Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Evaluation of Red Blood Cell Indices for Prediction of Glycemic Control in People Living with Type 2 Diabetes

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Background: Achieving target glycemic control in people living with diabetes lessens most of the diabetes-related complications and hemoglobin A1C is the gold standard test for assessing the long-term glycemic control. However, the expensive cost, and its limited access are problematic for routine use in developing countries, demanding an urgent alternative solution. Hence, this study aimed to assess the potential of red blood cell indices in predicting glycemic control among people living with type 2 diabetes at Dilla University Hospital.

Methods: A cross-sectional study was conducted among 207 participants with type 2 diabetes. Red blood cell indices were compared between the poor and good glycemic control groups using the *t*-test. Performance of red cell indices in discriminating poor glycemic control was assessed using receiver operating characteristic analysis.

Results: The prevalence of poor glycemic control among the participants was 91.8%. Higher mean hemoglobin (p=0.002) and mean corpuscular hemoglobin (p=0.015) was found in the good glycemic control group. An inverse correlation was also observed between hemoglobin A1C and mean corpuscular hemoglobin (r=−0.158; *P*=0.023). The area under the curve to discriminate poor glycemic control from good was statistically significant for hemoglobin, hematocrit, and mean corpuscular hemoglobin. However, hemoglobin had the highest discrimination ability at a cutoff ≤14.8 g/dL; area under the curve was 0.738 (*P*=0.001), sensitivity, specificity, and positive predictive value were 68.95%, 82.35%, and 97.77%, respectively.

Conclusion: This study revealed that hemoglobin can potentially be used to assess glycemic control among people with type 2 diabetes but the interpretation needs to be cautious.

Keywords: red blood cell indices, hemoglobin A1C, Ethiopia

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia.[1] The underlying chronic hyperglycemia seen in patients with DM is associated with damage to various target organs of our body. In that essence, management of diabetes fundamentally constitutes good glycemic control.[2] Evidence from randomized trials has shown that intensive glycemic control can clearly reduce the microvascular complications of DM. The evidence toward reducing macrovascular complications, although less compelling, supports an early and better glycemic control in type 2 DM.[3]

When it comes to assessing the glycemic control in people with DM, hemoglobin A1C(HbA1C) remains the preferred tool.[2] Nevertheless, the routine use of HbA1C in developing countries is limited by its high cost and reduced availability.[4] This is especially highly concerning for Africa where an estimated 24 million adults (aged 20–79 years) were living with diabetes in 2021, and it was responsible for a total of 416,000 deaths that year.[5] The prevalence of DM in the adult population of Ethiopia in 2021 was also 3.3% with an estimated 1.92 million cases.[6]

In the sub-Saharan Africa, if the HbA1C test is available, it is mostly in the larger hospitals. This limits the access, especially for those living in the rural areas. Moreover, the test requires availability of trained staffs, supply of electricity

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and refrigeration and this creates a challenge for its routine use in this part of the continent. The expense required to purchase, maintain and replace reagents has also made the price quite costly for patients whenever available.[4] The point of care HbA1C measurement devices could possibly bring a solution to the abovementioned problems,[7] but these have not been widely introduced in Ethiopia and issues regarding calibration and standardization of the machines might also affect their utilization here.

Many studies have shown an association between the RBC indices and HbA1C.[8–17] From the red blood cell (RBC) indices, mean corpuscular volume (MCV) indicates the size of RBCs, while mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) define the hemoglobin content of RBCs. The red cell distribution width (RDW), however, indicates the variation in size of RBCs.[18] The effect of hyperglycemia in altering the aggregation and deformability of RBCs[19] and the reduction in the lifespan of RBCs,[20] is hypothesized to bring changes in these RBC indices. Moreover glycated hemoglobin levels vary depending on the level of exposure to blood glucose and life span RBCs. Similarly, the MCV, MCH and MCHC do get affected by the age of RBCs.[21] These physiologic changes could bring an understanding to interpretation of RBC indices in a diabetic patient and possibly use them for assessing glycemia.

Taking into account the expensive nature and inadequate availability of HbA1C in developing countries,[4] including Ethiopia, it would be expected that other cheaper and widely available alternatives of glycemic assessment will be looked for. But, we noticed scarcity of data with regards to addressing this problem. We considered, among the possible alternatives, to evaluate role of RBC indices in this regard. Therefore this study aimed to assess if RBC indices can be used to assess glycemic control, taking HbA1C as a reference, in Dilla University General Hospital, Dilla, Ethiopia.

Method and Materials

Study Area and Period

The study was conducted at Dilla University Hospital, one of the teaching hospitals found in Dilla town of Gedeo Zone, SNNPR Region. Dilla town is located at approximately 373 km, south of Addis Ababa. It provides services for patients coming to the hospital from the catchment population of about three million people.

