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Neutrophil–lymphocyte ratio as a reliable marker to predict pre-clinical retinopathy among type 2 diabetic patients

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Abstract

Background Diabetic retinopathy is now recognized as a neurovascular in lieu of a microvascular complication. Visual evoked potentials (VEPs) are greatly valuable in detecting early diabetic retinal functional changes before the occurrence of structural damage. Low-grade inflammation plays a fundamental part in the development and progression of retinopathy in diabetics. Detecting diabetic patients with early retinopathy before the occurrence of clinical symptoms provides a window of opportunity to ensure the best prognosis for these eyes. Neutrophil–lymphocyte ratio (NLR) has recently been introduced as a novel marker of inflammation in various diseases. Indeed, the presence of a cheap, available, and reliable marker of inflammation that is capable to detect pre-clinical diabetic retinopathy (P-DR) is crucial for early intervention to retard the progression of ocular damage. As far as we know no previous studies investigated the role of NLR in the detection of P-DR. The aim of this study was to investigate the quality of prediction of NLR in detecting pre-clinical retinopathy in type 2 diabetic patients.

Results In this case–control study, VEPs results showed a significant delay in P100 latencies of the patients' group compared to the control group. According to the VEPs results, the patient group was further subdivided into two: diabetic with VEPs changes (a group with P-DR) and diabetic without VEPs changes. NLR was significantly elevated in patients with P-DR ($p < 0.001$). NLR cut-off point ≥ 1.97 is able to predict P-DR with 89.29% sensitivity and 84.37% specificity. Linear regression model revealed that NLR is the only independent factor that predicts P-DR. (odds ratio 3.312; 95% confidence interval 1.262–8.696, $p = 0.015$*.

Conclusions Visual evoked potentials have an important role to evaluate the visual pathway in diabetics and to diagnose pre-clinical diabetic retinopathy before the occurrence of structural damage. Neutrophil–lymphocyte ratio is a reliable marker for the detection of pre-clinical diabetic retinopathy with good sensitivity (89.29%) and specificity (84.37%). Finding a reliable available laboratory test to predict P-DR could be of help to save diabetic patients from serious ocular complications.

Keywords Neutrophil-lymphocytic ratio, Pre-clinical diabetic retinopathy, Pattern reversal visual evoked potentials, Type 2 diabetes mellitus

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Background

Diabetes mellitus (DM) ocular complications are diabetic retinopathy, optic neuropathy, cataract, and dry eye. Many studies have been published to identify damage due to DM in the optic nerve and visual pathway [1–4]. Systematic review and meta-analysis estimated the

number of diabetic retinopathies worldwide by 132.12 million in 2020 and that number is expected to grow to 160.5 million in 2045. Africa has the highest prevalence of diabetic retinopathy (35.90%) [5].

Diabetic retinopathy (DR) was used to be defined as a microvascular complication of diabetes. Vascular changes such as alterations in retinal artery diameter, architectural indices, and blood flow have been observed. Elevated serum levels of different blood markers and cytokines have been documented as early signs of DR [6]. Later on, DR was recognized as a neurovascular impairment that is non-visible by the ophthalmoscope [7]. Hyperglycemia and its associated metabolic derangement induce various harmful effects on the retinal neurovascular structure including the optic nerve, glial and immune cells, in parallel with the induced microvascular damage. It was found that neural dysfunction across the retina of diabetic patients precedes clinical vasculopathy [8]. This might open up new possibilities for DR management [9].

Neurodegenerative changes have been reported in the retina of pre-clinical diabetic retinopathy (P-DR). A significant decrease in the thickness of the retinal nerve fiber layer especially at the edge of the optic disc, parapapillary, was reported due to apoptosis of retinal neuronal cells along with activation of glial cells [10–12]. Diabetic papilopathy, neovascularization of the optic disc, and optic nerve atrophy are clinical hallmarks of optic nerve alterations in DR [4].

Electrophysiological procedures are the best tools in the early detection of diabetic neural damage of the retina before the clinical vascular alterations are apparent on fundoscopy [13–15]. Visual evoked potentials (VEPs) test is a sensitive tool and superior to magnetic resonance imaging (MRI) as regards the functional integrity of the visual pathway. The pattern-reversal VEPs (PRVEPs) test is the technique of choice for most clinical situations as it shows less variability in timing and waveform than other VEP techniques. But its use in regular screening is still low [14].

