RESEARCH PAPER

Impact of Metformin Therapy on Anemia and Red Cell Parameters in Type 2 Diabetes Mellitus

Varghese A[1]*, Vaidya KA[2], Joseph D[3] and David A[4]

[1]Research Scholar, Department of Pathology, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India
[2]Professor, Department of Pathology, Srinivas Institute of Medical Sciences and Research Centre, Mukka, Mangalore, Karnataka, India
[3]Associate Professor, Department of Pathology, Believers Church Medical College Hospital, Thiruvalla, Kerala, India
[4]Head of Department, Medical Research, Epidemiology & Biostatistics, Believers Church Medical College Hospital, Thiruvalla, Kerala, India
***Email:** abinmlt@gmail.com*

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ABSTRACT

The aim of the study was to evaluate the impact of metformin on red cell parameters in type 2 diabetes by comparing metformin users with non-metformin users within the T2DM group, and both groups with non-diabetic controls. A retrospective cross-sectional study of 1,728 participants included 1,048 with T2DM and 680 non-diabetic controls. The T2DM group was stratified into metformin users (n=630) and non-metformin users (n=418). Red cell parameters, including hemoglobin, hematocrit, RBC count, MCV, MCH, MCHC, and RDW-CV, were analyzed. Sex-stratified comparisons were performed using the Mann-Whitney U test and independent t-test after Shapiro-Wilk normality assessment. Type 2 diabetes was associated with statistically lower hemoglobin, hematocrit, and RBC count in males compared to non-diabetic controls. However, the absolute differences were small and clinically non-significant. Female red cell indices remained largely comparable between groups. Within the T2DM group, metformin users showed no clinically significant alterations in red cell parameters. Our study found that metformin was not independently associated with significant alterations in red cell parameters or with resulting anemia.

Keywords: Metformin, Type 2 diabetes, Red cell indices, Anemia, Vitamin B12, Hemoglobin

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INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by insulin resistance and progressive beta-cell dysfunction [1]. In T2DM, normal erythropoiesis is disrupted by pathophysiological processes, including chronic inflammation, oxidative stress, iron dysregulation, and impaired erythropoietin response [2,3]. Metformin is a first-line oral biguanide antidiabetic drug used in type 2 diabetes mellitus that lowers blood glucose mainly by reducing hepatic glucose production and improving insulin sensitivity [4] Metformin, despite its efficacy, can inhibit vitamin B12 absorption in the gut. A deficiency of vitamin B12 can cause anemia, most often megaloblastic anemia 5. Although there is a theoretical basis, the evidence linking metformin to clinically meaningful anemia remains inconsistent. Some research indicates a slightly increased risk of anemia, especially with long-term use. Other studies found no significant overall association. Many existing studies compare metformin users to healthy controls, often reporting higher rates of anemia in individuals with diabetes. However, this may not be due to metformin. Diabetes alone can affect red blood cells and hemoglobin, resulting in anemia. To address this gap, our study compared red cell parameters between diabetic and non-diabetic subjects to determine the independent impact of diabetes mellitus on red cell indices. Additionally, red cell parameters were compared between metformin users and non-metformin users within the diabetic population to identify the specific impact of metformin on red cell indices and induction of anemia.

MATERIALS AND METHODS

Study Design & Setting

The study was a retrospective cross-sectional study conducted at Believers Church Medical College Hospital, Thiruvalla. Data were collected over 4 years (from 2021 to 2024).

Study Population

A total of 1728 participants were included, comprising 1048 with type 2 diabetes and 680 nondiabetic controls. The T2DM group was further stratified into metformin users (n=630) and non-metformin users (n=418). Patients

*Author for Correspondence: abinmlt@gmail.com

with active infectious diseases, inflammatory conditions, autoimmune diseases, malignant tumors, pregnant women, and known cases that affect red cell indices were excluded from the sampling.

Sample size calculation
Sample size was calculated to ensure adequate statistical power for two comparisons: type 2 diabetes versus non-diabetic controls and metformin users versus non-metformin users, resulting in a total of 1728 participants.

Sampling method
A retrospective consecutive sampling, where eligible patients were systematically included

Data Collection
Patient data were retrieved from the hospital information system, laboratory information system, and medical records.

Laboratory Investigation
Parameters evaluated included hemoglobin (Hb, g/dL), hematocrit (HCT, %), RBC count ($\times 10^6/\mu L$), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), and red cell distribution width-coefficient of variation (RDW-CV, %), all processed on a DxH 900 hematology analyzer.

Ethical Clearance
This study was approved by the Institutional Ethics Committee of Believers Church Medical College Hospital, Thiruvalla, Kerala, India (IEC No. IEC/2024/18/455).

Statistical Analysis
All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Normality was assessed using the Shapiro-Wilk test. Normally distributed continuous variables were compared using the independent-samples t-test. Non-normally distributed variables were analyzed using the Mann-Whitney U test. Sex-stratified analyses of red cell parameters were conducted for T2DM versus non-diabetic controls. Subgroup analyses comparing metformin users with non-metformin users within the diabetes mellitus cohort were performed separately.

