ORIGINAL ARTICLE

Prevalence of Undiagnosed Diabetes and Prediabetes Using HbA1c Levels and its Association with Hematological Parameters in Apparently Healthy Individuals

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Abstract

Background: HbA1c has been suggested as a diagnostic marker for diabetes, but it can be affected by hematological factors.

Objective: To find the prevalence of undiagnosed diabetes and prediabetes using HbA1c levels and its association with hematological parameters.

Methods: This cross-sectional study was conducted at the Noon Foundation Laboratory, Rawalpindi from August 2024 to January 2025. The blood pressure, anthropometric, and hematological parameters were measured. HbA1c was classified as normal (<5.7%), prediabetes (5.7-6.4%), and diabetes (≥6.5%). Data analysis was performed using SPSS version 24.

Results: The sample comprised 196 healthy adults with an average age of 38.8 ± 10.0 years. There were slightly more male participants (55.1%) than female participants (44.9%). The frequency of undiagnosed prediabetes and diabetes was 36.2% and 9.7%, respectively. The multivariable linear regression analysis showed age (β=0.011, 95% CI: 0.005 - 0.018, p=0.002), BMI (β=0.014, 0.007 - 0.022, p=0.001), hemoglobin (β=-0.070, 95% CI: -0.130 - -0.010, p=0.025), and RDW (β=0.082, 95% CI: 0.024 - 0.140, p=0.009) were significantly associated with HbA1c.

Conclusion: Screening for HbA1c in seemingly healthy adults revealed a significant burden of concealed dysglycemia. Nevertheless, hematological parameters such as hemoglobin and red cell distribution width significantly affect its interpretation. The routine hematological assessment should be incorporated into screening strategies to improve diagnostic accuracy and reduce misclassification in communities.

Keywords: *HbA1c, prediabetes, undiagnosed diabetes, hematological indices.*

INTRODUCTION

Diabetes mellitus is a significant public health issue. It has been increasing significantly over the past 10 years, and this trend is expected to continue in the adult population. The most significant causes of diabetes mellitus reported are demographic, urbanization, obesity, and sedentary lifestyles. Recently, global estimates have placed the prevalence of diabetes among adults in the hundreds of millions and have projected further increases in the coming decades [1, 2].

A significant proportion of diabetes cases remain undiagnosed, as early-stage disease is often asymptomatic and screening practices are inadequate or inconsistent in many settings. Population-based studies and national surveys have consistently documented substantial numbers of adults with elevated glycemic indices who were previously unaware of their condition, highlighting missed opportunities for early detection and intervention [3, 4].

Glycated hemoglobin (HbA1c) is widely used for long-term glycemic monitoring and for screening for diabetes and prediabetes because it reflects average glycemia over the preceding 8 to 12 weeks and does not require fasting [5]. Major diabetes authorities recognize HbA1c (commonly using cut-offs of ≥6.5% for diabetes and 5.7-6.4% for prediabetes, although thresholds and local validation may vary, as an acceptable diagnostic test when assay standardization and quality assurance are ensured. Nonetheless, HbA1c-based screening in population or workplace surveys offers pragmatic advantages for detecting both undiagnosed diabetes and prediabetes in apparently healthy adults [6].

However, important biological and analytical limitations affect the interpretation of HbA1c. Conditions that alter red blood cell (RBC) lifespan, hemoglobin structure, or erythropoiesis, including iron-deficiency anemia, hemoglobinopathies, recent transfusion, renal disease, and certain vitamin deficiencies, may spuriously increase

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or decrease HbA1c values independent of glycemia. Several recent observational and interventional studies have shown that iron deficiency, along with other anemias, can raise measured HbA1c in non-diabetic individuals and that HbA1c may fall after iron repletion, underscoring the potential for misclassification when hematological disorders are common in the screened population [7-9].

Beyond anemia, emerging research has explored associations between routine hematological parameters, such as hemoglobin concentration, mean corpuscular volume, red cell distribution width, and platelet counts, and glycemic status. Several cross-sectional studies from diverse populations have reported correlations between HbA1c and red blood cell indices or inflammatory hematologic markers. Some studies have also found that individuals with higher HbA1c, including those meeting prediabetes thresholds, exhibit distinct hematologic profiles compared with normoglycemic peers The findings pose methodological and pathophysiological questions in that hematological abnormality can bias measurements of HbA1c as a screening tool, and systemic inflammation and a related change in hematopoiesis in support of metabolic dysregulation might itself be biologically correlated with dysglycemia [10, 11].

