Section: General Medicine

Original Research Article

CORRELATION OF RED CELL DISTRIBUTION WIDTH–CV (RDW-CV) AND HbA1c IN TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH DIABETIC RETINOPATHY

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ABSTRACT

Background: Diabetic retinopathy (DR) is a common microvascular complication of diabetes and a leading cause of preventable blindness. Red cell distribution width (RDW-CV) has emerged as a potential biomarker for microvascular complications. This study aimed to assess the correlation between RDW-CV and glycated haemoglobin (HbA1c) levels and to evaluate its association with DR in patients with type 2 diabetes mellitus (T2DM). **Materials and Methods:** This hospital-based cross-sectional study included 125 patients with T2DM attending a tertiary care centre in Barpeta, Assam. All patients underwent a detailed clinical evaluation, including fundoscopy and routine blood investigations. Associations between RDW-CV, HbA1c levels and fundoscopy findings were analysed, and correlations with severity of DR was assessed. **Result:** RDW-CV showed significant associations with HbA1c levels (x^2= 56.2, p < 0.0001) and fundoscopy findings (x^2 = 139.72, p = 0.0001). HbA1c levels were also significantly associated with fundoscopy findings (x^2= 50.20, p = 0.0007). RDW-CV demonstrated a moderate-to-strong positive correlation with HbA1c (r = 0.69) and a strong positive correlation with DR severity (r_s = 0 .79). **Conclusion:** RDW-CV is significantly associated with glycaemic control and the presence and severity of DR, and may serve as a simple, cost-effective marker for identifying at-risk patients. Further longitudinal studies are needed to validate its predictive role.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease marked by abnormalities in the secretion, action, or a combination of both, of the hormone insulin. The latest International Diabetes Federation (IDF) Diabetes Atlas (2021) reports that 10.5% (537 million) of global adult population (20-79 years) is living with diabetes. Over 90% of people with diabetes have type 2 diabetes mellitus. By 2045, it is projected that approximately 783 million adults will be living with diabetes.[1]

Diabetic retinopathy is a common microvascular complication of diabetes caused by high blood sugar damaging the retinal blood vessels, which can cause potential blindness but can be prevented through early detection and proper management. It develops slowly, often without early symptoms, affecting both type 1 and 2 diabetics. DR affects approximately 22–35% of people with diabetes globally, with roughly 6–10% suffering from vision-threatening stages of DR (VTDR). It is a leading cause of blindness driven by rising diabetes rates, with projections estimating over 190 million people could have DR by 2030.[2] Key risk factors include long duration of diabetes, poor glycaemic control, and hypertension.

The main indicator of long-term glycaemic control is glycated haemoglobin (HbA1c), which gives an average reading of blood sugar levels over a period of 2-3 months.[3] While HbA1c is the gold standard for assessing long-term glycaemic control, relying solely on it can be restrictive. There is a clinical need for additional biomarkers to enhance risk stratification.

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Red cell distribution width (RDW) is a measure of variation in the size of circulating erythrocytes. It can be expressed as RDW-CV (coefficient of variation) and RDW-SD (standard deviation). The more commonly used RDW-CV, which is based on the coefficient of variation of the red blood cell distribution volume and is expressed as a percentage. RDW-SD which is a direct measure of the width of the RBC distribution curve, is expressed in femtolitres.[4] RDW has garnered attention as a potential biomarker for diabetes. Elevated RDW levels have been related to poor glycaemic control as well as diabetic complications.[5] Moreover, RDW-CV is a standard component of a complete blood count (CBC), making it a highly accessible, inexpensive, and non-invasive tool that is readily available in virtually all healthcare settings.[6]

MATERIALS AND METHODS

Study Design and Setting: This was a hospital-based cross-sectional study conducted over one year, from July 2023 to June 2024 at Fakhruddin Ali Ahmed Medical College and Hospital (FAAMCH, renamed Barpeta Medical College and Hospital), Barpeta, Assam. It aimed to assess the correlation between RDW-CV and HbA_1c levels in patients with type 2 diabetes mellitus and to evaluate the association between RDW-CV and diabetic retinopathy.