RESULTS

Baseline Characteristics

Table 1: Demographics

Variable	Type 2 Diabetes Mellitus (n = 1,048)	Non-Diabetic control (n = 680)
Age (years)		
Mean ± SD	68.51 ± 10.09	65.4 ± 11.6
Range	40 – 95	41 – 89
Sex, n (%)		
Male	656 (62.6%)	319 (46.9%)
Female	392 (37.4%)	361 (53.1%)

Red Cell Parameters T2DM vs Non-Diabetic Controls

Table 2: Sex-stratified red cell parameters:T2DM vs Non-Diabetic Controls

Parameter	Sex	T2DM (n: Male=656, Female=392)		Non-DM (n: Male=319, Female=361)		p-Value
		Mean ± SD	Range (Min–Max)	Mean ± SD	Range (Min–Max)	
Red Cell Indices						
Hemoglobin (g/dL)	Male	13.09 ± 1.91	7.5 – 19.7	13.78 ± 1.59	9.3 – 18.3	<0.001
	Female	12.06 ± 1.54	6.1 – 17.5	12.29 ± 1.19	8.3 – 14.9	0.034
Hematocrit (%)	Male	39.33 ± 5.40	22.2 – 51.9	41.36 ± 4.68	28.6 – 53.3	<0.001
	Female	36.88 ± 4.42	20.5 – 54.9	37.46 ± 3.62	24.2 – 58.3	0.100
RBC Count ($\times 10^6/\mu L$)	Male	4.53 ± 0.65	2.71 – 6.70	4.73 ± 0.63	2.89 – 6.83	<0.001
	Female	4.34 ± 0.52	2.77 – 6.85	4.34 ± 0.45	2.36 – 5.83	0.935

T2DM, Type 2 diabetes mellitus; Non-DM, non-diabetes mellitus; SD, standard deviation. p-values derived using the Mann-Whitney U test

Red Cell Indices: Metformin vs Non-Metformin Subgroup Analysis

A sex-stratified comparison of red cell parameters was conducted between metformin users and non-metformin users within the T2DM population.

Table 3. Sex-stratified red cell parameters: Metformin versus non-metformin users within the T2DM cohort

Parameter	Male			Female		
	Metformin users	Non-Metformin users	p-value	Metformin users	Non-Metformin users	p-value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Red Cell Indices						
Hemoglobin (g/dL)	13.30 ± 1.79	12.94 ± 1.97	0.041*	12.05 ± 1.56	12.07 ± 1.52	0.828
Haematocrit (%)	40.03 ± 5.05	38.83 ± 5.58	0.015*	36.87 ± 4.54	36.89 ± 4.33	0.870
RBC Count ($\times 10^6/\mu L$)	4.63 ± 0.60	4.46 ± 0.67	0.001**	4.36 ± 0.55	4.33 ± 0.51	0.710

*Normality was assessed using the Shapiro-Wilk test; the Mann-Whitney U test was applied for non-normally distributed variables. SD, standard deviation. * $p < 0.05$; ** $p < 0.01$; NS, not significant.*

Red Cell Indices in Type 2 Diabetes Mellitus: A Comparison between Metformin and Non-Metformin Users

Red cell morphology indices were compared between metformin users and non-users within the T2DM group.

Table 4. Red cell morphology indices: Metformin versus non-metformin users within the T2DM population

Parameter	Metformin (n = 630) Mean ± SD	Non-Metformin (n = 418) Mean ± SD	p-value	Statistical Test
Red Cell Indices				
MCV (fL)	85.93 ± 6.22	86.55 ± 5.73	0.167	Mann-Whitney U
MCH (pg)	28.43 ± 2.60	28.62 ± 2.33	0.386	Mann-Whitney U
MCHC (g/dL)	32.95 ± 1.21	33.03 ± 1.14	0.317	Independent t-test
RDW-CV (%)	14.50 ± 1.77	14.42 ± 1.73	0.586	Mann-Whitney U

Normality was assessed using the Shapiro-Wilk test. Mann-Whitney U test was used for non-normal distributions; independent t-test was used for normally distributed variables. No statistically significant differences were observed ($p > 0.05$ for all). SD, standard deviation; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width – coefficient of variation.

