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Research Article

TO STUDY THE NEUTROPHIL-TO-LYMPHOCYTE RATIO AS A QUICK AND RELIABLE PREDICTIVE MARKER TO DIAGNOSE DIABETIC RETINOPATHY AMONG DIABETES PATIENTS: A CASE–CONTROL STUDY

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ABSTRACT

Objectives: Diabetic retinopathy (DR) is a prevalent microvascular complication of diabetes mellitus (DM), and a leading cause of preventable blindness worldwide. Chronic hyperglycemia derives retinal neurovascular damage, and systemic inflammation contributes significantly to its pathogenesis. The neutrophil-to-lymphocyte ratio (NLR), a simple biomarker of inflammation, had shown promise in identifying DR risk. This study is to assess NLR levels in DM patients with and without DR, determine its association with DR presence and severity, and evaluate its diagnostic accuracy.

Methods: This hospital-based case–control study enrolled 100 Type 2 DM (T2DM) patients (50 with clinically confirmed DR and 50 without DR) from September 2023 to February 2025 at a tertiary-care center in rural Pondicherry. All patients underwent fundoscopic examination, glycemic profiling, and complete blood counts to calculate NLR. Statistical analyses comprised an independent t-test, the Chi-square test, Cox regression, and diagnostic accuracy using the Statistical Package for the Social Sciences software.

Results: The mean age of the participants was 57.4±10.8 years; 66% of them were males. Mean NLR was significantly higher in the DR group (3.33±0.59) than in controls (1.74±0.49; p<0.001). Each unit rise in NLR nearly tripled DR risk (Hazard ratio: 2.98; 95% confidence interval: 2.02–4.38; p<0.001). Receiver operating characteristics analysis yielded 96% for sensitivity, 92% for specificity with Youden's index of 0.880.

Conclusion: Thus, NLR is a robust, cost-effective biomarker for early DR detection in T2DM patients. Incorporating NLR into routine diabetes care may enhance screening, risk stratification, and timely intervention to prevent vision loss.

Keywords: Diabetic retinopathy, Neutrophil-to-lymphocyte ratio, Biomarker, Vision loss.

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INTRODUCTION

One of the most common microvascular complications of diabetes mellitus (DM) and a major global cause of avoidable blindness is diabetic retinopathy (DR) [1-3]. Neurovascular injury and retinal microvascular impairment are caused by chronic hyperglycemia, and the prevalence of DR is closely associated with inadequate glycemic management and a longer duration of diabetes [4-6]. In 2020, DR impacted 103 million people worldwide; by 2045, that number is expected to increase to 161 million [4], while in India, it affects 12.5% of diabetics, and 4% of them have vision-threatening DR (VTDR) that could cause blindness [6]. Based on the SMART India screening study, which involved 36 Indian states [7], it was found that the prevalence of DR was 8% in patients without a DM diagnosis and 15.5% in those with established DM.

To reduce the risk of vision loss from VTDR and to provide useful tools for screening, diagnosis, and disease supervision, it is essential to identify the trustworthy biomarkers for early diagnosis and development of DR [8,9]. Inflammation has emerged as a major factor in the pathophysiology of DR in modern times [10]. The neutrophil-to-lymphocyte ratio (NLR), one of the many inflammatory markers, has drawn a lot of attention because of its link to systemic inflammation [11-17].

NLR is a straightforward hematological metric that is computed by dividing the complete blood count (CBC) test's absolute neutrophil count by its absolute lymphocyte count [18-20]. As a result, NLR represents the balance between lymphocyte-mediated regulatory immunological responses and neutrophil-mediated pro-inflammatory activities [20]. The pathophysiology of DR involves a complicated interaction between persistent, low-grade systemic inflammation and metabolic stress brought on by hyperglycemia [1,10,21,22]. Therefore, by encouraging leukocyte adhesion, endothelial dysfunction, increased vascular absorptivity, and capillary blockage, inflammation significantly contributes to the microvascular damage seen in DR [10,23,24].

Proinflammatory neutrophilia and relative lymphopenia contribute to retinal microvascular damage, and a higher NLR is associated with the presence and severity of DR [19,20,25]. Crucially, NLR has shown promise as a non-invasive biomarker for identifying DM patients at high risk for DR, particularly in the early stages before ocular symptoms appear. NLR may serve as an extrapolative indicator of the occurrence and severity of the DR, according to this theory [12,18,25]. In addition, it can be a crucial factor in directing therapeutic decision-making and a monitoring metric to evaluate the responsiveness to anti-inflammatory treatment in the course of DR.

NLR's predictive usefulness in Indian cohorts is still poorly understood despite new data, with inconsistent cutoff values and scant prospective data [13,26]. Its frequent usage is hampered by variability in NLR cutoff values, disagreements over its prognostic significance, and a paucity of prospective evidence. To support early detection techniques and better patient outcomes, the current study was conducted to evaluate NLR as a quick and reliable predictive marker for diagnosing DR in patients with T2DM, aiming to contribute to early detection strategies and improved patient's outcomes.

