



(RESEARCH ARTICLE)



## Red blood cell morphology changes in peripheral blood smear of type 2 diabetes mellitus patients

Ngwu Amauche Martina <sup>1,\*</sup>, Ezigbo Eyiuche Doris <sup>2</sup> and Ikegwuonu Ifeoma Chinwe <sup>3</sup>

<sup>1</sup> Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.

<sup>2</sup> Thrombosis and Haemostasis Unit, Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria.

<sup>3</sup> Department of Medical Laboratory Science, College of Medicine, University of Nigeria Enugu Campus, Enugu State, Nigeria.

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### Abstract

**Background:** The prevalence of type 2 diabetes mellitus (T2DM) is rising steadily in Nigeria. Chronic hyperglycemia alters red blood cell (RBC) structure and function, which may contribute to complications.

**Aim:** To evaluate RBC morphology and hematological parameters in patients with T2DM.

**Methods:** A case-control study was conducted at Enugu State University Teaching Hospital from July to November 2024, involving 70 T2DM patients (31 males, 39 females; aged 40–60 years) and 30 age- and sex-matched healthy controls. Hematological indices (RBC count, hemoglobin, packed cell volume [PCV], mean corpuscular indices, red cell distribution width [RDW]) were measured using an automated analyzer. Peripheral blood smears were prepared and examined for morphological abnormalities.

**Results:** T2DM patients had significantly lower PCV ( $36.89 \pm 6.62\%$ ) compared with controls ( $39.55 \pm 3.75\%$ ,  $p=0.03$ ). No significant differences were observed in other hematological indices. Peripheral smear examination revealed hypochromasia in 10% of patients and anisocytosis in 6%, with rouleaux formation in one female subject. Increased RDW values were observed in females aged 40–45 years compared with older patients ( $p<0.05$ ).

**Conclusion:** Patients with T2DM exhibited reduced hematocrit, increased RDW in younger females, and morphological changes including hypochromasia and anisocytosis. These findings support the inclusion of simple, cost-effective hematological parameters in the routine monitoring of diabetes mellitus.

**Keywords:** Type 2 Diabetes Mellitus; Red Blood Cell Morphology; Anisocytosis; Hypochromasia; Nigeria

### 1. Introduction

In Nigeria, type 2 diabetes mellitus (T2DM) is a major health issue that is influenced by several risk factors [1, 2]. The country rapid urbanization, which encourages more sedentary lives and bad eating habits, is one of the major risk factors. According to results of study obtained from 138 countries, the International Diabetes Federation approximated that the overall prevalence of diabetes in developed countries, middle, and low-income countries was 10.4%, 9.5%, and 4.0%, respectively [3]. The International Diabetes Federation has approximated that 463 million persons live with