The chronic care and follow-up of patients with diabetes is given at the medical referral clinic of the hospital and this service is given by internal medicine specialists collaborating with nurses and pharmacists.

The study was conducted from March 6, 2023 to May 2, 2023.

Study Design

A hospital-based, cross-sectional study was conducted.

Population

Source Population

All patients with type 2 DM, aged ≥18 years, and having follow-up at Dilla University Hospital DM clinic were the source population.

Study Population

All selected patients with type 2 DM, aged ≥18 years of age, having follow-up at Dilla University Hospital, DM clinic during the data collection period.

Inclusion and Exclusion Criteria

Inclusion Criteria

All patients with type 2 DM, aged ≥18 years, having follow-up at Dilla University Hospital during the data collection period, who were willing to participate in this study were included.

Exclusion Criteria

Those with conditions that might affect the RBC indices in isolation and alter its association with HbA1C including:

- Pregnant (second or third trimester) or lactating mothers during data collection,
- Critically ill patients,
- Use of immunosuppressive therapy in the past 3 months,
- Blood loss/transfusion in the past 3 months,
- Those who are known to have hematologic disorders were excluded from this study.

Sample Size Determination

G*Power version 3.1.9.7 was used to calculate sample size. It was calculated by taking the smallest r (Pearson correlation coefficient) that was found in previous studies which had assessed the correlation between HbA1C and each of the RBC indices. The α and β values that were used are: α=0.05; β=0.20. The smallest r that was found was for the MCH: r=0.2.[8] The calculated sample size was: n=193. Taking an additional 10% for the non-response rate, the total sample size became 213.

Sampling Technique and Sampling Procedure

All eligible patients with type 2 DM that came for follow-up starting from the onset of data collection period were included in a consecutive manner. The recruitment of study participants continued until we reached the calculated sample size.

Variables of the Study

Dependent Variable

Glycemic Control/Hemoglobin A1C Level

Independent Variables

RBC indices: RBC count, Hgb, MCV, MCH, MCHC, RDW.

Sociodemographic characteristics: age, sex.

Other clinical factors: duration of DM, type of medication, comorbidities, BMI.

Fasting Blood Glucose.

Data Collection Technique

Data were collected using pretested data collection tool. A Structured and pretested data collection tool was used to collect the data from medical records, direct patient interviews and laboratory results.

Participants were interviewed to assess the sociodemographic, behavioral, clinical and treatment related factors. Afterward, blood sample was collected for the determination of complete blood count, fasting blood glucose and HbA1C.

Four licensed BSc nurses and two BSc laboratory technicians were recruited to collect the data. Before actual data collection, the data collection tool was pretested. One supervisor, together with the principal investigator followed the data collection closely.

Laboratory Assays

Approximately 5 mL of venous blood was collected and transferred into an ethylenediaminetetraacetic acid (EDTA)-containing two test tubes with each containing 2.5 mL of blood. Then samples were transferred to Dilla University laboratory for immediate analysis. Two BSc laboratory technicians performed the laboratory analysis of the collected blood. RBC indices were analyzed using a fully automated hematology analyzer, UniCel DxH 800 (Beckman Coulter, USA) which uses the Coulter principle impedance and multi-angle laser scatter. HbA1c level was determined by using a fully automated Cobas® 6000 chemistry analyzer (Roche Diagnostics, Germany) which is based upon the principle of electrochemiluminescence (ECL). Both tests were performed after proper calibration was done. Fasting blood glucose was determined on the spot from a finger spot using a HemoCue β-glucose analyzer.

Data Quality and Management

To ensure data quality, the data were pretested on 5% of the calculated sample size. The pretest was performed on willing patients with type 2 DM who came to Dilla University Hospital DM clinic in the week prior to onset of data collection and they were excluded from being part of the study population. After the pretest, the necessary tool amendment was done for the final data collection. Data collectors were given intensive three day training on how to extract the data from patient registry and lab reports as well as perform interview. All of the laboratory measurements were performed after passing through the necessary calibration procedure and the laboratory used was accredited by the Ethiopian Accreditation Agency. Moreover, as the data were entered using the KoboToobox, training about the software and how to use it was also given to data collectors. In preparation for data collection, data form in KoboToolbox was adjusted in a way it will not allow illegal values. Coding was performed carefully to increase accuracy and quality of data collected. After initiation of data collection, the collected data was daily being checked for completeness and consistency by the supervisor and principal investigator.

Statistical Analysis

Data were exported to SPSS version 26 for cleaning and analysis. The Kolmogorov–Smirnov test was used to assess normality of distributions of variables. Descriptive statistics was used to summarize data.

Comparison of the RBC indices between those with good and poor glycemic control was performed using the independent sample *t*-test after checking the conditions of normality and homogeneity of variance were fulfilled. For assessing homogeneity of variances the Levene's test for equality of variances was used.

The correlation of HbA1C with red cell indices was determined using Pearson's correlation coefficient after checking both variables are normally distributed.