VEPs' role in the detection of P-DR was previously confirmed [13, 16]. A recent recommendation to use VEPs as a screening tool was published [17]. The early detection of visual dysfunction in diabetics ensures a better prognosis and quality of life in these patients [18].

Although the role of VEPs in detecting P-DR was confirmed, finding a cheap, rapid, available, and convenient laboratory test is demanding especially in areas where VEPs is not available.

Chronic inflammation plays a crucial role in the pathogenesis of DR [19]. Neutrophils are connected to the occurrence and progression of microangiopathy and inflammation of the endothelial cell wall. The high serum neutrophil count in patients with DR is suggesting the role of neutrophil-mediated inflammation in the pathogenesis of DR [20]. The neutrophil–lymphocyte ratio (NLR) is a new marker of the inflammatory response [21] it reflects both innate immune response and adaptive immune response. It was reported to be a potential biomarker of inflammation in diabetes and especially its complications, such as microvascular complications and neuropathy [22]. NLR was reported to be higher in patients with DR and was correlated to the severity of DR [20, 23–25].

This work was designed to use VEPs in diagnosing P-DR. And the novel part of this study was to investigate the quality of prediction of NLR in detecting P-DR in type 2 diabetic patients.

Methods

Study participants

One hundred twenty subjects were enrolled in this case–control study. Sixty type 2 diabetic patients fulfilled the American Diabetes Association criteria (2020) [26]. In addition, sixty age and sex-matched healthy volunteers were enrolled as a control group. All study subjects provided written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee.

Inclusion and exclusion criteria

Patients with type 2 diabetes mellitus were enrolled if they had normal visual acuity or were corrected by glasses and had normal fundus examination. Patients were referred to an ophthalmologist to exclude any patient with significant ocular disorders such as optic atrophy, vitreous opacities, amblyopia, glaucoma, cataract, and retinopathy. Patients were also excluded if they had any condition other than diabetes mellitus that could have influenced NLR, such as malignancy, auto-immune disease, recent infection, cardiovascular disease, a history of cerebrovascular accidents, chronic alcoholics, hepatic or renal co-morbidity, or patients with peripheral nervous system disorders unrelated to diabetes.

Demographic data were recorded for all studied subjects. General and neurological examinations were performed. Laboratory investigations including glycated hemoglobin (HbA1C) and complete blood count were performed for the calculation of NLR by dividing neutrophil count by lymphocyte count [22].

Toronto clinical neuropathy scoring system (TCNS) [27] was used to assess peripheral neuropathy [28]. TCNS score of each patient was recorded out of 19. It was used to classify the severity of neuropathy as follows: no neuropathy (0 to 5), mild neuropathy (6 to 8), moderate (9 to 11), and severe diabetic neuropathy (12 to 19) [29].

All participants underwent a pattern reversal visual evoked potentials (PRVEPs) test [30]. The PRVEP was performed using Neuropack 2 electromyograph apparatus (MEB-9400) from Nihon Kohden (Japan) [31]. The test was explained to the studied subjects to ensure full cooperation. The room was made quiet and comfortable with a uniform temperature maintained. The recordings were done between 10 am and 12 noon in a sitting position. TV pattern stimulator was placed at a distance of 100 cm from the subject's eyes. The stimulus was a checkerboard with a reversal pattern (pattern reversal-VEPs). The frequency of the stimulus was 1 Hz. The check size used was 16 with a visual angle to the horizontal screen size of 20° and a visual angle to the horizontal length of the black/white checks on the screen at 1.25°.

Before placing the recording electrodes, the patient's hair was separated and the skin was cleaned and scrubbed to decrease any impedance at the site of electrode placement. The recording electrodes were placed on the scalp relative to bony landmarks according to the International 10/20 system [31]. The anterior/posterior midline measurements are based on the distance between the nasion and the inion over the vertex. The active electrode was placed on the occipital scalp over the visual cortex at O_z with the reference electrode at F_z. A ground electrode was placed on the wrist.

In each recording, 200 sweeps were averaged. The analysis time was 300 ms. The vertical gain was 2.5–5 μV (variable according to the response). The filter setting was set at a Low cut of 1 Hz and a high cut of 100 Hz.