Objective of the study

The objectives of the study are as follows:

1. To determine the NLR in diabetes patients with DR admitted under the General Medicine department of a tertiary-care teaching hospital in Pondicherry
2. To measure the grading of DR in patients with T2DM
3. To compare the NLR among T2DM patients with DR and without DR
4. To find the association and diagnostic efficacy of NLR and DR among patients with diabetes.

METHODS

Study design and setting

From September 2023 to February 2025, hospital-based case–control research was carried out at the Mahatma Gandhi Medical College and Research Institute (MGMCRI), located in rural Pondicherry, India, at the Department of General Medicine and Ophthalmology. After receiving approval from MGMCRI's Institutional Human Ethics Committee (IHEC), this study was carried out (MGMCRI/Res/01/2023/105/IHEC/107). All subjects gave their informed consent, and the study complied with ethical guidelines.

Study population

Inclusion criteria

According to the American Diabetes Association's (ADA) recommendations, study participants with T2DM (defined as FBS ≥126 mg/dL, 2-h post-prandial blood sugar [PPBS] ≥200 mg/dL, and glycosylated hemoglobulin [HbA1c] ≥6.5%) ranged in age from 18 to 75 years [27] who made a presentation to the General Medicine Department. An ophthalmologist evaluated the patients by performing a thorough fundus examination using both direct and indirect techniques, noting the existence or lack of DR in accordance with predetermined classification criteria [22]. After DR assessment, patients with clinically confirmed DR (all stages of the DR [22]) were categorized as cases (group 1: DR), and patients without DR were grouped as controls (Group 2: No diabetic retinopathy [nDR]).

Exclusion criteria

Patients with hematological disorders, severe liver, kidney, or heart problems, acute or chronic illnesses, hypertension, pregnant or lactating women, alcoholism, or usage of drugs that affect the neutrophil count were excluded from the study.

Sample size and sampling procedures

The sample size for the study was established based on the aforementioned study conducted by Ulu *et al*. [25], the mean NLR of 3.59±2.07 in DR patients and 1.96±0.86 in those without DR. using these values, the bare minimum required sample size was calculated to be 30 (15 in each group) with 80% power and 95% confidence interval (CI) using OpenEpi (ver_3.01) [28]. The formula used to calculate was

$$n \geq \frac{[z_{1-\frac{\alpha}{2}} + z_{1-\beta}]^2[\sigma_1^{\,2} + \frac{\sigma_2^{\,2}}{r}]}{(\mu_1 - \mu_2)^2}$$

Where Type 1 error rate (α)–0.05 (95%); power (β)–0.2 (80%); expected mean of group 1 (μ_1)–0.359; expected standard deviation (SD) of group 1(σ_1)–0.207; expected mean of group (μ_2)–0.196; expected SD of group 2 (σ_2)–0.086; ratio (r)–1. Although considering the feasibility and availability of patients at the study site, the final sample size was increased to 100 participants (50 in each group) to enhance the statistical validity and generalizability. Participants were chosen through a non-probability consecutive sampling method and were matched in a 1:1 ratio.

Study procedure and data collection procedure

Fig. 1 provides a summary of the study process. Following the acquisition of informed consent, clinical data (diabetes history) and demographic data were gathered using a pre-formed Performa. Vital signs were collected, and a clinical and physical examination was performed. Venous blood samples were drawn for CBC and glycemic profile analysis in the lab. The samples are kept at room temperature, and the patient's name, age, sex, and identification number have been recorded. Testing was done within an hour following sample collection to reduce variations caused by sample ageing. Following the acquisition of the laboratory results, NLR was computed. According to the study by Forget *et al*., the typical range of NLR obtained for our investigation is 0.78–3.53 [29]. In addition, patients who did not meet the inclusion criteria received care in accordance with ADA guidelines without transgressing any ethical standards.

Statistical analysis of data

MS Excel (Ver_2007) was used to enter the data, and the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019) software was used for analysis. The Shapiro–Wilks and Kolmogorov–Smirnov tests were used to assess the data's normality. Depending on their distribution, continuous data are presented as mean and SD or median without interquartile range (IQR); categorical variables are reported as frequencies and percentages. Inferential statistics were conducted using the Mann–Whitney U-test for non-normally distributed continuous data, the independent t-test for regularly distributed continuous data, and Pearson's the Chi-square test for categorical variables, with significance set at $p<0.05$. After controlling for age, HbA1C, and body mass index (BMI), the independent predictive value of NLR for DR was calculated using a multivariate Cox-proportional hazard regression analysis. The risk connection was depicted by the hazard regression plot. The diagnostic performance of NLR and HbA1C was analyzed using a receiver operating characteristics (ROC) curve, which computed the area under curve (AUC) and diagnostic accuracy metrics, such as Youden's index (J), where 1 denotes a perfect test (a complete separation) and 0 denotes a random test (complete overlap) [30].

RESULTS

The sociodemographic details, characteristics of the diabetes, and comorbidities of the study participants were presented in Table 1. Among 21 (42%) of the patients had DR in bilateral eye, while 14 patients (28%) and 15 patients (30%) had left and right eye DR. The grading of DR among the study participants in DR group was presented in Fig. 2.