Assumption of normality and homoscedasticity of residuals required for using linear regression were checked using residual plot. Multiple linear regression was used to develop a model that predicts HbA1C from RBC indices. Multicollinearity among independent variables was checked using the variance inflation factor (VIF) values taking 10 as a cut-off.

The diagnostic ability of the RBC indices in predicting glycemic control was assessed by calculating a receiver operating characteristic (ROC) curve derived from MedCalc® Statistical Software version 22.001. Different values of each of the RBC indices were compared for identifying a diagnostic cutoff and area under the ROC curve (AUC) with 95%CI was calculated. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at the identified cutoff is described. Statistical significance was declared with at $P<0.05$.

Operational Definition

Body mass index classification.[22]

Underweight: <18.5 kg/m^2

Normal weight: 18.5–24.9 kg/m^2

Pre-obesity: 25.0–29.9 kg/m^2

Obesity: ≥30 kg/m^2

Critically ill patients: those patients the physician deemed necessary for them to be sent to the Emergency Room for urgent intervention.

Good glycemic control: HbA1C level less than 7%.[2]

Poor glycemic control: HbA1C level greater than or equal to 7%.[2]

Type 2 DM: A patient with a physician diagnosis of type 2 DM.

Result

In this study, 207 patients with type 2 DM were included. The response rate was 97.2%.

Sociodemographic Characteristics

In this study, males comprised 54.1% of the participants (Figure 1). More than half (52.7%) of the study participants were in the age group of 40–59, while participants less than 30 years were only 2 (1%) (Figure 2).

Clinical and Anthropometric Factors

A higher proportion 88 (43.1%) of the participants had a normal BMI between 18.5 and 24.9 kg/m^2 followed by those in the pre-obesity range of 25–29.9 kg/m^2 accounting to 35.3%. History of hypertension was reported in 96 (46.4%) of participants (Table 1).

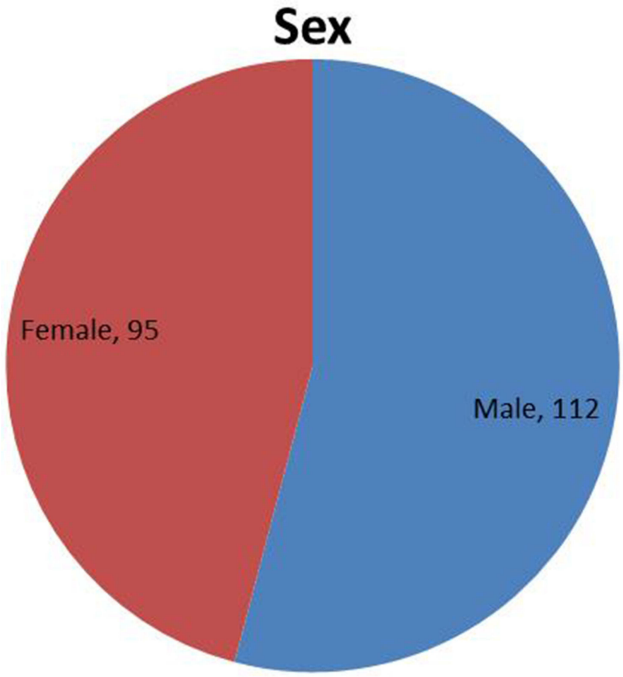

Figure 1 Sex distribution of patients with type 2 DM in Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023.

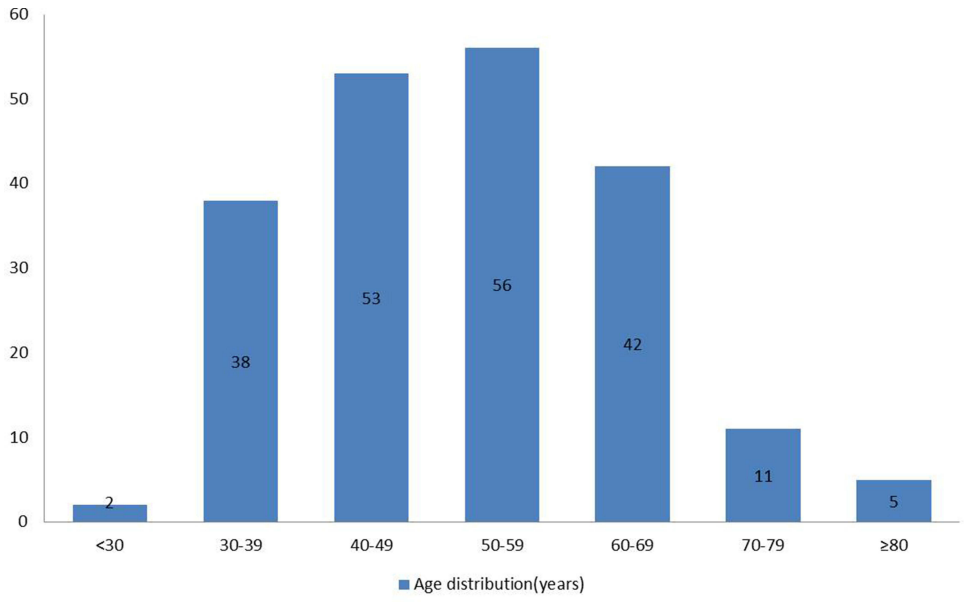

Figure 2 Age distribution of patients with type 2 DM in Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023.