Study Population: Type 2 diabetes mellitus patients aged 40 years and above attending the Medicine and Ophthalmology outpatient departments (OPD) or admitted to the medicine ward, who provided informed consent, were included in the study. Patients with conditions affecting red blood cells or HbA_1c levels (such as hemoglobinopathies, anaemia, splenectomy, pregnancy and hypothyroidism) and those with severe systemic illnesses, including chronic liver disease, end-stage renal disease, rheumatological disorders, malignancy, or active infections (e.g., malaria, tuberculosis), were excluded from the study.

Sample Size: A total of 125 patients were included in the study. The sample size was calculated using a standard statistical formula assuming a 9% national prevalence of diabetes, a 95% confidence interval, and a 5% allowable error. The formula used is $N = \frac{Z^2 p\,(1-p)}{d^2}$ where, Z is the statistical constant (1.96 for a 95% confidence interval), p is the expected incidence (attained from previous studies), and d is the allowable error.

Data Collection and Investigations: A diagnosis of diabetes was made based on the American Diabetes Association (ADA) 2017 criteria requiring a Fasting Blood Sugar (FBS) ≥ 126 mg/dL, 2-hour postprandial sugar (PPBS) ≥ 200 mg/dL or $HbA_1c \geq 6.5\%$[6]. Data were collected using a structured proforma. Detailed history was obtained and thorough clinical examination including fundoscopy was performed for every patient. Routine blood tests (CBC that included RDW-CV, renal function tests, liver function tests, routine urine examination) in addition to FBS, 2-hour PPBS and HbA_1c was carried out in each patient. The HbA_1c levels were measured using a chromatography-based HPLC (High-Performance Liquid Chromatography) assay. DR was diagnosed through fundoscopy examination.

Data Analysis: Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) version 23 and RStudio (2024.04.2). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as frequencies and percentages. Chi-square (x^2) test was used to assess associations between categorical variables such as RDW-CV categories, HbA_1c levels, and fundoscopy findings. A p-value (probability value) < 0.05 was considered significant. Karl Pearson correlation analysis was used to measure the linear relationship between RDW-CV and HbA_1c. Pearson correlation coefficient (r) > 0.7 was considered a strong correlation, 0.3–0.7 as moderate, and < 0.3 as weak. Spearman's rank correlation analysis was applied to observe the relationship between RDW-CV and the severity of diabetic retinopathy. Spearman's rank correlation coefficient (r_s) +1 was considered a positive association, 0 as no association, and -1 as a negative association. Results were visually represented through scatter plots and heatmaps with colour gradients representing the magnitude of the correlation coefficients.

Ethical Considerations: The study was approved by the Institutional Ethics Committee of Barpeta Medical College and Hospital, Barpeta, Assam. Informed written consent was taken from each participant in a language they understood. Confidentiality was maintained throughout the study.

RESULTS

The study included a total of 125 patients with type 2 diabetes mellitus aged 40 years and above. The participants' mean age was 59.27 ± 11.74 years. Majority of the participants, 28.8% (36) of the study population were in the age group 60-69 years. 26.4% (33) were between ages 40-49 years, 23.2% (29) were above 70 years, and 21.6% (27) were between 50-59 years. [Table 1]

Out of 125 participants, 49.6% (62) were male and 50.4% (63) were female. [Table 2]

Amongst the study population, the mean duration of diabetes was 10.64 ± 6.31 years. 44% (55) of the participants had comorbidities while 56% (70) did not have any. Of the total 125 participants, 24.8% (31) were on oral hypoglycaemic agents (OHA), 38.4% (48) were on insulin, and 36.8% (46) were on both OHA and insulin. [Table 3 and 4]

Routine urine examination of the participants showed no protein in 39.2% (49), trace amount in 28.8% (36), 1+ in 21.6% (27), and 2+ in 10.4% (13). Whereas 28% (35) showed no urine sugar, 36% (45) had trace

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amounts, 20% (25) had 1+, and 16% (20) had 2+. [Table 5]

The fundoscopy examination revealed no signs of diabetic retinopathy in 32% (40), non-proliferative diabetic retinopathy (NPDR) in 12.8% (16), proliferative diabetic retinopathy (PDR) in 45.6% (57), and diabetic macular oedema (DME) in 9.6% (12). [Table 6]

The mean RDW-CV values of the participants according to the duration of diabetes were 15.64 ± 1.85% for patients with duration < 5 years, 15.72 ± 1.53% for those with duration 5-9 years, and 15.19 ± 1.88% for those with duration ≥ 10 years. [Table 7]