Table 2 shows BP and laboratory profile of the study participants with and without DR. The mean HbA_{1C} levels were 8.95±2.43%, where it was 10.21±2.38% in DR group and 7.68±1.74% in nDR group which was found to be statistically significant (t=6.065; 95% CI: 1.706–3.365; $p<0.001$). Thus, it indicates that poor long-term glycemic control is strongly associated with the presence of DR. The mean neutrophil was 63.65±28.35%, with a significantly higher mean in DR group (66.93±9.60%) compared to the nDR group (60.38±12.47%) ($p=0.004$), which indicates a trend of elevated neutrophil count in patients with DR, suggesting an underlying proinflammatory state. Conversely, the mean lymphocyte (%) was significantly lower in the DR group 20.59±4.14% than in the nDR group 36.12±7.41% ($p<0.001$).

The mean NLR among all participants was 2.53±0.96, with a significantly higher observed in the DR group (3.33±0.59) compared to the nDR group (1.74±0.49) ($p<0.001$) suggests a strong association between the systemic inflammation and the DR development. Furthermore, DR patients 18 (36%) had abnormal NLR values, while none of the nDR patients had elevated NLR ($p<0.001$). In contrast, all 50 patients in the nDR group had NLR values within the normal range (Table 2).

Table 3 demonstrates the association of various variables with normal and abnormal NLR among patients with DR (n=50). Out of 50 patients, 18 (38%) had abnormal NLR levels. In the analysis, patients with abnormal NLR had significantly lower lymphocyte counts (17.73±2.33) compared to those with normal NLR (22.19±4.10) (t=4.228; 95% CI: 2.336–6.573; $p<0.001$), indicating enhanced systemic inflammation in

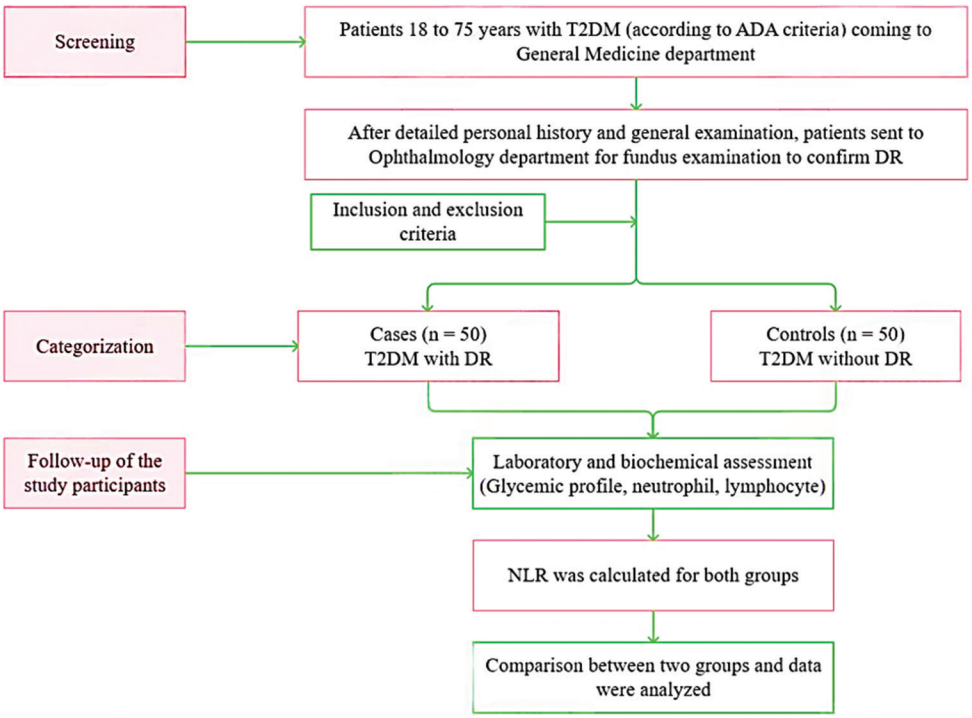

Fig. 1: Flow of the study procedure

Table 1: Baseline demographic, diabetes characteristics, and comorbidity of the study participants based on DR in patients with T2DM (n=100)

Variables	Total (n=100)	DR (n=50)	nDR (n=50)	p-value
Age (in years)	57.39±10.84	58.98±10.61	55.80±10.95	0.143[a]
Gender				
Male	66 (66.0)	35 (70.0)	31 (62.0)	0.398[b]
Female	34 (34.0)	15 (30.0)	19 (38.0)	
Diabetes symptoms				
No symptoms	38 (38.0)	21 (42.0)	17 (34.0)	0.410[b]
Symptoms (nocturia, polyuria, and neuropathy)	62 (62.0)	29 (58.0)	33 (66.0)	
Medications				
OHA	70 (70.0)	35 (70.0)	35 (70.0)	0.831[b]
Insulin	27 (27.0)	13 (26.0)	14 (28.0)	
Both	3 (3.0)	2 (4.0)	1 (2.0)	
Adherence to treatment				
Regular	49 (49.0)	25 (50.0)	24 (48.0)	0.841[b]
Irregular	51 (51.0)	25 (50.0)	26 (52.0)	
Duration of diabetes (in years)				
Median (IQR)	6 (3.25–10)	7 (4–11.25)	5 (3–8)	0.095[c]
Newly diagnosed	2 (2.0)	2 (4.0)	0 (0)	0.018[b]
1–5	45 (45.0)	16 (32.0)	29 (58.0)	
≥6	53 (53.0)	32 (64.0)	21 (42.0)	
BMI (kg/m^2)				
Mean±SD	26.39±4.15	26.74±4.32	26.04±3.98	0.399[a]
Underweight (<18.5)	2 (2.0)	0 (0)	2 (4.0)	0.661[b]
Normal (18.5–22.9)	21 (21.0)	10 (20.0)	11 (22.0)	
Overweight (23–24.9)	15 (15.0)	7 (14.0)	8 (16.0)	
Obesity–I (25–29.9)	42 (42.0)	22 (44.0)	20 (40.0)	
Obesity–II (≥30)	20 (20.0)	11 (22.0)	9 (18.0)	
Other comorbidity				
CAD	7 (7.0)	2 (4.0)	5 (10.0)	0.580[b]
Cataract	5 (5.0)	3 (6.0)	2 (4.0)	
CVA	3 (3.0)	2 (4.0)	1 (2.0)	
Fibroid	1 (1.0)	0 (0)	1 (1.0)	
No comorbidity	84 (84.0)	43 (86.0)	41 (82.0)	