Table 1 Clinical and Anthropometric Characteristics of Patients with Type 2 DM in Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

Variable	Hemoglobin A1C(%)			Chi Squared *P*-value
	<7	≥7	Total (%)	
BMI (kg/m²)				
<18.5 (underweight)	0	4	4 (2.0)	0.726
18.5–24.9 (normal weight)	9	79	88 (43.1)	
25.0–29.9 (pre-obesity)	5	67	72 (35.3)	
≥30 (obesity)	3	37	40 (19.6)	
Hypertension history				
Yes	11	85	96 (46.4)	0.092
No	6	105	111 (53.6)	
Fasting blood glucose (mg/dL)				
≤130	11	50	61 (29.5)	0.002
>130	6	140	146 (70.5)	
Type of medication for DM				
Insulin only	8	80	88 (42.5)	0.97
Oral hypoglycemics only	6	79	85 (41.1)	
Insulin with oral hypoglycemics	3	31	34 (16.4)	
Median duration since diagnosis of DM (months)	72 (20–103)	72 (36–120)	Median (IQR) = 72 (36–120)	

The fasting blood glucose was 130 mg/dL or less in 61 (29.5%) of participants, whereas the HbA1C was less than 7% in only 17 (8.2%). Insulin alone was prescribed in 88 (42.5%) of participants, while it was prescribed in combination with oral hypoglycemic in 34 (16.4%). The median duration since diagnosis of DM for the participants was 72 months with an IQR of 36–120 months (Table 1).

Red Blood Cell Indices

The median hemoglobin for the participants was 14.2 g/dL, with an interquartile range between 13.4 g/dL and 15.3 g/dL. The median was found to be 84.6 fL, 31.8 pg, 37.7 g/dL and 13.5% for MCV, MCH, MCHC, and RDW, respectively among the participants (Table 2).

Mean Difference in RBC Indices

All of the RBC indices were found to have a significant *P*-value on the Kolmogorov–Smirnov test failing the assumption of normality needed to use the Student's *t*-test. For this reason, their values were transformed using common logarithmic transformation and the mean difference was reported after back transformation.

The mean hemoglobin was 8% higher in the group with good glycemic control (HbA1C<7) compared to those with poor glycemic control (μ_1-μ_2 =1.08, 95%CI:1.028–1.131). Similarly, there was a statistically significant mean difference in MCH values between those with good versus poor glycemic control groups. The group with good glycemic control had a 4% higher mean MCH level compared to the group with poor glycemic control (μ_1-μ_2=1.04; 95%CI:1.008–1.071) (Table 3).

Table 2 Summary of RBC Indices for Patients with Type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

RBC Indices	Median (IQR)	Maximum	Minimum	Reference Interval
RBC (*10^6)	4.49 (4.24–4.82)	6.29	3.30	4.00–5.50
Hemoglobin (g/dL)	14.2 (13.4–15.3)	18.2	10.0	12.0–17.4
Hematocrit (%)	37.9 (35.5–40.7)	83.2	26.8	36.0–52.0
MCV (fL)	84.6 (81.4–87.1)	122.4	63.5	76.0–96.0
MCH (pg)	31.8 (30.6–32.9)	36.9	21.8	27.0–32.0
MCHC (g/dL)	37.7 (36.7–38.6)	41.0	25.6	30.0–35.0
RDW (%)	13.5 (13.0–14.2)	49.3	12.0	0.0–16.0

Table 3 Mean Difference in RBC Indices Between Those with HbA1C<7 versus HbA1C≥7 Patients with Type 2 DM in Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

Variables	Mean difference[a]	*P*-value
RBC (*10^6/μL)	1.04	0.141
Hemoglobin (g/dL)	1.08	0.002*
Hematocrit (%)	1.06	0.063
MCV (fL)	1.03	0.138
MCH (pg)	1.04	0.015*
MCHC (g/dL)	1.01	0.332
RDW (%)	0.97	0.424

Notes: [a]Values were back transformed from logarithmically transformed variables. *Statistical significance at $P<0.05$.

Correlation Between RBC Indices and HbA1C

Both the HbA1C and RBC indices had a skewed distribution so their values were logarithmically transformed to assess correlation. There was a statistically significant negative correlation between hemoglobin A1C and MCH values (r=−0.158; $P=0.023$). The correlation between HbA1C and the other RBC indices was not statistically significant (Table 4).

