic hypochromic anemia in the case group. The comparative hematological and glycemic parameters of study groups are summarized in Table 1.

Table 1: Comparison of various parameters between case group and control group

Parameters	Cases (n=500)			Controls (n=500)			p value*
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Age (years)	37.63 ± 13.47	18.00	60.00	36.38 ± 12.21	19.00	60.00	0.124
Hemoglobin (g/dL)	8.923 ± 2.241	2.50	11.80	15.36 ± 1.378	13.00	17.80	< 0.001
PCV (%)	26.78 ± 6.924	6.90	40.40	46.30 ± 6.064	33.00	60.80	< 0.001
RBC count ($\times 10^6/\mu L$)	4.715 ± 0.3710	3.69	5.50	5.841 ± 0.2425	4.79	6.00	< 0.001
MCV (fL)	77.86 ± 2.669	67.00	80.00	98.53 ± 1.945	91.00	100.00	< 0.001
MCH (pg)	23.88± 1.214	20.27	26.36	30.02 ± 1.517	26.00	34.00	< 0.001
MCHC (g/dL)	30.87 ± 1.046	28.00	33.00	33.46 ± 1.216	30.00	36.00	< 0.001
HbA1c (%)	5.766 ± 0.4383	4.60	6.40	5.273 ± 0.6468	4.10	6.40	< 0.001

*p-value calculated using independent sample t-test for comparison between case and control

The comparison of HbA1c levels between both the groups demonstrated a *statistically significant* difference. The average HbA1c in the case group was 5.766 ± 0.4383%, with values ranging from 4.60% to 6.4%, while in the control group, the mean HbA1c was lower at 5.273± 0.6468%, with a range of 4.10% to 6.4%. This difference proved to be highly significant upon analysis ($p<0.001$). Additionally, analysis of HbA1c distribution based on ADA criteria for normal (≤5.6%) and prediabetic (HbA1c 5.7-6.4%), revealed a surplus share of participants in the control group (67.6%) had HbA1c values ≤5.6%, while only 40.8% of the case group fell into this category. Conversely, a significantly greater proportion of individuals in the case group (59.2%) exhibited HbA1c levels between 5.7–6.4%, compared to 32.4% in the control group. This variation in distribution also reached *statistical significance* ($p< 0.001$), indicating a potential upward shift in HbA1c values among anemic individuals despite the absence of diabetes. Further analysis of data revealed that relative risk of being classified as prediabetic in the anemic group was 1.717 and odds ratio was 3.027. The distribution of HbA1c values between the both study arms is displayed in Table 2.

Table 2: Distribution of Hba1c between both groups

Hba1c Range	Cases (n=500)	Control (n=500)
Pre Diabetic (5.7-6.4%)	59.2% (296)	32.4% (162)
Normal (<5.7%)	40.8% (204)	67.6% (338)
p-value	<0.001	

*p-value calculated using Chi-square test

To investigate the interrelationship between hemoglobin and HbA1c in both the groups, Pearson correlation analysis was performed. In the case group (individuals with microcytic hypochromic anemia), a moderate negative with correlation coefficient (r) was -0.5833 which was statistically significant (p<0.001) was observed, indicating that lower hemoglobin were associated with higher HbA1c values. In contrast, the control group (non-anemic individuals) demonstrated very weak negative correlation with r value of –0.0550 and a statistically non-significant p-value of 0.220. These relationships are visually represented in Figure 2, where the scatter plots show a nearly flat trend line in the control group and a clear downward-sloping trend line in the case group, corresponding to the respective correlation values.

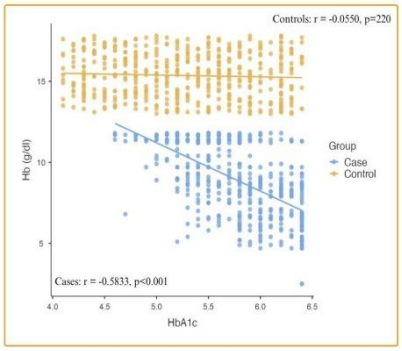

Figure 2: Scatter plots depicting the concordance among hemoglobin value and HbA1c in the case and control groups.