Pattern reversal visual evoked potentials (PRVEPs) were recorded monocular bilaterally and binocular for each subject. Two reproducible responses have to be obtained. Measurements of P100 latency, amplitude, and interocular P100 latency difference (IOLD) were obtained.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented by numbers and percentages. Categorical variables comparison was done by chi-square test (χ^2). The normality of data was done by the Kolmogorov–Smirnov and Shapiro–Wilk tests. For normally distributed data mean and standard deviation (SD) were used. While median and range (minimum and maximum) were used for non-parametric data. Student *t*-test (*t*) was used for two groups comparison for normally distributed data, while Mann–Whitney (*U*) test was performed for non-parametric data. Pearson coefficient (*r*) was used to correlate between two normally distributed quantitative variables. The definition of the diagnostic value of NLR in the prediction of preclinical retinopathy was obtained by receiver operating characteristic (ROC) analysis. An area of more than 50% gives acceptable performance and an area of about 100% is the best performance for the test. The significance of the obtained results was judged at the 5% level. Logistic regression was used to detect the independent variables affecting pre-clinical diabetic retinopathy.

Results

In both groups, the number of females exceeded the number of males. In the patient group (65% ♀, 35% ♂) and in the control group (66.7% ♀, 33.3% ♂). The mean age was 50.48 ± 7.98 years in the patient group and 52.47 ± 8.85 years in the control group. The mean BMI in the patient group was 31.07 ± 5.76 kg/m^{2}and in the control group was 29.92 ± 3.82 kg/m^2. Eleven of the studied patients were smokers and 9 were smokers in the control group. There was no statistically significant difference between the studied groups as regards gender ($\chi^2 = 0.037$, $p = 0.847$), age ($t = 1.290$, $p = 0.200$), BMI ($t = 1.286$, $p = 0.201$), and smoking ($\chi^2 = 0.240$, $p = 0.624$).

The pattern-reversal VEPs (PRVEPs) results are demonstrated in Table 1. There was a significant delay in P100 latencies of the patients' group compared to the control group ($p < 0.001$) in binocular, right monocular, and left monocular fields. The IOLD was significantly longer in patients as compared to the control. 2.5 standard deviations above the mean value of the control group were used to define delayed P100 and IOLD latencies. The Cut-off value for binocular field P100 latency was 106.78 ms, right monocular latency was 106.77 ms, left monocular latency was 106.75 ms, and IOLD was 3.62 ms (Fig. 1).

According to PRVEPs results the studied diabetic patients were further subdivided into two groups: the first group included 28 patients (46.66%) with P-DR and the second group contained 32 patients (53.33%) without P-DR (Table 2). No statistically significant difference could be found between the 2 groups as regards gender ($\chi^2 = 0.424$ at $p = 0.515$), and BMI (t = 1.577 at $p = 0.120$). However, patients with P-DR were older ($t = 2.67$ at $p = 0.010$) and had a longer diabetic duration ($U = 283.0$ at $p = 0.014$) than those without P-DR. Smoking was significantly associated with P-DR ($\chi^2 = 6.687$ at $p = 0.010$). A significant association was found between the use of oral hypoglycemic drugs and P-DR, while this was not the situation among insulin users ($\chi^2 = 4.115$ at $p = 0.042$).

In addition, the patient group with P-DR had a higher score on the Toronto clinical neuropathy score system ($U = 220.0$, $p = 0.001$). The Toronto severity grade was higher in the group with P-DR ($\chi^2 = 11.292$, $p = 0.001$).

The diabetic group with P-DR was compared to the diabetic group without P-DR regarding the performed

Table 1 Comparison between the patients and control group according to P100 wave and IOLD of PRVEPs

PVEPs P100 wave	DM ($n=60$)	Control ($n=60$)	Test of Sig	*p*
Latency (msec)				
Binocular field				
Mean ± SD	106 ± 8.8	99.3 ± 3.0	$t=5.486^{*}$	$<0.001^{*}$
Right monocular				
Mean ± SD	107 ± 9.0	99.4 ± 2.9	$t=5.978^{*}$	$<0.001^{*}$
Left monocular				
Mean ± SD	108 ± 9.4	100 ± 2.7	$t=6.364^{*}$	$<0.001^{*}$
IOLD (msec)				
Median (Min.–Max.)	2.0 (0–16)	1.0 (0–4)	$U=1237.5^{*}$	0.003^{*}
Amplitude (μV)				
Binocular field				
Median (Min.–Max.)	8.25 (3.0–20.0)	8.50 (5.0–22.5)	$U=1665.0$	0.478
Right monocular				
Median (Min.–Max.)	7.50 (2.50–22.0)	8.0 (4.50–21.5)	$U=1564.0$	0.214
Left monocular				
Median (Min.–Max.)	7.0 (2.10–17.5)	7.50 (5.0–21.0)	$U=1508.5$	0.125