\*Corresponding author: Ngwu Amauche Martina

diabetes globally (9.3%) in 2019, with an expected increase of about 800 million (10.9%) by 2045 [4, 5]. In Nigeria, it has been estimated that 3.9 million individuals lived with diabetes in 2019 and expected to reach 6.0 million by 2045 [6]. The prevalence of diabetes mellitus has risen to unusual levels globally, demanding a comprehensive mechanism to identify reliable biomarkers for early diagnosis, prognosis and management [7]. Bone marrow, which is the main site of hematopoiesis produces red blood cells every day through erythropoiesis [8]. Red blood cells (RBCs) have a biconcave disk with flattened centre. Red blood cells lack nucleus and can easily change shape making them fit to pass through blood vessels [9]. Typical red blood cells are circular in shape with a central pallor due to its biconcave shape. Red blood cell size can be described as macrocytic (large), microcytic (small), normocytic or anisocytosis. Red blood cells color can be evaluated as hyperchromic, hypochromic or polychromasia. The shape can appear in different form such as ancanthocytes, spherocytes, stomatocytes, tear drops or poikilocytes [10]. In some pathological conditions, cytoplasmic or nuclear finding like Howell jolly bodies, Heinz bodies, pappenheimer bodies and basophilic stippling can be found in red cells [11]. In diabetic conditions, red blood cells present certain changes such as reduced flexibility which slow down their ability to pass through narrow capillaries and microvessels [12]. Persistent exposure to hyperglycaemia and oxidative stress can quicken the aging process of red blood cell, thereby leading to hemolysis or premature cell death [13]. Other changes like alterations in the red blood cell membrane composition and function, such as alterations in lipid content and membrane proteins as a result of glycation and oxidative stress, can eventually lead to increased aggregation in RBCs. The aggregated RBC can obstruct blood flow through microvasculature, which lead to tissue hypoxia and complications seen in diabetes [14]. Diabetes also causes alterations in endothelial cell structure and function, leading to increased vascular permeability. Diabetes again contributes to changes in endothelial cell structure and function, which causes increased vascular permeability in organs like kidney (nephropathy) and eyes (diabetic retinopathy) respectively [15]. Morphology changes can occur in RBCs of diabetics' patients, which elevate oxidation-reduction sensitive fragility of cell membrane, leading to hemolysis [16]. Evaluating the morphological changes on red blood cells of diabetics' patients is crucial for management and treatment of the disease. This study was undertaken to assess red blood cell morphology and hematological indices in patients with T2DM attending a tertiary hospital in Enugu, Nigeria.

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## 2. Materials and Methods

### 2.1. Study Design

This case-control study was conducted at the Hematology and Blood Transfusion Laboratory and the Diabetes Outpatient Clinic of Enugu State University Teaching Hospital, Enugu, Nigeria, from July to November 2024. A total of 70 patients with T2DM (31 males and 39 females, aged 40–60 years) and 30 apparently healthy, non-diabetic controls were recruited. Participants with hematological disorders (e.g., hemoglobinopathies) or chronic illnesses such as renal disease, liver cirrhosis, or cancer were excluded. Demographic and clinical data were collected using structured questionnaires.

### 2.2. Sample Collection and Laboratory Analysis

Five milliliters of venous blood were collected from each participant into ethylenediaminetetraacetic acid (EDTA) tubes. Red cell indices—including RBC count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width-coefficient of variation (RDW-CV), and red cell distribution width-standard deviation (RDW-SD)—were determined using a five-part hematology analyzer (Mindray BC 5150).

Peripheral blood smears were prepared, air-dried, and stained with Leishman stain according to standard protocols [17]. Morphological abnormalities such as hypochromasia, anisocytosis, rouleaux formation, and poikilocytosis were graded according to published criteria [18, 19].

### 2.3. Ethical Considerations

The study was approved by the Research Ethics Committee of Enugu State University Teaching Hospital. Written informed consent was obtained from all participants.

### 2.4. Statistical Analysis

Data were analyzed using SPSS version 25.0. Results were expressed as mean  $\pm$  standard deviation (SD) or percentages, as appropriate. Comparisons between groups were performed using independent samples t-test or one-way ANOVA, while associations between categorical variables were assessed using chi-square tests. A p-value of  $<0.05$  was considered statistically significant.

### 3. Result

The mean PCV was significantly lower in T2DM patients compared with controls ( $36.89 \pm 6.62\%$  vs.  $39.55 \pm 3.75\%$ ,  $p=0.03$ ). Other hematological indices, including RBC count, Hb, MCV, MCH, and MCHC, showed no significant differences between groups (Table 3.1).

**Table 1** Hematological Parameters of T2DM Patients vs. Controls

Parameters	test (N=70)	control (N=30)	p-value
RBC count (1012/L)	$4.51 \pm 0.29$	$4.56 \pm 0.46$	0.88
HGB (g/dl)	$12.31 \pm 2.19$	$13.19 \pm 1.25$	1.00
PCV (%)	$36.89 \pm 6.62$	$39.55 \pm 3.75$	0.03*
MCV (fl)	$87.37 \pm 9.04$	$88.30 \pm 4.24$	0.76
MCH (pg)	$29.17 \pm 3.04$	$29.15 \pm 1.72$	1.00
MCHC (g/dl)	$33.32 \pm 0.25$	$32.85 \pm 0.78$	0.99
RDW-CV (%)	$14.23 \pm 1.81$	$13.95 \pm 1.24$	0.65
RDW-SD (fl)	$44.83 \pm 6.76$	$43.38 \pm 4.88$	0.45
Age (years)	$49.99 \pm 7.99$	$26.87 \pm 3.81$	<0.001*