The mean HbA1c values according to the duration of diabetes were 8.72 ± 2.83% for patients with diabetes duration < 5 years, 8.18 ± 2.71% for those with duration 5-9 years, and 8.04 ± 3.64% for patients with duration ≥10 years. [Table 8]

Further analysis of RDW-CV categories in relation to HbA1c levels demonstrated that higher RDW-CV values were more frequently observed in patients with higher HbA1c levels. Most patients with HbA1c > 9% had RDW-CV values ≥ 15%, whereas patients with HbA1C < 7% were more commonly distributed in the lower RDW-CV categories [Table 9]. A statistically significant association was found between RDW-CV categories and HbA1c levels (x^2= 56.2, p < 0.0001) [Figure 1]. Furthermore, Pearson correlation coefficient (r) analysis showed a moderate to strong positive correlation between RDW-CV and HbA1c levels (r = 0.69) suggesting that higher RDW-CV values are associated with poorer glycaemic control in patients with T2DM, which is also evident in the correlation heat map [Figure 2].

The comparison of laboratory parameters with fundoscopic findings showed that RDW-CV and HbA1c levels increased with increasing severity of DR. Patients without retinopathy had the lowest RDW-CV values (13.24 ± 0.8%) while those with PDR showed the highest values (17.01 ± 0.84%). Similarly, HbA1c levels were lowest in patients without retinopathy (6.68 ± 2.21%) and highest in those with PDR (10.23 ± 2.61%). [Table 10]

The fundoscopy findings in relation to RDW-CV levels revealed that patients with advanced stages of DR had RDW-CV ≥ 15%, 32 patients with PDR had RDW > 17%, whereas patients with no retinopathy and NPDR had lower RDW-CV (< 15%) with 40 patients showing no DR. [Table 11 and Figure 3]. A statistically significant association was found between RDW-CV and fundoscopy findings (x^2 = 139.72, p = 0.0001). The correlation between RDW-CV levels and fundoscopic findings was further evaluated using Spearman's rank correlation coefficient (r_s). A positive correlation was noted between RDW-CV and severity of DR (rs = 0 .79), as was also evident in the correlation heat map [Figure 4].

The relationship between fundoscopy findings and HbA1c levels showed that higher HbA1c levels were found in patients with advanced diabetic retinopathy with 30.4% (38) of them with PDR having HbA1c levels ≥ 9%, whereas those with no retinopathy, 23.2% (29) had HbA1c levels < 7% [Table- 12, Figure- 5]. A statistically significant association was found between HbA1c levels and the fundoscopy findings (x^2= 50.20, p = 0.0007) reflecting that poorer glycaemic control leads to advanced stages of retinopathy.

Table 1: Age distribution of the study population

Age interval (years)	Frequency (n)	Percentage (%)
40-49	33	26.4
50-59	27	21.6
60-69	36	28.8
≥70	29	23.2
Total	125	100

Table 2: Gender distribution of the study population

Sex	Frequency (n)	Percentage (%)
Male	62	49.6
Female	63	50.4
Total	125	100

Table 3: Presence of other co-morbidities

Presence of co-morbidities	Frequency (n)	Percentage (%)
Yes	55	44
No	70	56
Total	125	100

Table 4: Treatment received for diabetes

Treatment for diabetes	Frequency (n)	Percentage (%)
Insulin	48	38.4
OHA	31	24.8
Both	46	36.8
Total	125	100

Table 5: Presence of protein and sugar in routine urine examination

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Parameter	Negative	Trace	1+	2+	Total
Urine protein	49 (39.2%)	36 (28.8%)	27 (21.6%)	13 (10.4%)	125 (100%)
Urine sugar	35 (28%)	45 (36%)	25 (20%)	20 (16%)	125 (100%)

Table 6: Fundoscopy findings of the study population

Fundoscopy finding	Frequency (n)	Percentage (%)
No retinopathy	40	32
NPDR	16	12.8
PDR	57	45.6
DME	12	9.6
Total	125	100

Table 7: Distribution of RDW-CV by duration of diabetes

Duration of Diabetes	RDW-CV (mean ± SD) %
< 5	15.64 ± 1.85
5-9	15.72 ± 1.53
≥ 10	15.19 ± 1.88