[a]Independent t-test: Ordinal and parametric, [b]Pearson's Chi-square: Nominal and non-parametric, [c]Mann–Whitney U test – Ordinal and non-parametric. p<0.05 is statistically significant indicated by boldface. Values given in brackets were percentage. Values of 0 were considered as 0.5 in the cells. SD: Standard deviation, IQR: Interquartile range, DR: Diabetic retinopathy, nDR: No diabetic retinopathy, CAD: Coronary artery disease, Cerebrovascular accident: BMI: Body mass index

the elevated NLR group. In addition, the PPBS levels were significantly higher in patients with abnormal NLR (305.21±36.51 mg/dL) compared to those with normal NLR (275.45±43.06 mg/dL), (t=−2.472;95% CI: −53.968−−5.553; p=0.017).

Table 2: Vitals and the laboratory profile of the study participants with and without DR (n=112)

Variables	Total (n=100)	DR (n=50)	nDR (n=50)	p-value
SBP (mmHg)	119.90±8.22	119.00±8.14	120.80±8.29	0.276[a]
DBP (mmHg)	73.40±6.06	72.40±5.91	74.40±6.11	0.100[a]
FBS (mg/dL)	188.12±38.92	185.76±37.63	190.48±40.40	0.547[a]
2-h PPBS (mg/dL)	281.25±43.62	286.16±42.94	276.34±44.18	0.262[a]
HbA_{1C} (%)				
Mean±SD	8.95±2.43	10.21±2.38	7.68±1.74	**<0.001[a]**
Pre-DM (≥5.7–6.4)	14 (14.0)	1 (2.0)	13 (26.0)	**0.001[b]**
DM (≥6.5)	86 (86.0)	49 (98.0)	37 (74.0)	
Neutrophil (%)	63.65±28.35	66.93±9.60	60.38±12.47	**0.004[a]**
Lymphocyte (%)	11.55±9.83	20.59±4.14	36.12±7.41	**<0.001[a]**
NLR				
Mean±SD	2.53±0.96	3.33±0.59	1.74±0.49	**<0.001[a]**
Normal (0.78–3.53)	82 (82.0)	32 (64.0)	50 (100.0)	**<0.001[b]**
Abnormal (≥3.54)	18 (18.0)	18 (36.0)	0 (0)	

[a]Independent t-test: Continuous and parametric, [b]Chi-square test: Ordinal and parametric, p<0.05 is statistically significant indicated by boldface. SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, PPBS: Post-prandial blood sugar, DR: Diabetic retinopathy, nDR: No diabetic retinopathy, HbA_{1C}: Glycosylated hemoglobulin

Table 3: Association of NLR levels in patients with DR (n=50)

Variables	Normal (0.78–3.53) (n=32)	Abnormal (≥3.54) (n=18)	p-value*
Age (in years)	58.69±10.75	59.50±10.63	0.798[a]
Gender			
Male	23 (71.9)	12 (66.7)	0.700[b]
Female	9 (28.1)	6 (33.3)	
Duration of diabetes (in years)			
Newly diagnosed	2 (6.3)	0 (0)	0.557[b]
1–5	10 (31.3)	6 (33.3)	
≥6	20 (62.5)	12 (66.7)	
Median (IQR)			0.935[c]
Medication			
OHA	22 (68.8)	13 (72.2)	0.555[b]
Insulin	8 (25.0)	5 (27.8)	
Both	2 (6.3)	0 (0)	
DM compliance			
Regular	19 (59.4)	6 (33.3)	0.077[b]
Irregular	13 (40.6)	12 (66.7)	
BMI (kg/m^2)			
Normal (18.5–22.9)	6 (18.8)	4 (22.2)	0.902[b]
Overweight (23–24.9)	4 (12.5)	3 (16.7)	
Obesity–I (25–29.9)	14 (43.8)	8 (44.4)	
Obesity–II (≥ 30)	8 (25.0)	3 (16.7)	
Mean±SD	27.17±4.76	25.98±3.38	0.356[a]
SBP	118.13±8.20	120.56±8.02	0.316[a]
DBP	72.19±6.08	72.78±5.74	0.738[a]
FBS	186.17±39.45	185.04±35.26	0.920[a]
PPBS	275.45±43.06	305.21±36.51	**0.017[a]**
HbA1C	10.41±2.43	9.86±2.32	0.440[a]
Neutrophils	65.09±10.13	70.20±7.79	0.071[a]
Lymphocytes	22.19±4.10	17.73±2.33	**<0.001[a]**
DR severity			
Mild NPDR	11 (34.4)	12 (66.7)	0.175[b]
Moderate NPDR	9 (28.1)	3 (16.7)	
Severe NPDR	7 (21.9)	2 (11.1)	
PDR	5 (15.6)	1 (5.6)	