The results showed that there was an increase in the female T2DM compared to the male T2DM with respect to the mean values of the RDW-CV and RDW-SD (values indicated): RDW-CV ( $14.53 \pm 2.08$ ) and RDW-SD ( $45.77 \pm 7.06$ ), for the female T2DM (Table 3.2).

**Table 2** Gender-Based Comparison

Parameters	female (N=39)	male (N=31)	p-value
RBC count (1012/L)	$4.22 \pm 0.65$	$4.22 \pm 0.71$	0.999
HGB (g/dl)	$12.31 \pm 2.17$	$12.31 \pm 2.27$	1.000
PCV (%)	$36.85 \pm 6.56$	$36.93 \pm 6.81$	0.999
MCV (fl)	$87.52 \pm 9.28$	$87.16 \pm 8.87$	0.985
MCH (pg)	$29.20 \pm 3.09$	$29.12 \pm 3.02$	0.994
MCHC (g/dl)	$33.33 \pm 0.33$	$33.31 \pm 0.05$	0.905
RDW-CV (%)	$14.53 \pm 2.08$	$13.83 \pm 1.29$	0.203
RDW-SD (fl)	$45.77 \pm 7.06$	$43.59 \pm 6.25$	0.365
FBS (mmol/L)	$6.45 \pm 0.92$	$7.30 \pm 1.05$	0.942
Age(years)	$49.18 \pm 8.68$	$51.07 \pm 6.41$	0.549

The mean value of RDW-CV and RDW-SD for the female T2DM between the ages of 40-45 years ( $15.46 \pm 2.31$  and  $49.34 \pm 7.73$ ) were significantly higher compared to those that are 50 years and above ( $13.29 \pm 1.16$  and  $41.62 \pm 2.66$ ) ( $p=0.04;0.01$ ) (Table 3.3).

**Table 3** Age-Based Comparison

Male									
Age (yrs)	RBC	HGB	PCV	MCV	MCH	MCHC	RDW-CV	RDW-SD	FBS
40-45	4.37±0.36	12.18±1.02	36.55±3.05	84.28±11.09	28.08±3.72	33.30±0.01	13.67±0.42	41.02±3.89	7.00±1.16
46-50	4.15±0.85	11.77±2.79	35.29±8.38	84.11±10.00	28.23±3.52	33.33±0.09	14.11±1.45	44.00±8.15	7.55±1.05
51-55	3.85±0.64	11.78±2.10	35.34±6.31	91.68±6.94	30.56±2.32	33.30±0.01	13.82±0.54	45.14±2.77	7.32±0.82
56-60	4.46±0.72	13.49±2.16	40.46±6.49	90.68±4.18	30.24±1.39	33.30±0.01	13.56±1.83	43.99±6.62	7.18±1.22
p-value	0.46	0.40	0.39	0.22	0.28	0.65	0.83	0.73	0.76
Female									
40-45	4.07±0.57	12.03±2.29	36.01±6.92	88.30±10.26	29.52±3.42	33.38±0.29	15.46±2.31	49.34±7.73	6.25±0.87
46-50	4.09±1.09	12.37±3.52	37.10±10.57	90.40±4.66	30.13±1.55	33.30±0.01	13.98±2.15	44.57±4.29	6.48±0.97
51-55	4.51±0.59	12.23±1.64	36.50±4.99	81.45±7.10	27.18±2.12	33.37±0.64	13.78±1.26	41.62±2.66	6.93±0.71
56-60	4.95±0.46	13.23±1.38	39.70±4.14	88.91±10.25	29.57±3.52	33.22±0.23	13.29±1.16	41.88±5.49	6.74±0.93
p-value	0.29	0.63	0.61	0.34	0.35	0.67	0.04*	0.01*	0.32