Table 8: Distribution of HbA_{1c} by duration of diabetes

Duration of Diabetes	HbA1c (mean ± SD) %
< 5	8.72 ± 2.83
5-9	8.18 ± 2.71
≥ 10	8.04 ± 3.64

Table 9: Frequency distribution of RDW-CV and HbA_{1c}

RDW-CV	HbA1c < 7%	HbA1c 7-8.9%	HbA1c > 9%
11-12.9	12	4	0
13-14.9	18	10	2
15-16.9	13	13	22
>17	1	5	25

Table 10: Comparison of laboratory parameters with fundoscopy findings

Parameter	No Retinopathy	NPDR	PDR	DME
RDW-CV	13.24 ± 0.8	15.03 ± 0.31	17.01 ± 0.84	15.98 ± 0.26
HbA1c	6.68 ± 2.21	7.56 ± 1.83	10.23 ± 2.61	9.16 ± 1.97
FBS	181.3 ± 45.01	173.93 ± 31.27	176.91 ± 43.18	172.44 ± 41.26
PPBS	225.59 ± 43.2	198.31 ± 57.06	208.28 ± 44.17	219.32 ± 46.43

Table 11: Association between RDW-CV and fundoscopy findings

RDW-CV (%)	No Retinopathy	NPDR	PDR	DME
11-12.9	13	0	0	0
13-14.9	27	5	1	0
15-16.9	0	11	24	12
> 17	0	0	32	0

Table 12: Association between HbA_{1c} levels and fundoscopy findings

HbA1c	No Retinopathy	NPDR	PDR	DME
< 7.0	29 (23.2%)	7 (5.6%)	6 (4.8%)	2 (1.6%)
7.0 - 8.9	9 (7.2%)	4 (3.2%)	13 (10.4%)	4 (3.2%)
≥ 9.0	2 (1.6%)	5 (4.0 %)	38 (30.4%)	6 (8%)

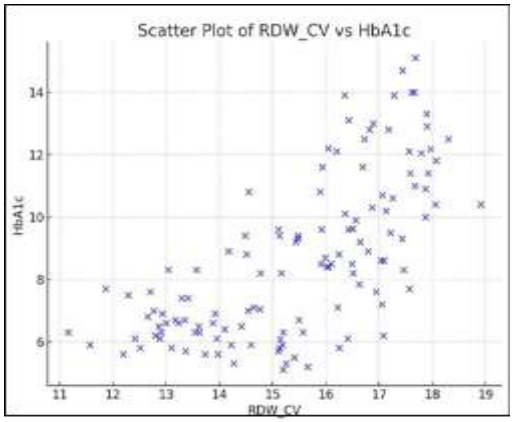

Figure 1: Scatter plot showing the correlation between RDW-CV and HbA_{1c} in type 2 diabetes mellitus

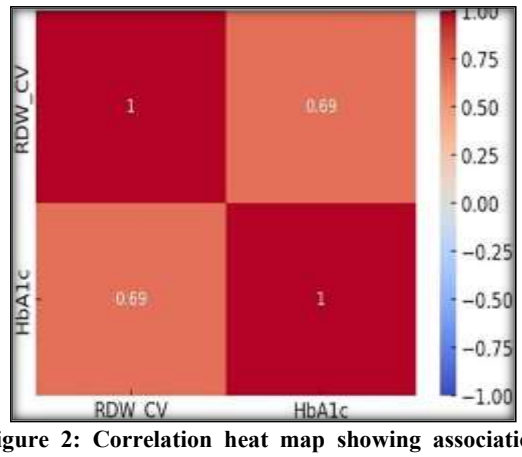

Figure 2: Correlation heat map showing association between RDW-CV and HbA_{1c}

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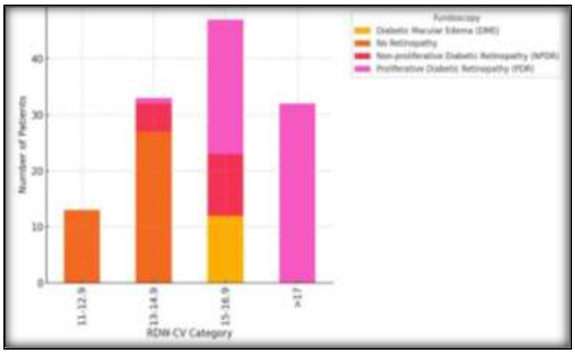