These findings collectively highlight notable differences in hematological and glycemic indices between anemic and non-anemic individuals and underscore the potential confounding influence of anemia on HbA1c interpretation in clinical settings.

DISCUSSION

The study sought to achieve the objective of evaluating the repercussions of microcytic hypochromic anemia on HbA1c levels in non-diabetic individuals. Cohort of a thousand participants was recruited out of which 500 were assigned to the case group and 500 to the control group. Both study arms were demographically aligned in terms of age and gender, minimizing selection bias.

Critical finding of the study was a statistically significant elevation in mean HbA1c levels among the anemic group in juxtaposition with the non-anemic control group, irrespective of diabetic status among participants. Additionally, red blood cell indices (MCV, MCH, MCHC) showed a statistically meaningful reduction in the anemic group, confirming the microcytic hypochromic nature of anemia.

A notable observation in this study was that a significantly larger subset of individuals in the anemic group had HbA1c values in prediabetic range, compared to the non-anemic group ($p<0.001$)which supports the notion that HbA1c can be increased in the clinical background of microcytic hypochromic anemia. Notably 59.2% anemic individuals were misclassified as prediabetic whereas only 32.4% of controls were in this range. The relative risk(RR) of being classified as prediabetic in the anemic group comes out to be 1.717, approximately 72 percent higher risk of anemic being misclassified as prediabetic than non anemic individuals. The odds ratio further reinforces the strength of this association suggesting that odds of false prediabetic labeling were over 3 times in the anemic group which may lead to unwanted clinical decisions and therapeutic interventions.

The Pearson correlation analysis, illustrated by scatter plots, demonstrated a clear distinction in the pattern of association between hemoglobin and HbA1c levels across the two groups. Among anemic, non-diabetic individuals, a modest and statistically significant inverse relationship was observed, indicating that lower hemoglobin levels were generally associated with slightly higher HbA1c values. This trend aligns with existing literature suggesting that even within normal hemoglobin ranges, variations in red blood cells such as turnover, lifespan, or iron status can subtly influence HbA1c measurements. In contrast, this relationship was weak and non-significant statistically in individuals without anemia and diabetes. The scatter plot for the non anemic group displayed wide dispersion of data points and an almost flat regression line, indicating minimal to no correlation between hemoglobin concentration and HbA1c levels. Importantly, the presence of significant inverse correlation between hemoglobin and HbA1c levels in anemic individuals shows that even mild reductions in hemoglobin may result in disproportionately high HbA1c values. This suggests that the presence of anemia itself may lead to spurious elevation of HbA1c, likely due to altered red cell kinetics or structural changes that enhance glycation susceptibility and supports the hypothesis that the distortion in HbA1c may be more qualitative than quantitative, driven by cellular and biochemical changes rather than a simple function of hemoglobin concentration. These findings highlight the need for cautious interpretation of HbA1c in anemic individuals and call into question its standalone use as a marker of glycemic control in such settings.

Several studies have corroborated the present study's finding that microcytic hypochromic anemia can lead to spuriously elevated HbA1c levels, even in cases who do not have diabetes. Alzahrani et al. reported significantly higher mean HbA1c in individuals with iron deficiency anemia (5.75%) and sickle cell anemia (5.83%) compared to non-anemic controls (5.32%), highlighting the confounding effect of anemia on glycemic markers[12]. Gharde et al. similarly observed a higher mean HbA1c (6.04 ± 0.74%) in anemic individuals versus 4.91 ± 0.65% in non-anemic subjects, with a greater proportion of the anemic group falling within the 5.6–6.5% range[13]. An inverse association between serum iron and HbA1c was identified by Reddy et al, providing a plausible explanation for elevated HbA1c in anemic individuals[14]. Meta-analysis by Katwal et al. also concluded that iron deficiency anemia was associated with falsely high HbA1c values, which reduced following iron supplementation[15]. The consistency of these findings with the current study underscores the need for caution when interpreting HbA1c results in individuals with coexisting anemia.