SD Standard deviation, *IOLD* Interocular latency difference, *t* Student *t*-test, *U*Mann-Whitney test, *PP*-value for comparing the two studied groups

* Statistically significant at $P \leq 0.05$

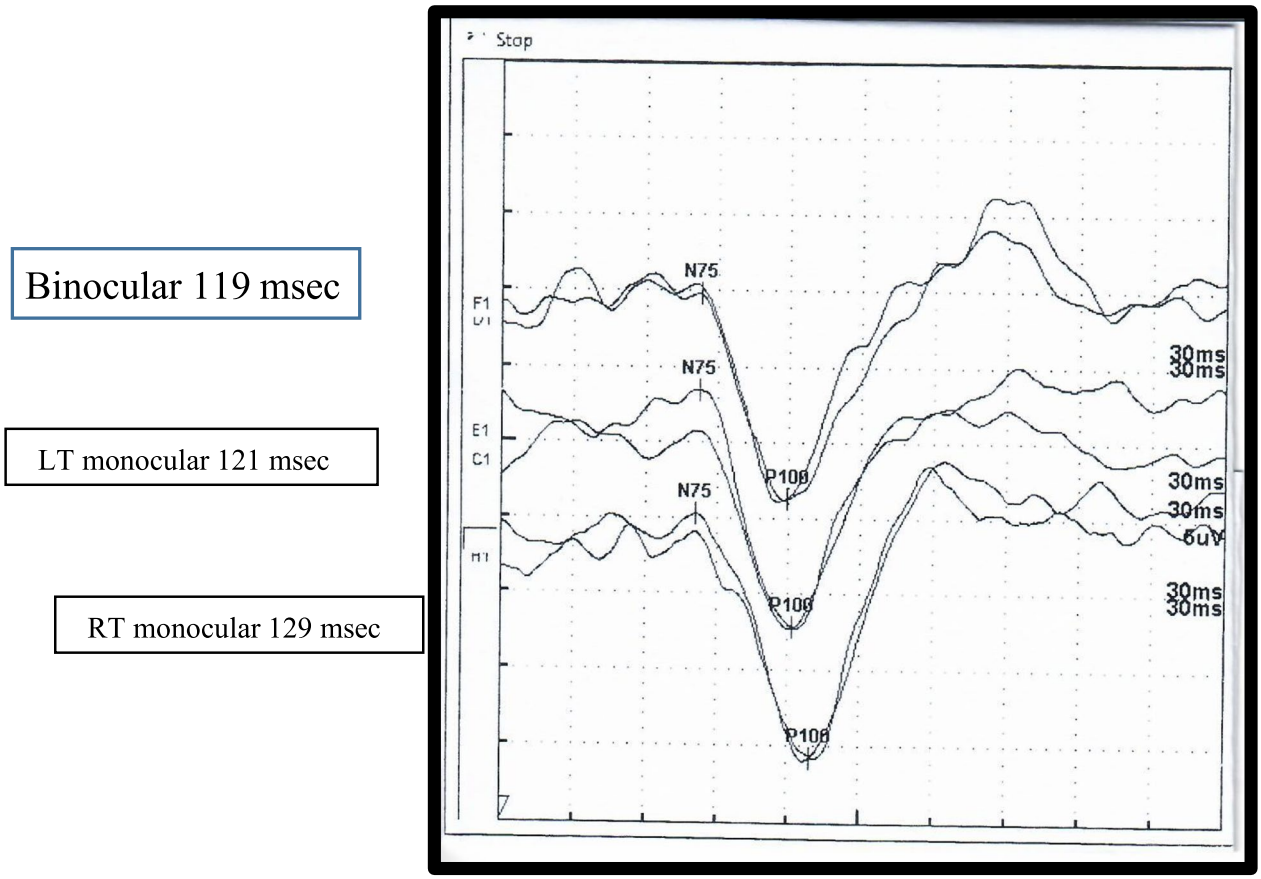

Fig. 1 PR-VEP study of a 51-year-old diabetic female patient showing delayed P100 latency of binocular (119 ms), RT monocular (129 ms), LT monocular (121 ms), and IOLD (8 ms). NLR (3.25)