*[a]Independent t-test: Continuous and parametric, [b]Pearson's Chi-square test: Nominal and non-parametric, [c]Mann–Whitney U test – Ordinal and non-parametric, p<0.05 is statistically significant indicated by boldface. Values given in brackets were percentage. Values in any cell are "0" it is considered as 0.5. SD: Standard deviation, IQR: Interquartile range, DM: Diabetes mellitus, OHA: Oral hypoglycemic agents, BMI: Body-mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, PPBS: Post-prandial blood sugar, HbA_{1C}: Glycosylated hemoglobulin, NLR: Neutrophil-to-lymphocyte ratio

While Table 4 shows the status of NLR among the DR severity in patients with DR and were statistically non-significant (p=0.401).

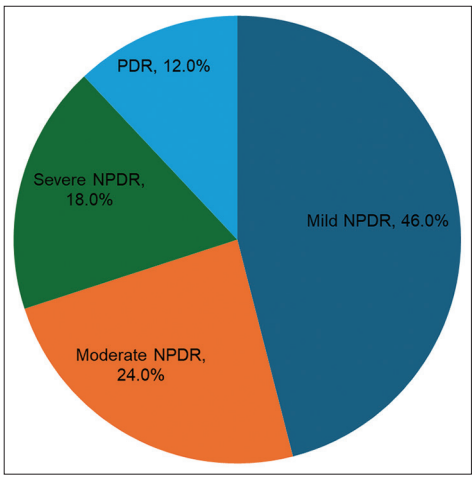

Fig. 2: Grading of diabetic retinopathy (DR) among the study participants with DR (n=50). NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

Table 5 represents the correlation between NLR with other variables, which shows that a moderate positive correlation was observed for HbA_{1C} (r=0.467; p<0.001) (Fig. 3) indicating that higher NLR values were associates with poorer long-term glycemic control, and a strong negative correlation was found for the presence of DR (r_{pb}–0.824; p<0.001). This reflects that as NLR increases, the likelihood of developing DR group increases significantly, further validating NLR as a predictive marker for DR.

The analysis demonstrated that NLR was significantly associated with an increased risk of DR (Hazard ratio [HR]: 2.98; 95% CI: 2.02–4.38; p<0.001), indicating that for every unit increase in NLR, the risk of DR nearly tripled. Age was also found to be a significant factor inversely associated with DR risk (HR: 0.95; 95% CI: 0.92–0.98; p=0.003), while HbA_{1C} and BMI did not show statistically significant associations in the adjusted model (Table 6 and Fig. 4). The hazard regression plot demonstrated a clear upward trend, indicating a positive association between increasing NLR values and the hazard of developing DR.

The ROC curve for NLR demonstrated an excellent diagnostic ability, with an AUC of 0.986 (p<0.001), indicating near-perfect discrimination between DR and nDR patients. A cutoff value of 2.38 for NLR yielded 96% sensitivity, and 92% specificity, with a positive predictive value (PPV) of 92.31% and a negative predictive value (NPV) of 95.83%. The J for NLR was 0.880, confirming its high diagnostic utility. J value closer to 1 signifies a better discriminatory test, and the value of 0.880

Table 4: Status of NLR among DR severity in patients with DR (n=50)

Grading of DR	n(%)	Mean±SD	Range (min-max)	p-value*
Mild NPDR	23 (46.0)	3.48±0.69	2.26–4.51	**0.401**
Moderate NPDR	12 (24.0)	3.20±0.47	2.38–4.07	
Severe NPDR	9 (18.0)	3.23±0.60	2.57–4.57	
PDR	6 (12.0)	3.12±0.31	2.171–3.64	

*ANOVA: Analysis of variance, p<0.05 is statistically significant and indicated in boldface. NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, SD: Standard deviation, min: Minimum, max: Maximum, NLR: Neutrophil-to-lymphocyte ratio, DR: Diabetic retinopathy

Table 5: Correlation between the NLR and the factors for DR among the study participants (n=100)

Variables	Correlation (r or rho)	p-value[#] (2-tailed)
With diabetes duration	0.190	0.058[a]
With BMI	0.031	0.761[b]
With FBS	−0.049	0.628[b]
With PPBS	0.165	0.100[b]
With HbA_{1C}	0.467	<0.001[b]
With DR	−0.824	<0.001[c]