Estimation of HbA1C Level

To estimate HbA1C level, a model was developed using multiple linear regression. A common logarithmic transformation of the dependent variable was applied to fulfil assumption of normality. The Durbin–Watson test for assessing independence of residuals was checked on the transformed HbA1C level and it gave a result of 1.50 (acceptable if within range of 1.5–2.5). The Cook's distance for each observation was less than 1, demonstrating the absence of outliers.

A scatterplot of "standardized residuals" against the 'standardized predicted values' was applied and showed equal variance of the residuals (Figure 3).

The regression model used included 11 independent variables: age, sex, duration of DM, type of treatment for DM, presence of hypertension, BMI, fasting blood glucose, hemoglobin, MCH, MCHC, and RDW. The model explained 28.5% of the total variation in HbA1C [coefficient of determination (R^2)=0.285]. The analysis of variance was

Table 4 Correlation Between HbA1C Level and the RBC Indices Among Patients with Type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

RBC Indicesa	HbA1C (%)a	
	Pearson Correlation (r)	*P*-value
RBC	0.013	0.851
Hemoglobin	−0.088	0.209
Hematocrit	−0.027	0.705
MCV	−0.086	0.216
MCH	−0.158	0.023*
MCHC	−0.076	0.274
RDW	0.085	0.226

Notes: [a]The RBC indices and HbA1C were logarithmically transformed to fulfill the assumptions for using Pearson's correlation. *Statistical significance at $P<0.05$.

statistically significant ($P<0.001$) implying the ability of the independent variables to predict the dependent variable in a linear fashion.

The RBC count, HCT and MCV were removed from the model because multicollinearity was observed with the other independent variables (VIF>50). The VIF for each of the included variables, afterward, was less than 10 (maximum was 1.497) and the tolerance was greater than 0.2 (minimum was 0.668), showing the absence of multicollinearity (Table 5).

The multiple regression model showed that for an increase in BMI by 1 kg/m^2, there was a statistically significant decrement of HbA1C level by 4.1% [B=0.959($10^{-0.018}$), 95%CI: 0.9268–0.9954]. Similarly, for every 1 pg increment of MCH, HbA1C level decreased by 1.8% [B=0.982($10^{-0.008}$), 95%CI: 0.9661–0.9977] (Table 5).

In contrast, the absence of history of hypertension was associated with a 6.9% higher level of HbA1C compared to those who have history of hypertension which is seen to be statistically significant [B=1.069($10^{0.029}$), 95%CI: 1.0023–1.1376]. The use of insulin was also associated with a 3.99% higher level of HbA1C compared to those taking only oral

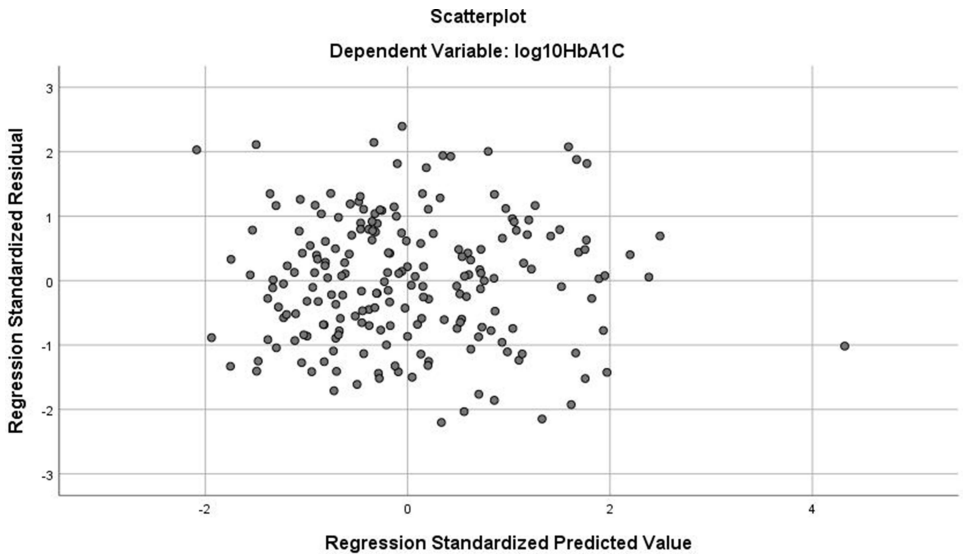

Figure 3 Scatterplot of standardized residual against standardized predicted values.