In the blood film of male subjects, 3 (9.6%) blood films out of 31 showed hypochromasia and among 3, one blood film showed slight hypochromasia (1+), one showed moderate hypochromasia (2+) and one blood film showed marked hypochromasia (3+). In the blood film of female subjects, 4 (10.2%) blood films out of 39 showed hypochromasia and among 4, two blood films showed slight hypochromasia (1+) and two blood films showed marked hypochromasia (3+). Again in the blood film of male subjects, 1 (3.2%) blood film out of 31 showed anisocytosis. While in the female subject, 3 (7.8 %) blood films out of 39 showed anisocytosis and among 3, one blood film showed slight anisocytosis (1+), one showed moderate anisocytosis and one blood film showed marked anisocytosis (3+). However, one blood film (2.6%) of female subjects showed rouleaux formation. In all, the Chi square (X<sup>2</sup>) test exhibited no statistically significant effects of gender on RBC morphology (X<sup>2</sup>=12.500, p-value=0.052) (Table 3.4).

**Table 4** Grading of red cell morphology according to gender

Grading	Male	Female	X <sup>2</sup>
	N (%)	N (%)	
1+ (slight hypochromasia)	1 (3.2)	2 (5.1)	
2+ (moderate hypochromasia)	1 (3.2)	0 (0)	
3+ (marked hypochromasia)	1 (3.2)	2 (5.1)	X <sup>2</sup> = 12.500, p-value=0.052
1+ (slight anisocytosis)	0 (0)	1 (2.6)	
2+ (moderate anisocytosis)	1 (3.2)	1 (2.6)	
3+ (marked anisocytosis)	0 (0)	1 (2.6)	
1+ (rouleaux formation)	0 (0)	1 (2.6)	
Normocytic normochromic	27 (87.1)	31 (79.5)	
Total	31 (100%)	39 (100%)	

Among the blood film of subjects less than 45 years, 3 (12 %) films out of 25 showed hypochromasia and among 3, two films showed slight hypochromasia (1+) and one film showed marked hypochromasia (3+). Among the blood film of subjects between 46-60 years, 5 (11%) blood films out of 45 showed hypochromasia and among 5, two blood films showed slight hypochromasia (1+), one blood film showed moderate hypochromasia (2+) and two blood films showed marked hypochromasia (3+). Again in the blood film subjects less than 45 years, 2 (8%) blood films out of 25 showed anisocytosis and among 2, one blood film showed slight anisocytosis and one blood film showed moderate anisocytosis. While in the subject between 46-60 years, 2 (4.4 %) blood films out of 45 showed anisocytosis and among 2, one showed moderate anisocytosis (2+) and one blood film showed marked anisocytosis (3+). However, one blood film (4%)

of subjects less than 45 years showed rouleaux formation. In all, the Chi square ( $X^2$ ) test exhibited no statistical significant effects of age on RBC morphology ( $X^2=12.500$ ,  $p\text{-value}=0.187$ ) (Table 3.5).

**Table 5** Grading of red cell morphology according to Age (years)

Grading	< 45 years	46-60	$X^2$
	N(%)	N(%)	
1+ (slight hypochromasia)	2 (8)	2 (4.4)	
2+ (moderate hypochromasia)	0 (0)	1 (2.2)	
3+ (marked hypochromasia)	1 (4)	2 (4.4)	$X^2 = 12.500$ , $p\text{-value}=0.187$
1+ (slight anisocytosis)	1 (4)	0 (0)	
2+ (moderate anisocytosis)	1 (4)	1 (2.2)	
3+ (marked anisocytosis)	0 (0)	1 (2.2)	
1+ (rouleaux formation)	1 (4)	0 (0)	
Normocytic normochromic	19 (76)	38 (84.4)	
Total	25 (100 %)	45 (100%)	