[#a]Spearman's correlation, [b]Pearson's correlation, [c]Point-biserial correlation. Correlation is significant at the 0.01 level (2-tailed). BMI: Body-mass index, FBS: Fasting blood sugar, PPBS: Post-prandial blood sugar, DR: Diabetic retinopathy, HbA_{1c}: Glycosylated hemoglobulin, NLR: Neutrophil-to-lymphocyte ratio

Table 6: The adjusted multivariable Cox regression for DR according to NLR among the study participants (n=100)

Variables	HR	95% CI	p-value*
NLR	2.98	2.02–4.38	**<0.001**
HbA_{1C}	1.02	0.91–1.15	0.707
BMI	0.93	0.86–1.01	0.089
Age	0.95	0.92–0.98	**0.003**

*Multivariable Cox regression, p<0.05 is statistically significant and indicated in boldface. NLR: Neutrophil-to-lymphocyte ratio, HbA_{1C}: Glycosylated hemoglobulin, BMI: Body mass index, HR: Hazard ratio, CI: Confidence interval

strongly supports the clinical usefulness of NLR as a diagnostic marker (Table 7 and Fig. 5).

DISCUSSION

This hospital-based case–control study was carried out at MGMCRI in Pondicherry, India, between September 2023 and February 2025, with the goal of assessing NLR as a rapid and accurate prognostic marker for DR in patients with Type 2 diabetes. Fifty DR patients and fifty non-DR patients were selected from a total of 100 patients. We computed NLR for both groups. In comparison to individuals without DR (1.74±0.49), our results showed that patients with DR had a considerably higher mean NLR (3.33±0.59) (p<0.001). In addition, multivariate Cox proportional hazard analysis showed that the probability of DR approximately quadrupled with each unit increase in NLR (HR: 2.98, 95% CI: 2.02–4.38, p<0.001).

Our study's glycemic profile showed notable variations, especially with regard to HbA1C levels. The literature review continuously highlights how prolonged hyperglycemia damages the retinal vasculature, where high HbA1C levels cause inflammation, oxidative stress, and endothelial dysfunction [21,31]. The hallmarks of DR, capillary leakage and neovascularization, are ultimately caused by these pathophysiological mechanisms [31-33]. Our results showed that patients with DR had significantly higher HbA1C values than those without DR, which strongly

Table 7: Diagnostic characteristics of the NLR

Variables	NLR	HbA_{1C}
Cutoff point	2.38	8.3
Sensitivity	96	80
Specificity (%)	92	74
PPV (%)	92.31	75.47
NPV (%)	95.83	78.72
Youden's index	0.880	0.824
AUC	0.986	0.824
p-value	**<0.001**	**<0.001**

*ROC: Receiver operating characteristic curve, p<0.05 is statistically significant and indicated in boldface. NLR: Neutrophil-to-lymphocyte ratio, HbA_{1C}: Glycosylated hemoglobulin, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under curve

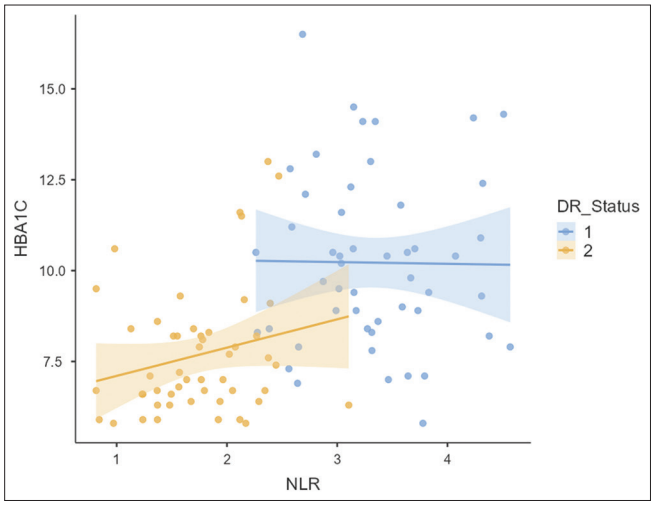

Fig. 3: Scatterplot showing the neutrophil-to-lymphocyte ratio with glycosylated hemoglobulin among the study participants (n=100) (diabetic retinopathy [DR] status: 1-with DR, 2-without DR)

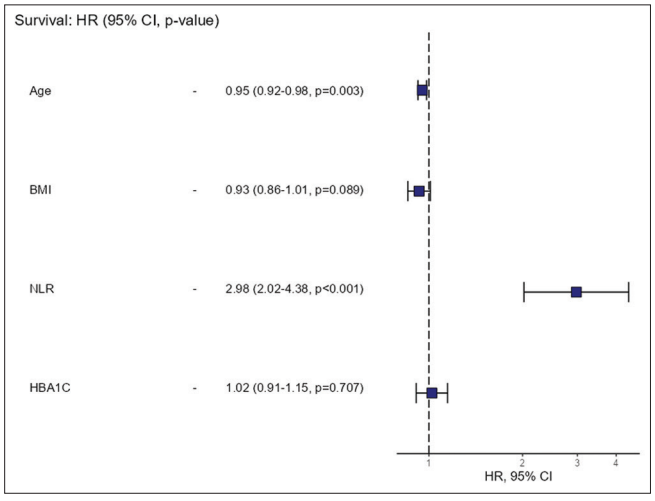

Fig. 4: Hazard regression plot

suggests that chronic hyperglycemia contributes to the development of retinal issues [34,35], provide compelling empirical evidence in favor of the inflammatory and metabolic models of DR that have been previously reported in the literature [13,36,37]. Numerous investigations have shown that inadequate long-term glycemic management is a major contributing element to the pathophysiology of DR, and this connection supports those findings [11,15,21,31,38,39].