Table 5 Multiple Regression Analysis for Predictors of HbA1C Among Patients with Type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

Variable	Unstandardized Coefficients		95%CI for B		*P*-value	Collinearity Statistics	
	B[a]	Std Error[a]	Lower Bound[a]	Upper Bound[a]		Tolerance	VIF
Intercept	1.024	0.170	0.689	1.358	<0.0001*		
Sex	−0.004	0.014	−0.032	0.024	0.794	0.711	1.407
Age	0.000	0.001	−0.001	0.001	0.704	0.668	1.497
Duration of DM (months)	0.0002	0.000	0.000	0.000	0.587	0.789	1.267
BMI	−0.018	0.008	−0.033	−0.002	0.027*	0.903	1.108
Hypertension history	0.029	0.014	0.001	0.056	0.042*	0.729	1.373
Type of DM treatment	0.017	0.006	0.004	0.030	0.010*	0.783	1.276
Hemoglobin	−0.001	0.005	−0.011	0.009	0.773	0.734	1.363
MCH	−0.008	0.004	−0.015	−0.001	0.033*	0.697	1.435
MCHC	0.001	0.004	−0.006	0.009	0.717	0.701	1.427
RDW	0.004	0.002	−0.001	0.008	0.098	0.761	1.314
FBG	0.001	0.000	0.000	0.001	<0.0001*	0.972	1.029

Notes: [a]Values are for logarithmically transformed dependent variable, Log_{10}HbA1C. *Statistical significance at $P<0.05$.

hypoglycemic agents with a statistical significance [B=1.0399($10^{0.017}$), 95%CI: 1.0093–1.0715]. The HbA1C level increased by 0.23% for every 1 gm/dL increment of the fasting blood glucose (B=1.0023($10^{0.001}$), $P<0.0001$) (Table 5). The derived regression equation from the model was:

Log (HbA1C) = 1.024 + 0.001 (FBG) + 0.29 (HTN) + 0.017 (Type of DM treatment) – 0.018 (BMI) – 0.08 (MCH)

Performance of RBC Indices in Discriminating Poor Glycemic Control

The hemoglobin level at a cutoff of 14.8 g/dL or less has accurately predicted 95.65% of those with poor glycemic control with an area under the curve (AUC) of 0.738 which is statistically significant ($P=0.0011$). The positive and negative predictive values at the identified cutoff were 97.77% and 19.16% respectively (Figure 4 and Table 6).

Similarly, the hematocrit at a cutoff of ≤41% yielded a sensitivity of 82.11% and a specificity of 58.82% yielding a statistically significant AUC of 0.689 ($P=0.0103$). The positive and negative predictive values at the identified cutoff were 95.70% and 22.72% respectively (Figure 5 and Table 6).

At a cutoff of ≤32.8, the AUC for MCH was 0.688 with a sensitivity of 77.89 and specificity of 58.82 which is statistically significant ($P=0.0103$). The positive and negative predictive values at the identified cutoff were 95.49% and 19.23%, respectively (Figure 6 and Table 6).

Discussion

This study found that the prevalence of poor glycemic control among patients with type 2 DM at Dilla University Hospital, as assessed by HbA1C level, was 91.8%. The mean hemoglobin and MCH levels were higher in those with good glycemic control compared to the group with poor glycemic control. An inverse correlation was also observed between HbA1C and MCH levels. In additio, Hb, HCT, and MCH were able to distinguish between poor and good glycemic control, with Hb showing the highest discrimination ability. All of the RBC indices exhibited an excellent positive predictive value (PPV) of over 90%.

The glycemic control among patients with type 2 DM in this study was very poor in comparison to other Ethiopian studies conducted in Addis Ababa, Jimma, and Mekele where prevalence of poor glycemic control defined by HbA1C

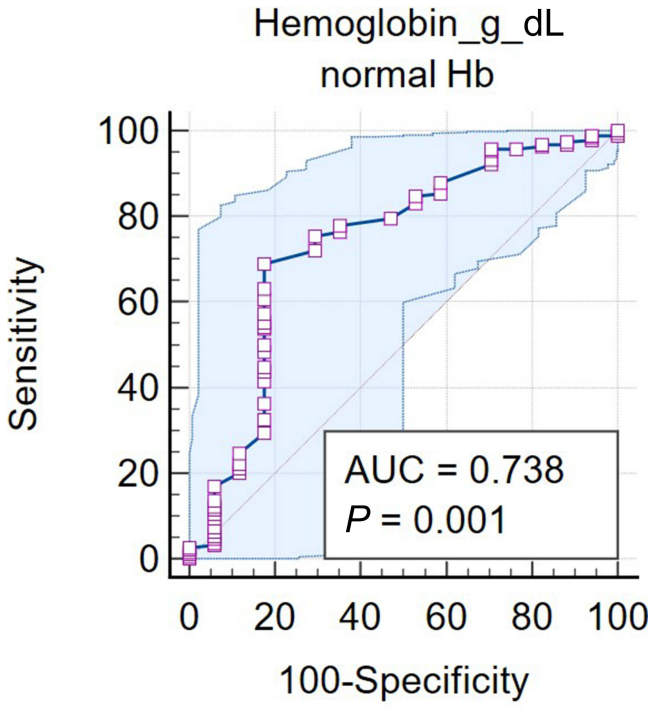

Figure 4 ROC curve for performance of hemoglobin to assess glycemic control among patients with type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023.