Figure 3: Bar diagram showing association between RDW-CV and fundoscopy findings

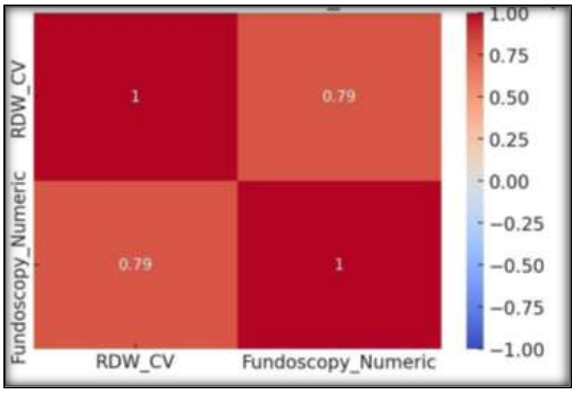

Figure 4: Heatmap of Spearman correlation between RDW-CV and fundoscopy findings

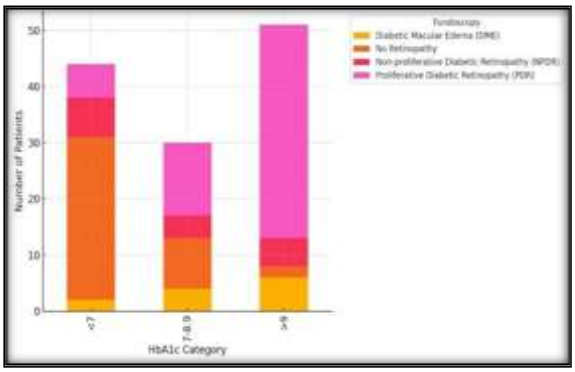

Figure 5: Bar diagram showing association between HbA1c and fundoscopy findings

DISCUSSION

This hospital-based cross-sectional study included 125 patients aged above 40 years and above with type 2 diabetes mellitus. The mean age of the study population was 59.27 ± 11.74 years, with the majority of the participants (28.8%) in the 60-69 years age group. A large proportion of patients aged 60 years and above highlights that older patient has a higher risk of complications like DR, consistent with previous studies showing that retinopathy risk increases with age and duration of diabetes.[7] Gender distribution in our study was fairly balanced (49.6% male and 50.4% female) which reduced gender bias in our results ensuring broader applicability amongst both male & female diabetics. Chaturvedi N et al found that women diabetics have a greater likelihood of developing cardiovascular disease that can also influence the occurrence and progression of DR.[8]

The mean duration of diabetes was 10.64 ± 6.31 years amongst the participants. 24.8% were on OHA, 38.4% were on insulin and, 36.8% were on both OHA and insulin. Routine urine examination of the participants showed proteinuria in 60.8% cases, whereas sugar was present in 72% cases. Previous studies by Keane WF et al[9] and Goldberg RB et al,[10] had highlighted proteinuria as an early indicator of diabetic kidney disease and glycosuria as an indicator of poor glycaemic control. The fundoscopy examination showed no signs of retinopathy in 32%, NPDR in 12.8%, PDR in 45.6% and DME in 9.6%. The ETDRS (Early Treatment Diabetic Retinopathy Study Research Group) had reported that without proper treatment, NPDR can advance to PDR leading to severe visual impairment or blindness.[11] Presence of PDR in one third of our study participants aligned with the finding of the study by You JW et al.[12]

The mean RDW-CV values were 15.64 ± 1.85% for patients with diabetes duration less than 5 years, 15.72 ± 1.53% for those with duration between 5-9 years, and 15.19 ± 1.88 % for those with duration 10 years and above. Existing literature supports the observation that prolonged diabetes is associated with increased RDW-CV due to chronic inflammation & oxidative stress.[13] The mean HbA_1c values were 8.72 ± 2.83% for patients with diabetes duration below 5 years, 8.18 ± 2.71% for those with duration between 5-9 years, and 8.04 ± 3.64 % for patients with duration 10 years and above.