The mean neutrophil count in our study was significantly higher (66.93±9.60%) in DR patients than in non-DR patients (60.38±12.47%), and the difference was statistically significant (p=0.004). An increased proinflammatory response in DR is suggested by this rise in neutrophils [40-42]. As the study by Ulu *et al*. [25] showed, in diabetic patients with DR, higher neutrophil numbers significantly contribute to an elevated NLR. Similarly, Tang *et al*. [15], highlighted that endothelial dysfunction and retinal microvascular injury in DR are frequently linked to an increase in neutrophils. These results support the notion that neutrophilia is a crucial marker of systemic inflammation, which fuels the etiology of DR.

With a mean of 20.59±4.14%, the lymphocyte count was significantly lower in the DR groups than in the non-DR groups (p<0.001). A decrease in lymphocytes indicates a weakened regulatory and anti-inflammatory immune response, which intensifies the inflammatory cascade that results in DR [43,44]. This observation is in consistent with the study by Ulu *et al*. [25], claimed that DR patients had a significant reduction in lymphocytes, which raised their NLR. In addition, studies by Tang *et al*. [15], and Chittawar *et al*. [13], also unearthed that decreased lymphocyte counts were linked to the advancement and severity of DR, indicating that a reduction in regulatory control over inflammation could potentially expedite microvascular damage.

In our study, patients with DR had a significantly higher mean of NLR 3.33±0.59 when compared to 1.74±0.49 in those without DR (p<0.001), yielding an overall mean of 2.53±0.96 that indicates an elevated systematic inflammatory state. This elevation in the DR group is consistent with several previous reports. For instance, Ulu *et al*. [25], observed mean NLR values of 3.59±2.07 in DR patients versus 1.96±0.86 in non-DR patients, which is closely comparable to our study findings despite a slightly greater variability in their study. Similarly, Rajendrakumar *et al*. [14], reported a median NLR of 2.04 (IQR: 1.5–2.7) in Scottish patients, with an optimal cutoff value of 3.04 for predicting DR incidence, suggesting that an elevated NLR, in the range observed in our DR group, is associated with a higher risk of retinopathy. These studies reinforce the association between a heightened inflammatory state, as reflected by NLR, and the presence of DR.

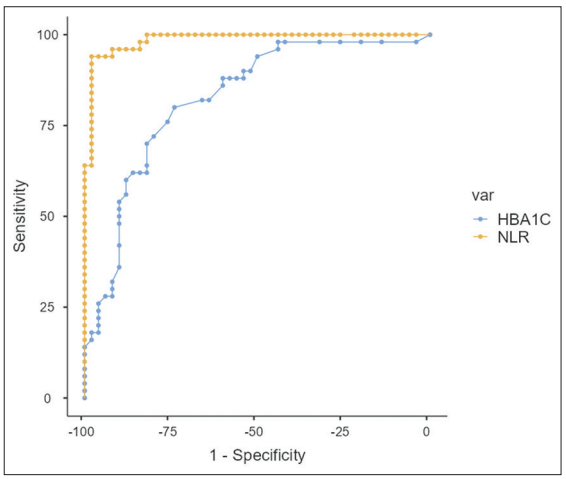

Fig. 5: Receiver operating characteristics curve for neutrophil-to-lymphocyte ratio and glycosylated hemoglobulin on diabetic retinopathy

In contrast, other studies have reported relatively lower NLR values. Study by Khandare *et al*. [45], in contrast to our cohort, mean NLR levels in 2018 were 2.49±0.94 for DR patients and 2.06±0.56 for non-DR patients, indicating a more moderate inflammatory response. Similarly, PDR patients' mean NLR was 2.67±1.02 compared to 1.85±0.49 in the control group, according to Ilhan *et al*. [46] Variations in glycemic management, disease duration, and population demography may be the cause of the disparities amongst studies (Table 8). The trend across the research is consistent, suggesting that a higher NLR is associated with the existence and severity of DR, regardless of variations in absolute values. The findings suggest that NLR is a reliable and accessible biomarker for DR prediction. This is consistent with the paradigm of inflammatory etiology presented in a number of research [11,15,47].

The multivariable Cox proportional hazards regression analysis indicated that an increased NLR serves as a strong, independent predictor of DR. Each unit increase in NLR was linked to an almost threefold increase in the hazard of DR (HR: 2.98; 95% CI: 2.02–4.38; p<0.001), even after controlling for confounding factors including HbA1C, BMI, and age. These findings are consistent with previous literature, where studies like those by Ulu *et al*. [25], and Tang *et al*. [15], have similarly demonstrated the independent prognostic value of NLR in predicting DR.