level was 73.8%,[23] 60.5%,[8] and 61.9%,[24] respectively. This finding can be explained by the fact that routine use of HbA1c for glycemic control assessment is not well-practiced in the study area due to reagent shortages and difficulties in maintaining chemistry machines. Moreover simpler technologies like point of care A1C measurement devices are not also available. As a result, clinicians rely on measuring fasting blood glucose levels, despite its limitations in assessing chronic hyperglycemia. This reliance has led to inaccurate labeling of patients as having good glycemic control, delaying further interventions to achieve glycemic targets.

The mean hemoglobin, in our study, was lower in those with poor glycemic control (HbA1C≥7) compared to those with good glycemic control (HbA1C<7). This finding has also been reported in studies coming from India[11,12] and Jimma, Ethiopia.[8] The lower hemoglobin associated with poor glycemic control can be explained by the altered structure and function of erythrocyte membranes, as well as the altered hemoglobin structure associated with chronic hyperglycemia. This hyperglycemia results in

Table 6 Diagnostic Performance of RBC Indices to Discriminate Poor Glycemic Control from Good Among Patients with Type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023

	AUC	95%CI	*P*-value	Cutoff value	Sensitivity	Specificity	PPV	NPV
RBC (*10^6/μL)	0.614	0.544–0.681	0.1180	≤4.57	57.89	70.59	95.65	13.05
Hemoglobin (g/dL)	0.738	0.672–0.796	0.0011*	≤14.8	68.95	82.35	97.77	19.16
Hematocrit (%)	0.689	0.621–0.752	0.0128*	≤41	82.11	58.82	95.70	22.72
MCV (fL)	0.596	0.525–0.663	0.21	≤88.7	86.84	35.29	93.75	19.35
MCH (pg)	0.688	0.621–0.751	0.0103*	≤32.8	77.89	58.82	95.49	19.23
MCHC (g/dL)	0.554	0.484–0.623	0.5175	≤39	90.00	29.41	93.44	20.83
RDW (%)	0.562	0.491–0.631	0.3924	>13.3	58.42	64.71	94.87	12.22

Note: **P*-value significant at <0.05.

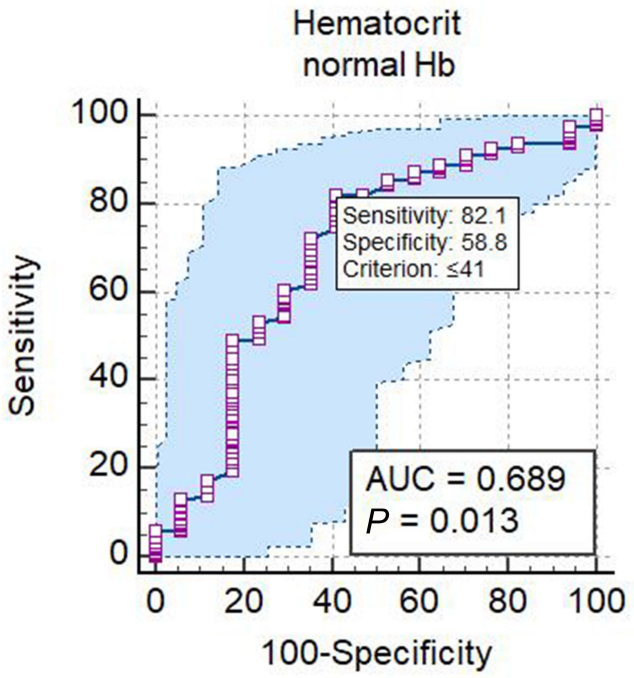

Figure 5 ROC curve for performance of hematocrit to assess glycemic control among patients with type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023.

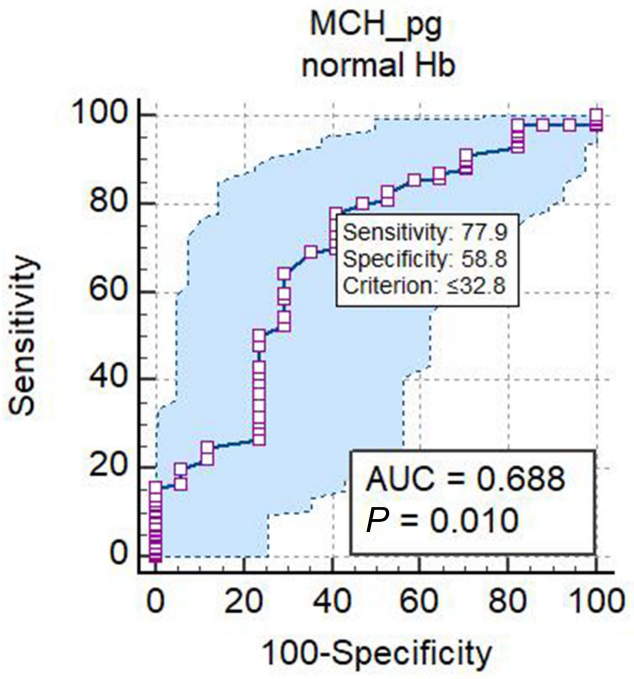

Figure 6 ROC curve for performance of MCH to assess glycemic control among patients with type 2 DM at Dilla University General Hospital, Dilla, Ethiopia (n=207), 2023.