#### 4. Discussion

In diabetes mellitus, chronic hyperglycemia can lead to severe life-threatening complications and such complications heighten damages to many organs, such as the kidneys, cardiovascular systems and nervous systems [20, 21]. Apart from this, changes in various haematological parameters such as red blood cells are frequently encountered in individual with diabetes mellitus [22]. In non-diabetic patients, RBCs have biconcave shape, with elevated plasma membrane integrity and cytoskeleton stability. This allows the red cell membrane to deform considerably in order to survive the shear stress witnessed when the cells pass through vessels of different diameters and blood streams of different turbulence, within the circulatory system [23, 24]. But the RBCs of diabetic patients flow within the blood in a hyperglycemic environment in almost their entire lives. This leads to alterations in the specific morphology, such as shape, color, size inclusion and arrangement. These alterations may deleteriously affect the RBCs function [25]. In diabetic patients with variations in RDW, low PCV, hypochromasia, anisocytosis, poikilocytosis, spherocytosis or normocytic normochromic anemia, the peripheral blood smear can be used to interpret the diagnostic significant of red blood cell findings. Increasing evidence suggests that alterations in red blood cell distribution width (RDW), an index of heterogeneousness in circulating red blood cell size, clearly serve as a diagnostic marker to gauge diabetes mellitus complications and mortality risk [26]. Earlier studies have evaluated RDW changes in diabetes mellitus in respect of either RDW-Standard Deviation or RDW-Coefficient of Variation. And it has been observed that there is significantly increased risk of developing diabetes mellitus in association with low RDW, demonstrating its certainty as a surrogate marker for reduced red blood cell survival [27, 28]. However, increased RDW have been described to be associated with an increased risk and a poor diagnosis for diabetic nephropathy and capable of assisting as an instrument to evaluate the influence of therapy [29, 30]. The present study observed increased RDW in female subjects between the ages of 40-45 years, which is in agreement with study done by Tsuboi *et al.*, [31] in DM patients. Anemia is one of the frequent and wide spread blood associated disorder that occurs in patients with diabetes [32]. It basically occurs in diabetes mellitus patients who developed renal deficiency [33, 34]. Growing evidence suggests that the prevalence of anemia among type 2 diabetes mellitus is usually related with the failure of the kidney to produce proper erythropoietin [35, 36]. In this study, PCV of 36.9% was found in type 2 diabetes mellitus patients. This is in line with study done in Ethiopia that reported hemoglobin level of 9.4g/dl-17.5gdl in T2DM patients [37]. In this finding, 82.9% of the DM patients had normocytic and normochromic blood picture. This is in line with study conducted in India, Iraq, China and Malaysia [38-41]. The normocytic normochromic blood picture could suggest anemia of chronic disease in diabetes mellitus patients [42]. Again, normocytic normochromic anemia could be indicating the implication of the renal origin of anemia in diabetes mellitus patients [42, 43-46]. Hypochromasia and anisocytosis (variation in size) was found in this study. Hypochromasia and anisocytosis of red blood cells are known to be common laboratory findings. The commonest cause of hypochromasia is iron deficiency [47]. When diagnosed properly, red blood cell morphology can be a vital tool for laboratory hematology professionals to recommend proper laboratory follow-up and to determine the best tests for definitive diagnosis.

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## 5. Conclusion

This study demonstrated that patients with type 2 diabetes mellitus show reduced hematocrit levels, increased red cell distribution width particularly among younger females and morphological abnormalities such as hypochromasia and anisocytosis. Although most patients retained a normocytic normochromic blood picture, the observed changes highlight subtle hematological alterations associated with diabetes. Given the accessibility and low cost of these tests, incorporating routine hematological screening into the monitoring of T2DM could provide valuable clinical insights, especially in resource-limited settings. Future studies with larger, age-matched cohorts are needed to confirm these findings and explore their implications for early detection and management of diabetic complications.

### *Limitations*

A key limitation of this study is the significant age difference between cases (mean age ~50 years) and controls (mean age ~27 years). Since age independently influences hematological indices, this mismatch may have confounded results. Larger studies with age- and sex-matched controls are recommended.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

Authors declared that no competing interests exist.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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