The diagnostic performance of NLR was excellent, as evidenced by a ROC curve area of 0.986 (p<0.001), indicating nearly perfect discrimination between patients with and without DR. A cutoff value of 2.38 yielded 96% sensitivity and 92% specificity, with corresponding PPV and NPV of 92.31% and 95.83%, respectively, and a Youden's index of 0.880. Study done by Upadhayula *et al,* in India (2024) also showed that the AUC of 0.87 and 85% accuracy in 130 T2DM patients [48]. Furthermore, El-Tawab *et al*. [12], identified a cutoff value of ≥1.97 for predicting pre-clinical DR in T2DM patients, indicating that even modest increased in NLR could have diagnostic significance. These findings suggest that NLR outperforms as a predictive marker

Table 8: Comparison of NLR values with our study results

Studies	Population	NLR
Our study	100 T2DM patients (50 with DR and 50 without DR)	DR: 3.33±0.59 Without DR: 1.74±0.49
He *et al*., 2022, US [11]	2772 patients from NHANES data	Diabetes patients: 2.4±1.5 DR patients: 2.7±1.7
El-Tawab *et al*., 2023, Egypt [12]	120 patients with T2DM	PDR 2.33 (IQR 1.48–6.79) without PDR: 1.48 (IQR 1.08–5.30)
Rajendrakumar *et al*., 2023, Scotland [14]	23,531 T2DM Scottish patients	2.04 (IQR 1.5–2.7)
Abdullah, 2021, India [16]	160 patients with T2DM (80 with DR and 80 without DR)	DR 2.15±0.5 DM 1.91±0.61
Ilhan *et al*., 2019, Turkey [46]	80 patients with DR, 40 patients had PDR, 40 patients NPDR	PDR 2.67±1.02 Severe NPDR 2.16±0.58 Control 1.85±0.49
Khandare *et al*., 2018, India [45]	115 T2DM patients	DR 2.49±0.94 No DR 2.06±0.56

NHANES: National health and nutrition examination survey, NLR: Neutrophil-to-lymphocyte ratio, T2DM: Type 2 diabetes mellitus, DR: Diabetic retinopathy, NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, IQR: Interquartile range

Table 9: Diagnostic accuracy of NLR in various studies compared to our study findings

Studies	Population	NLR
Our study	100 T2DM patients (50 with DR and 50 without DR)	Cutoff: 2.38 Sensitivity 96% Specificity 92%
He *et al*., 2022, US [11]	2772 patients from NHANES data	Inflection point 4.778
El-Tawab *et al*., 2023, Egypt [12]	120 patients with T2DM	Cutoff≥1.97 Sensitivity 89.29% Specificity 84.37%
Chittawar *et al*., 2017, India [13]	298 T2DM patients for DR	AUC: 0.708 Sensitivity 64.2% Specificity 63%
Rajendrakumar *et al*., 2023, Scotland [14]	23,531 T2DM Scottish patients	Cutoff 3.04
Gollakota *et al.*, 2022, India [17]	98 T2DM patients	Cutoff 2.032 Sensitivity 89.1% Specificity 81.2%
Ilhan *et al*., 2019, Turkey [46]	80 patients with DR, 40 patients had PDR, 40 patients NPDR	Cutoff 2.11 Sensitivity 76% Specificity 80%
Upadhyayula *et al.*, 2024, India [48]	130 T2DM patients	AUC 0.87 accuracy 85%

NHANES: National health and nutrition examination survey, NLR: Neutrophil-to-lymphocyte ratio, T2DM: Type 2 diabetes mellitus, DR: Diabetic retinopathy, NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, AUC: Area under curve

for DR, corroborating previous studies which report similar high diagnostic accuracy for NLR in detecting retinal microvascular damage [11-14,17,25] (Table 9).

Strengths of this work include a well-matched case–control design, thorough clinical and laboratory evaluations, a novel focus on a rural Pondicherry T2DM community, and the application of strong statistical techniques to validate NLR as an independent predictive biomarker. Its small sample size and single-center setting, however, might restrict its generalizability. In addition, possible confounders, such as other inflammatory disorders, were not completely controlled for, and the absence of longitudinal follow-up makes it impossible to evaluate long-term results or draw conclusions about causality.

CONCLUSION

This study found a substantial correlation between the presence and severity of DR in patients with Type 2 diabetes and an elevated NLR. In light of these findings, it is advised that NLR be included in diabetes management routing protocols as an easily accessible and reasonably priced biomarker for early DR screening and risk lamination. This could enable prompt intervention and lessen the burden of complications that could endanger vision.

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AUTHOR'S CONTRIBUTIONS

Conceptualization, VR, SS; Methodology, VR, SS; Software, ER, VR; Validation, SS, VR; Formal Analysis, ER; Investigation, ER; Resources, ER, VR; Data Curation, VR; Writing – Original Draft Preparation, ER; Writing – Review and Editing, SS, VR; Visualization, VR, SS; Supervision, SS; Project Administration, ER, VR, SS.

CONFLICTS OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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USE OF AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No AI-assisted technologies were used in the writing process.

DATA AVAILABILITY STATEMENT

All data generated during or analyzed during this study are included in this published article.

ETHICAL APPROVAL

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for the publication of this case report and accompanying images. This study was approved by the Institutional Ethical Committee (IHEC number mentioned in the study).

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