poor deformability of erythrocytes with structural alterations which makes them fragile and more prone to hemolysis and/or eryptosis, lowering the hemoglobin level. The ongoing hemolysis of glycated red blood cells (RBCs) also contributes to oxidative stress on vascular structures, increasing the risk of developing atherosclerosis and its complications.[25–27] Therefore,

lower hemoglobin levels in the context of poor glycemic control can serve as an indicator of the risk of developing DM-related vascular complications.

Similarly, this study revealed a significant difference in MCH levels between the groups with good and poor glycemic control, with higher MCH levels seen in the group with good glycemic control (HbA1c <7%). We also found a weak, yet, statistically significant negative correlation between MCH and HbA1c levels. This correlation between MCH and HbA1c levels was also reported in a study conducted in Jimma, Ethiopia.[8] MCH level is a measure of the amount of hemoglobin per RBC.[18] The secondary structure of hemoglobin is altered at high blood glucose level, with an increase in β-sheet contents of hemoglobin that might affect the MCH.[25]

Furthermore, in this study, a hemoglobin level cutoff of less than 14.8 g/dL could reasonably distinguish between poor and good glycemic control, with a sensitivity of 68.95%, specificity of 82.35%, and an excellent PPV of 97.77%. A report from India, although using a different cutoff, also suggested that hemoglobin levels perform well in diagnosing poor glycemic control.[11] The same mechanisms that explain the lower hemoglobin levels in the group with poor glycemic control, as described earlier, can be applied here.

However, this study did not find a significant association between RDW and HbA1C levels, which is in contrast to studies conducted in Pakistan[17] and Bangladesh.[13] Chronic hyperglycemia associated with DM affects the deformability of RBCs and reduces their life span. A shorter life span of RBCs leads to lower HbA1c levels because the duration of exposure to hyperglycemia is reduced for newly replaced erythrocytes. Conversely, RDW increases with a shorter life span of RBCs.[9] The balance between these factors may determine the relationship between RDW and HbA1c levels and could explain the absence of association observed in this study. In addition, hypertension is known to cause an increase in RDW,[9] and differences in the hypertensive populations between this study and others may also influence the association between glycemic control and RDW.

In general, in our study we found an association between some of the RBC indices and the HbA1C level. But this finding should be interpreted cautiously. Even if we tried to exclude patients with conditions that might affect the RBC indices, there might be some with undiagnosed hematologic conditions that could have affected the RBC indices and thereby altered the association.

Conclusion and Recommendation

The findings of this study have shown that the hemoglobin value can potentially be utilized to evaluate glycemic control among people with type 2 DM. However, it is very important to interpret these results cautiously.

We suggest clinicians, in situations where the use of HbA1c is impractical, consider using the hemoglobin level of individual patients, along with repeated measurements of blood glucose levels, to assess the chronic glycemic control. This information can help guide treatment decisions. Moreover, we recommend that researchers in this field conduct future studies which are multicentered, involving larger sample sizes, and with a longitudinal design.

Strength and Limitations of the Study

Strength

This study has extended beyond assessing the mere relationship between HbA1C levels and RBC indices to assessing whether RBC indices can be used as a diagnostic tool to evaluate glycemic control among people with type 2 DM.

Limitations

The main limitation of this study is its cross-sectional design, which limits its utility in assessing the cause-effect nature of associations. Moreover, the fact that it's a single-center study might make it difficult to generalize the finding to a larger population with different backgrounds.

Ethical Consideration

Ethical clearance was obtained from Dilla University, College of Medicine and Health Sciences Ethical Review Board, Protocol Unique Number: duirb/019/23-02. Both written and verbal informed consent to take part in the research was

taken from all participants before the commencement of the study. Information collected was kept confidential and never disclosed to others without consent of participants and the hospital. Medical record numbers rather than name of patients were recorded. Abnormal results were communicated to the treating physician. The study was performed in accordance with the principles stated in the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work in the conception, study design, execution, acquisition of data, analysis, interpretation, and writing/critically reviewing the article. All authors have agreed on the journal to which the article will be submitted and on all versions of the article submitted. The authors agree to take responsibility and be accountable for the contents of the article.

Disclosure

The authors report no conflicts of interest in this work.

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