Further analysis of RDW-CV in relation to HbA1c levels demonstrated that higher RDW-CV values were more frequently observed in patients with higher HbA1c levels. Most patients with HbA1c above 9% had RDW-CV values 15% and above, whereas patients with HbA1c below 7% had lower RDW-CV. A statistically significant association was found between RDW-CV and HbA1c levels ($x^2 = 56.2$, $p < 0.0001$). Pearson correlation coefficient analysis had showed a moderate to strong positive correlation between RDW-CV and HbA1c levels ($r = 0.69$) suggesting that higher RDW-CV levels are associated with poorer glycaemic control in patients with type 2 diabetes mellitus. Studies conducted by Lippi G et al,[14] Förhecz Z et al,[15] Alis R et al,[11] also found that patients with higher RDW-CV had higher HbA1c levels. This relationship may be explained by increased erythrocyte turnover caused by hyperglycaemia induced oxidative stress and inflammation, which disrupt erythropoiesis and lead to variability in RBC size. Additionally, nutritional deficiencies such as iron, vitamin B12 and folate, commonly seen in diabetics can impair erythrocyte production and further contribute to elevated RDW-CV.

The comparison of laboratory parameters like RDW-CV, HbA1c, FBS, PPBS with fundoscopic findings showed that RDW-CV and HbA1c levels increased with increasing severity of DR which suggest a positive association between elevated RDW-CV,

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poor glycaemic control and the severity of DR in patients with Type 2 DM. However, FBS and PPBS levels showed a less pronounced but still a notable trend. While the differences in FBS are relatively small across groups with values ranging from 172.44 ± 41.26 mg/dl to 181.3 ± 45.01 mg/dl, PPBS levels are slightly higher in the no retinopathy (225.59 ± 43.2 mg/dl) and DME group (219.32 ± 46.43mg/dl). The overall trend suggested that higher blood sugar levels are associated with the development and progression of retinopathy, although the association may be less direct than that observed with RDW-CV and HbA1c.

The fundoscopy findings in relation to RDW-CV levels revealed that patients with advanced stages of DR had RDW-CV ≥ 15%, whereas patients with no retinopathy had lower RDW-CV. A statistically significant association was found between RDW-CV categories and fundoscopy findings ($x^2 = 139.72$, $p = 0.0001$). The correlation between RDW-CV levels and fundoscopic findings was further evaluated using Spearman's rank correlation coefficient. A positive correlation was noted between RDW-CV and severity of DR ($r_s = 0.79$) suggesting that higher RDW-CV levels were associated with more advanced retinal changes. This finding aligned with several studies. Lippi G et al,[16] and Malandrino N et al,[17] had observed that higher RDW-CV was associated with increased risks of microvascular complications including DR. In a study by Zeng M et al,[18] it was shown that higher baseline RDW-CV levels were linked to a higher risk of retinopathy development and a quicker rate of disease progression.

The relationship between fundoscopy findings and HbA1c levels showed that higher HbA1c levels were found in patients with advanced diabetic retinopathy with 30.4% of them with PDR having HbA1c levels ≥ 9%, whereas those with no retinopathy (23.2%) had HbA1c levels below 7%. A statistically significant association was also found between HbA1c levels and the fundoscopy findings ($x^2=50.20$, $p = 0.0007$), reflecting that poorer glycaemic control leads to advanced stages of retinopathy. The literature has ample evidence of the association between elevated HbA1c level & DR. A study by Klein R et al,[14] had established a link between increased HbA1c levels and a higher risk of DR progression.

CONCLUSION

Our study demonstrated a significant positive correlation between RDW-CV and HbA_1c levels in patients with Type 2 DM. Furthermore, higher RDW-CV values were found to be associated with the presence and severity of DR. As a simple and routinely available haematological parameter, RDW-CV may serve as a potential adjunctive marker for identifying patients at risk of diabetic retinopathy. This will allow timely intervention and prevent worsening of retinopathy. However, large-scale longitudinal studies are needed to confirm the predictive value of RDW-CV over time.

Limitations: The cross-sectional design of the study limits the ability to establish a definitive temporal relationship between rising RDW and the onset of complications, and its relatively small and less diverse population restricts its generalizability. Potential confounders like anaemia, chronic inflammation and cardiovascular diseases were not fully controlled. Unaccounted nutritional and lifestyle factor may have influenced the results.

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