Screening apparently healthy adults with HbA1c, therefore, serves a dual role: it may uncover a silent burden of undiagnosed diabetes and prediabetes, and it can reveal associations between glycemic markers and hematological indices that are clinically and epidemiologically important. Yet, despite growing evidence, gaps remain. Many population studies report prevalence figures without systematically accounting for hematological confounders; others examine hematologic-glycemic links in clinical or diabetic cohorts rather than in apparently healthy, community-based samples. Consequently, the true magnitude of misclassification risk when using HbA1c for community screening in populations with a high prevalence of anemia or hemoglobin variants remains incompletely defined [12].

Early detection of undiagnosed diabetes and prediabetes is crucial for preventing microvascular and macrovascular complications and for enabling timely lifestyle or therapeutic interventions. HbA1c is an attractive tool for community and workplace screening because it is convenient and reflects chronic glycemia; however, its accuracy can be affected by common hematological disturbances. Understanding the prevalence of undiagnosed dysglycaemia in apparently healthy adults, and the influence of routine hematological parameters on HbA1c results, can help clinicians and policy-makers interpret screening outcomes more reliably, tailor screening strategies in populations with high rates of anemia or hemoglobinopathies, and avoid both false reassurance and unnecessary labeling. The present study aimed to determine the prevalence of undiagnosed diabetes and prediabetes using HbA1c in apparently healthy adults, and to evaluate the association between HbA1c categories and routine hematological parameters.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted at the Noon Foundation Laboratory, Rawalpindi, from August 2024 to January 2025. Ethical approval for the study was obtained from the Ethics Review Committee of the Noon Foundation Laboratory, Rawalpindi (Approval No. NFL/ET/R/001, dated 01-08-2024). Sample size was calculated using the OpenEpi software with an anticipated prevalence of 15% for undiagnosed diabetes/prediabetes in middle-aged populations, a 95% confidence level, and a 5% margin of error; the required sample size was 196 participants [13].

Participants were recruited using non-probability consecutive sampling. Inclusion criteria comprised of adults of either gender of age ≥18 years, apparently healthy, attending the laboratory for routine health checks or employer/ pre-employment screening with no prior diagnosis of diabetes or prediabetes. Participants were excluded if they had known diabetes mellitus or were on glucose-lowering therapy; had previously diagnosed hemoglobinopathies such as sickle cell disease or thalassemia major; had received a blood transfusion within the past three months; were currently pregnant; had chronic kidney disease stage 3 or higher; were receiving iron-replacement or erythropoietin therapy; had an acute infection or hospitalization within the past two weeks; or had any condition that, in the investigator's opinion, could interfere with HbA1c interpretation or safe phlebotomy.

Data were collected from routine outpatient consultations conducted at the study center during the pre-set study period. Individuals who appeared healthy and were attending the outpatient department for general health assessment or minor, non-chronic complaints were consecutively approached to participate. After written informed consent, a short interviewer-administered pro forma was completed, capturing demographics (age, sex), anthropometrics (height in cm, weight in kg, BMI in Kg/m2), and relevant medical history (hypertension, dyslipidemia, smoking, and medication history). A single venous blood draw (approximately 5-7 mL) was performed using standardized aseptic technique. One EDTA tube for a complete blood count (CBC) and one tube (or appropriate tube) for HbA1c measurement. Measured CBC parameters were hemoglobin (Hb, g/dL), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), red cell distribution width (RDW, %), and platelet count (×10⁹/L); analyses were done using the automated hematology analyzer of the laboratory based on the manufacturer's calibration and internal quality control. HbA1c was measured

using a certified NGSP-traceable method (*e.g.*, HPLC or a standardized assay) according to the laboratory's routine protocols. All assays were subjected to internal and external quality-assurance measures.

Laboratory personnel were blinded to the participant questionnaire data during the analytical phase. The primary outcome was the prevalence of abnormal HbA1c categories among apparently healthy adults: normoglycaemia (<5.7%), prediabetes (5.7-6.4%), and diabetes (≥6.5%) according to standard diagnostic cut-points. Secondary analyses examined the relationship between continuous HbA1c values and CBC indices (Hb, MCV, MCH, RDW, platelet count) and compared CBC indices across the three HbA1c categories.

Data were entered into and analyzed using SPSS version 24. Descriptive statistics are presented as mean ± SD for normally distributed variables. Assumption of normality was assessed using the Shapiro-Wilk test. Group comparisons were made using a one-way ANOVA with post hoc testing using Tukey's test. Correlations between HbA1c and each hematological index were assessed using Pearson's correlation. Multivariable linear regression models were performed considering HbA1c as the dependent variable and hematological indices as independent variables. Variables with $p<0.10$ in univariable testing were included in the multivariable linear regression model. The variance inflation factor (VIF) and tolerance statistics were used to assess multicollinearity among the independent variables. VIF of <5 and a tolerance of > 0.2 was acceptable. Statistical significance was set at $p\leq0.05$ in the multivariable regression model.