The observed elevation of HbA1c levels in anemic individuals, despite normoglycemia, may be attributed to several pathophysiological mechanisms. One proposed explanation involves altered red blood cell (RBC) turnover. In microcytic hypochromic anemia, the lifespan of erythrocytes is often shortened due to their smaller size and reduced hemoglobin content. This leads to a compensatory increase in erythropoiesis, resulting in a higher proportion of younger RBCs in circulation, which have a greater tendency for glycation due to higher metabolic activity. Another contributing factor may be structural alterations in hemoglobin caused by iron deficiency, particularly affecting the quaternary structure of the β-globin chain, thereby enhancing glycation susceptibility. Additionally, anemia has been associated with impaired tissue oxygenation and oxidative stress, which may promote non-enzymatic glycation of hemoglobin. These mechanisms collectively contribute to disproportionately elevated HbA1c levels in the absence of true hyperglycemia. Such findings reinforce the need to interpret HbA1c cautiously in anemic patients and consider concurrent hematological parameters before making clinical decisions based solely on glycemic markers.

These findings are of uttermost clinical importance in regions with a highest burden of anemia, such as India. Relying solely on HbA1c for the diagnosis or monitoring of diabetes in such populations may result in overdiagnosis or unnecessary intensification of therapy in individuals who are, in fact, euglycemic. This has the potential to expose patients to the risks of hypoglycemia, psychological stress, and unwarranted treatment costs. Moreover, public health screening

programs that use HbA1c as a standalone tool may misclassify individuals with anemia, leading to inaccurate epidemiological data and misallocation of healthcare resources. Hence, it is advisable that clinicians take into account the patient's hematological profile while analyzing HbA1c, particularly in non-diabetic individuals. When anemia is suspected or confirmed, alternative diagnostic tools like fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), or continuous glucose monitoring (CGM) facilitate more consistent and precise reflection of glycemic status.

CONCLUSION

The present study highlights that microcytic hypochromic anemia can lead to a robust, spurious elevation in HbA1c levels among non-diabetic individuals, and have approximately 72 percent higher likelihood of misclassification in prediabetic category. These findings underscore the importance of evaluating hematological parameters when interpreting HbA1c results, especially in populations with high anemia prevalence like India. These alterations occur due to altered erythrocyte lifespan and glycation kinetics. In such contexts, it is advisable to evaluate the hematological parameters before interpretation of HbA1c and correcting anemia may be essential to ensure accurate diagnosis and avoid potential overtreatment or misclassification of glycemic status.

Strengths

The key strength of this study lies in its focused comparison between non-diabetic individuals with and without microcytic hypochromic anemia, with careful age- and sex-matching to minimize confounding. Exclusion of systemic illnesses further enhanced the methodological rigor. Moreover, the use of standardized hematological and biochemical methods added robustness and reproducibility to the assessment of HbA1c levels in relation to anemia. Inclusion of sub group analysis added depth to interpretation of relationship between anemia and glycemic markers.

Limitations

This study also presents several limitations. Single center setting despite large sample size leads to limited extrapolation of findings to the general populations. Additionally, relevant variables such as iron profile (serum ferritin, transferrin saturation), nutritional status, and body mass index (BMI) were not assessed, potentially overlooking contributors to HbA1c variability. The cross-sectional design limits causal inference regarding the relationship between anemia and glycemic markers. Subsequent research should be designed as a multi-center study with larger cohorts and long term follow-up to assess HbA1c before and after anemia correction. Including iron studies and a wider spectrum of anemia types may help clarify specific mechanisms by which anemia affects HbA1c and enhance diagnostic accuracy in diverse clinical settings.

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