DISCUSSION

In males, mean hemoglobin was lower in the T2DM group (13.09 ± 1.91 g/dL) than in non-diabetic controls (13.78 ± 1.59 g/dL). However, females showed minimal intergroup differences, with 12.06 ± 1.54 g/dL in T2DM versus 12.29 ± 1.19 g/dL in non-diabetic controls. Male T2DM patients had a lower mean hematocrit (39.33 ± 5.40%) than male controls (41.36 ± 4.68%). In females, hematocrit values were closely comparable between groups (36.88 ± 4.42% vs. 37.46 ± 3.62%). Male T2DM subjects demonstrated a modestly reduced RBC count (4.53 ± 0.65 × $10^6/\mu L$) relative to non-diabetic males (4.73 ± 0.63 × $10^6/\mu L$), and in females, the values were identical (4.34 ± 0.52 vs. 4.34 ± 0.45 × $10^6/\mu L$). A Calabar study reported hemoglobin values of 11.9 ± 2.2 g/dL in diabetes versus 13.3 ± 0.9 g/dL in controls, directionally consistent with the male T2DM hemoglobin of 13.09 ± 1.91 g/dL [6]. Studies from Ethiopia, Nepal, and India further demonstrate that poor glycaemic control, reflected by elevated HbA1c, amplifies reductions in RBC count, hemoglobin, and hematocrit within diabetic populations, suggesting a dose-dependent erythrosuppressive effect of chronic hyperglycemia [7–9]. However, the literature is not uniformly concordant. An Ethiopian study found lower hemoglobin in T2DM but no significant difference in RBC count. In contrast, Pakistani and Indian studies reported no statistically significant intergroup differences in RBC count or hemoglobin before

sex-stratification [10–12]. These discrepancies likely reflect heterogeneity in disease duration and baseline anemia prevalence across study populations. A Korean study similarly demonstrated a stronger association between diabetes and anemia prevalence in men than in women [13]. This may reflect relative protection in females through hormonal or hematopoietic compensatory mechanisms, or the confounding effect of pre-existing iron-deficiency anemia.

Sex-stratified analysis of red cell indices revealed a significant association between metformin use and higher hemoglobin, hematocrit, and RBC count in male patients ($p = 0.041$, 0.015, and 0.001, respectively) compared to non-metformin users. No significant differences were observed in females across all three parameters. Although statistically significant differences were observed in male patients, the absolute between-group differences were small, and overlapping standard deviations suggest that these findings lack clinical significance. No significant differences in MCV, MCH, MCHC, or RDW-CV were observed between metformin users and non-users. This indicates that metformin use doesn't alter red cell morphology. Conflict findings have been reported from existing studies. Lingam et al. (2023) reported that metformin users had lower RBC counts alongside higher hematocrit, MCV, MCH, and RDW compared to non-users [14]. Similarly, Abdullah et al. (2023) observed significant declines in PCV, hemoglobin, and RBC count following three months to one year of metformin exposure [15]. Husain et al. (2022) reported lower hemoglobin and MCHC levels in North Indian patients on metformin compared with non-diabetic non-users. In contrast, many studies reported a non-significant correlation between metformin use and red cell parameters [4]. Qasim et al. (2023) found no significant differences in RBC count, hemoglobin, MCV, MCH, MCHC, or hematocrit between metformin users and controls [16]. Smaoui et al. (2022) found no significant correlation between metformin use and red cell parameters [17]. Many studies used non-diabetic controls as a comparator group, whereas our study compared non-diabetic controls with metformin users and non-metformin users within the T2DM group. The findings suggest that metformin does not show an independent adverse effect on red cell parameters. The statistically significant elevations in hemoglobin, hematocrit, and RBC count observed among male metformin users are unlikely to be clinically relevant. Metformin exposure may be associated with subtle reductions in red cell parameters in females. The lack of significant changes in females may partly reflect hormonal variability over 40 years, including the menopausal transition and metformin-associated alterations in sex hormones, which could modify erythropoiesis [18,19]. Metformin alone does not affect red cell indices, and her confounding factors, such as dietary habits, iron deficiency, and comorbid nutritional deficiencies, cannot be entirely excluded. 67.5% of the study population had long-standing diabetes with microvascular and macrovascular complications. Being a retrospective study, data on vitamin B12 supplementation status were not uniformly available for the study population, and confounding by supplementation status could therefore not be adjusted for. Even among metformin users who may have received vitamin B12 supplementation, serum vitamin B12 assay is not routinely performed as a standard clinical practice. While glycemic parameters such as fasting glucose and HbA1c are monitored regularly in diabetic patients, haemogram parameters and vitamin B12 status are not subjected to comparable routine surveillance. The present study therefore also draws attention to an existing gap in anemia screening practices among metformin-treated diabetic patients. Also the study underscores the importance of incorporating haemogram parameters a relatively low-cost investigation alongside vitamin B12 assessment, despite its additional cost, into the routine follow-up of patients on long-term metformin therapy.

CONCLUSION

Diabetes mellitus was associated with lower hemoglobin, hematocrit, and RBC count in males compared with non-diabetic controls. However, the absolute differences were small and clinically non-significant. Female red cell indices remained largely comparable between groups. Within the T2DM population, metformin users had marginally higher hemoglobin, hematocrit, and RBC count among males, though these differences were not clinically relevant. No differences in MCV, MCH, MCHC, or RDW-CV were detected, confirming that metformin does not alter red cell morphology. These findings suggest that reduced hemoglobin and red cell parameters may reflect the disease burden of diabetes mellitus rather than metformin therapy. Future longitudinal studies incorporating Vitamin B12 assays and metformin exposure duration would clarify the temporal relationship between metformin exposure and the hematological changes.

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