RESULTS

The sample comprised 196 healthy adults with an average age of 38.8 ± 10.0 years. There were slightly more male participants (55.1%) than female participants (44.9%), and the mean BMI showed that the population is mostly overweight. About 15% were smokers. Blood pressure readings were within normal limits, and hematological data, including hemoglobin, MCV, MCH, RDW, and platelet counts, were mostly within normal limits. The average HbA1c was 5.73 ± 0.68, indicating that most participants had normal glycemia, but some were predisposed to dysglycemia (**Table 1**).

Table 1: Baseline characteristics of study participants (n=196).

Variables	Mean ± SD / n (%)
Age (years)	38.8 ± 10.0
Gender	
Male	108 (55.1)
Female	88 (44.9)
BMI (kg/m²)	26.4 ± 3.8
Smokers	29 (14.8)
Systolic BP (mmHg)	122.5 ± 13.2
Diastolic BP (mmHg)	79.2 ± 8.3
Hemoglobin (g/dL)	13.7 ± 1.3
MCV (fL)	86.8 ± 5.1
MCH (pg)	28.9 ± 2.0
RDW (%)	13.4 ± 1.2
Platelet count (×10⁹/L)	263 ± 56
HbA1c (%)	5.73 ± 0.68

When classified by HbA1c levels, just over half of the participants had normal glycemia, while 36% fell into the prediabetes range. A smaller proportion had undiagnosed diabetes. Overall, nearly 46% of participants exhibited abnormal HbA1c (prediabetes plus diabetes), highlighting a substantial prevalence of unrecognized dysglycemia in this apparently healthy cohort (**Table 2**).

Table 2: Distribution of participants according to HbA1c categories (n=196).

HbA1c Category	HbA1c Range (%)	n (%)
Normal Glycemia	<5.7	106 (54.1)
Prediabetes	5.7 - 6.4	71 (36.2)
Diabetes (Undiagnosed)	≥ 6.5	19 (9.7)

Hemoglobin level, MCV, and RDW significantly differed across three HbA1c categories, with a trend of higher values in the normal to diabetic range. Post-hoc analysis showed that for hemoglobin, a significant difference was observed between theetween the normal and group sets. For MCV, significant differences were observed between the normal and diabetes groups, and between the diabetic and prediabetic groups. For RDW, significant differences were observed between normal and pre-diabetes, and between normal and diabetes (**Table 3**).

Table 3: Comparison of CBC indices across HbA1c categories (n=196).

Parameters	Normal mean±SD	Prediabetes mean±SD	Diabetes mean±SD	p-value
Hemoglobin (g/dL)	13.9 ± 1.2	13.4 ± 1.4	12.9 ± 1.5	*0.031
MCV (fL)	87.4 ± 4.8	86.0 ± 5.2	84.3 ± 5.9	*0.048
MCH (pg)	29.2 ± 2.0	28.6 ± 2.1	27.9 ± 2.0	0.067
RDW (%)	13.0 ± 0.9	13.7 ± 1.3	14.3 ± 1.5	**0.004
Platelet Count (×10⁹/L)	255 ± 48	266 ± 57	277 ± 62	0.288

*Significant at p<0.05, **Significant at p<0.01

Correlation analysis showed that HbA1c was negatively correlated (moderately) with hemoglobin and MCV and positively correlated with RDW, which means that participants with better HbA1c had more red cell mass and more red cell variability. MCH and platelet count exhibited weak or insignificant correlations with HbA1c, indicating limited effects on glycemic indicators. Although statistically significant, the observed correlations were moderate in magnitude, indicating modest linear associations rather than strong predictive relationships. Therefore, these findings should be interpreted as indicative of potential interaction rather than definitive clinical determinants of glycaemic status (**Table 4**).

Multivariable linear regression analysis showed that age, BMI, hemoglobin, and RDW were significantly associated with HbA1c, accounting for more than 40%

Table 4: Correlation between HbA1c and Hematological Indices (n=196)

Variables	r (Correlation Coefficient)	p-value	Direction
Hemoglobin (g/dL)	-0.33	0.002 **	Negative
MCV (fL)	-0.27	0.008 *	Negative
MCH (pg)	-0.20	0.061	Negative (NS)
RDW (%)	+0.36	0.001 **	Positive
Platelet count (×10⁹/L)	+0.15	0.120	NS

*Significant at p<0.05, **Significant at p<0.01

of the variance. The association between HbA1c and hemoglobin was negative, whereas that with RDW, age, and BMI was positive. The adjusted model did not find sex to have a significant impact on HbA1c. The multicollinearity (VIF < 2.0; tolerance > 0.5) was acceptable, indicating that the regression estimates were stable. The general model accounted for the variance in HbA1c, with a moderate $R^2 = 0.42$ (**Table 5**).

Table 5: Multivariable linear regression model for factors associated with of HbA1c (n=196).

Variables	Unstandardized β	95% CI	p-value
Age (years)	0.011	0.005-0.018	**0.002
BMI (kg/m²)	0.014	0.007-0.022	**0.001
Hemoglobin (g/dL)	-0.070	-0.130-0.010	*0.025
RDW (%)	0.082	0.024-0.140	**0.009
Sex (male)	0.039	-0.080-0.160	0.528
Constant	4.21	-	<0.001

CI: Confidence interval, *Significant at p<0.05, **Significant at p<0.01

DISCUSSION

In this study of 196 apparently healthy adults, a substantial burden of previously unrecognized dysglycaemia was observed. Approximately 36.2% of participants had HbA1c values within the prediabetic range, while 9.7% met the diagnostic threshold for diabetes despite having no prior diagnosis. Overall, the combined prevalence of abnormal HbA1c (prediabetes plus undiagnosed diabetes) was 45.9%, indicating that nearly half of these otherwise asymptomatic adults exhibited clinically relevant glycaemic abnormalities.

An analysis of U.S. data and national reports showed undiagnosed diabetes affecting roughly 1 to 4% of adults, with higher absolute prediabetes prevalence reported separately, reflecting intensive screening and detection programs in those settings [3, 14]. Similarly, studies and regional analyses have documented wide global variation and sizable pockets of missed dysglycaemia, especially in low and middle-income settings. The present study observed a high combined prevalence, consistent with studies that found major undiagnosed disease burdens in populations with less systematic population-level screening [4, 15].

Several studies reported particularly high diabetes prevalence in Pakistan and neighboring populations, supporting the validity of the high screening rate in our study. Recent reviews have emphasized that Pakistan's adult diabetes burden is among the highest worldwide, and community screening studies across South Asia have consistently revealed large proportions of previously undiagnosed dysglycaemia, findings that are consistent with the elevated prevalence observed in our study [16, 17].

The main aim of the present study was to evaluate the association between routine hematological indices and HbA1c. The present study found statistically significant negative correlations between HbA1c and hemoglobin and MCV, and a positive correlation with RDW. Multivariable modeling demonstrated that lower hemoglobin and higher RDW independently predicted higher HbA1c even after adjustment for age and BMI. These results are consistent with several investigations that reported either an inverse relationship between hemoglobin and HbA1c or a positive association between RDW and dysglycaemia. A study by Janice *et al.* (2022), along with other studies, has documented that iron deficiency and related changes in erythropoiesis alter HbA1c concentrations and that correcting iron deficiency can alter measured HbA1c, implying that anemia and iron status are important confounders of HbA1c interpretation [9, 18]. Similarly, a study by Hassan *et al.* (2023) has shown positive associations between RDW and prediabetes or higher HbA1c, suggesting that increased red cell size heterogeneity marks metabolic stress or low-grade inflammation that accompanies dysglycemia [18, 19].

Iron deficiency anemia, other causes of reduced erythrocyte turnover, or altered hemoglobin glycation can raise or lower HbA1c independent of true glucose exposure. Conversely, metabolic dysfunction and systemic inflammation associated with insulin resistance may directly affect erythropoiesis and red cell indices, raising RDW and producing biologically meaningful correlations. Recent syntheses and primary studies evaluating RBC indices as predictors of HbA1c or diabetes risk echo our mixed pattern. Some reports showed stronger hemoglobin-HbA1c effects, while others emphasized RDW or MCV as discriminators, depending on the population, prevalence of iron deficiency/hemoglobinopathy, and analytic methods [20, 21].

Our findings showed that age and BMI were independent positive predictors of HbA1c, consistent with other studies that show an association between increasing age and higher adiposity, higher average glycemia, and greater diabetes risk. Several analyses have shown that the steady increase in HbA1c with age and the strong positive association between BMI and HbA1c or incident dysglycaemia support the external validity of our regression results [22, 23]. These findings suggest a clinically relevant association between hematological parameters and HbA1c; however, given the moderate strength of correlations, the results should be interpreted with caution and confirmed in larger prospective studies.

In general, our findings reinforce an emerging body of literature that points out that HbA1c screening reveals a high incidence of prediabetes and undiagnosed diabetes in most populations. Nevertheless, the interpretation of HbA1c must always take into account the hematological condition, especially hemoglobin concentration and RDW. To enhance diagnostic precision and prevent false reassurance or unjustified labeling, screening programs and clinicians must consider routine hematologic indices, or, in some cases, iron studies, in addition to HbA1c measurements.

The results of the present study showed the possible necessity of a regular screening of HbA1c even in seemingly healthy people because the percentage of individuals having abnormal levels of glucose was substantial (46.2%). There is a strong correlation between HbA1c and hemoglobin, MCV, or RDW, underscoring the importance of clinicians interpreting HbA1c results with caution in patients with anemia or abnormal red cell indices. When complete blood count (CBC) parameters are added to current screening programs, such as the inclusion of HbA1c, the misclassification of glycemic status and metabolic risk in individuals can be detected at an earlier stage. Additionally, hematologic screening for diabetes can be a cost-effective measure in resource-constrained environments. These results also justify the use of hematological evaluation in preventive health checkups to enhance early diagnosis and prioritize diabetes screening.

There are some limitations to this study. First, the cross-sectional nature of the study does not allow for causal inference regarding hematological parameters and HbA1c levels. Second, the sample size (n = 196) was small and based on a single center, which might not allow generalizing the results to the rest of the population. Third is the use of a single HbA1c test without confirmatory fasting plasma glucose (FPG) or an oral glucose tolerance test (OGTT). Despite HbA1c being internationally recognized as a screening and diagnostic tool when standardized assays are used, single-measurement classification can introduce misclassification, especially among those near diagnostic boundaries. Since this research was a community-based screening evaluation rather than a confirmatory diagnostic evaluation, a parallel glycemic evaluation was not systematically conducted. Further research using a combination of HbA1c and glucose-based measures would enable a more robust distinction between true dysglycemia and measurement error.

Fourth, despite hematological indices being the central aspect of our hypothesis, iron studies (*e.g.*, serum ferritin, transferrin saturation, or total iron-binding capacity) or a systematic anemia classification were not conducted. Therefore, we were unable to determine whether apparent associations between HbA1c and hemoglobin or RDW are indicative of a true metabolic interaction or confounded by iron deficiency or changes in erythrocyte turnover. Given the high prevalence of iron deficiency in most populations, especially in South Asians, subsequent research that includes comprehensive iron profiling and hemoglobinopathy screening would greatly enhance interpretability and assist in optimizing the HbA1c-based screening algorithm. Finally, lifestyle variables such as nutrition, exercise, and subclinical inflammation were not considered, which may affect glycemic regulation and hematologic variables.

CONCLUSION

HbA1c is a useful and effective community-level tool for detecting dysglycaemia, although it does not function alone without supporting hematological processes. The interaction between red blood cell indices and HbA1c in this research has a clinically significant implication: changes in erythrocyte parameters can potentially affect the glycemic sub-classification, even in seemingly healthy individuals. The use of HbA1c itself in populations with common anemia and red cell defects might be associated with diagnostic misclassification. Including complete blood count parameters as part of regular screening interpretation would enhance clinical decision-making and improve the accuracy of early diabetes detection procedures. Future studies that incorporate both iron research and parallel glycemic measurements will be necessary to refine screening algorithms and ensure that the HbA1c diagnosis is accurate and fair across different populations.

ETHICS APPROVAL

Ethical approval for the study was obtained from the Ethics Review Committee of the Noon Foundation Laboratory, Rawalpindi (Approval No. NFL/ET/R/001, dated 01-08-2024). All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written consent was obtained from the participants.

AVAILABILITY OF DATA

The data can be shared after obtaining consent from all concerned authors, as needed.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Declared none.

AUTHORS' CONTRIBUTION

Umbreen Hashim: Conception, design of study, data collection, data analysis, manuscript writing, and final editing.

Amina Zulfiqar: Study supervision, critical review, and intellectual input in manuscript editing.

Muhammad Javaid Iqbal: Data entry, literature search, and contribution to manuscript writing.

Shahneela Shakoor: Data entry, and assistance in manuscript drafting.

Aqsa Noureen: Data analysis, interpretation of results, and review of manuscript.

Humera Javed: Critical review and revision of manuscript.

All authors read and approved the final manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of the present work, the authors used ChatGPT (GPT-4, OpenAI) sparingly to generate language suggestions and perform minor proofreading in some parts of the manuscript. After using this tool/ service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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