Table 2 Comparison between diabetics with and without P-DR according to PRVEPs P100 wave latency and IOLD

PRVEP P100 (Latency)	Diabetics with P-DR ($n=28$)	Diabetics without P-DR ($n=32$)	Test of Sig	*p*
Binocular field				
Mean ± SD	113 ± 7.70	99.63 ± 3.20	$t=8.565^{*}$	$<0.001^{*}$
Right monocular				
Mean ± SD	114 ± 8.83	101 ± 2.92	$t=7.254^{*}$	$<0.001^{*}$
Left monocular				
Mean ± SD	116 ± 6.91	101 ± 3.14	$t=10.967^{*}$	$<0.001^{*}$
IOLD				
Median (Min.–Max.)	4.0 (0–16)	1.4 (0–3)	$U=213.5^{*}$	$<0.001^{*}$

SD Standard deviation, *t* Student *t*-test, *U*Mann-Whitney test, *IOLD* Interocular latency difference, *pp*-value for comparing diabetics with and without P-DR

* Statistically significant at $p \leq 0.05$

laboratory results shown in Table 3. The HbA1C level and the NLR were significantly higher in the patients with P-DR.

Receiver operator characteristic (ROC) curve analysis was performed to identify the optimal cut-off point of NLR for predicting P-DR in diabetic patients. The cut-off point of NLR was 1.97 with sensitivity = 89.29% and specificity = 84.37%. The area under the curve (AUC) was 0.874 (Fig. 2).

To specify the factors that predict P-DR, 9 factors were selected to enter a univariate logistic regression model. The significant factors by univariate logistic regression were NLR, age, smoking, BMI, DM duration, PN, and HbA1C ($p \leq 0.001$). A multivariate logistic regression model was further done to identify independent factors predicting P-DR. It revealed that NLR is the only independent factor that predicts P-DR. (odds ratio 3.312; 95% confidence interval 1.262–8.696, $p=0.015^{*}$ (Table 4).

Discussion

In this study, PRVEPs were used for the diagnosis of P-DR at an early stage when the patient's fundus showed no signs of retinal involvement [13, 16–18]. About half of the studied patients (46.66%) had abnormalities in PRVEPs. Denoting that pre-clinical retinopathy is not uncommon in type 2 diabetes mellitus (T2DM) patients. A higher percentage of abnormal PRVEPs was detected in previous studies [13, 18]. This is explained by differences in diabetic clinical characteristics such as the duration of the disease and type of treatment.

In this study, the significant delay in P100 and IOLD latencies compared to the control reflects the dysfunction of the retina's ganglion cells and demyelinating changes in the optic nerve pathway, which is caused by the microvascular insult induced by the hyperglycemic state [32]. Low-grade inflammation in T2DM with the recruitment of different mediators was also accused of the delay in the conduction of the visual pathway [16]. The undetected significant difference in P100 amplitude among diabetics and non-diabetics in this study is attributed to the normal or corrected visual acuity of all patients which if reduced has a direct impact on the P 100 amplitude [16, 33].

The studied patients with P-DR showed no difference from the patients without P-DR in gender & BMI. Indeed, there is a contradiction in the literature regarding the association between P-DR and gender. Some studies reported male predisposition [34], others found female prevalence, [35] further was similar to our study [36]. The same contradiction was found as regards P-DR and BMI [37–40]. These contradictions could be explained by the different ethnic and racial backgrounds in different

Table 3 Comparison between diabetics with and without P-DR according to laboratory results

	P-DR ($n=28$)	Without P-DR ($n=32$)	Test of Sig	*p*
HbA1C (%)				
Mean ± SD	8.46 ± 1.66	7.27 ± 1.28	$t=3.152^{*}$	0.003^{*}
NLR				
Median (Min.–Max.)	2.33 (1.48–6.79)	1.48 (1.08–5.30)	$U=112.5^{*}$	$<0.001^{*}$

SD Standard deviation, *t* Student *t*-test, *U* Mann–Whitney test, p*p* value for comparing between diabetics with and without P-DR

* Statistically significant at $p \leq 0.05$

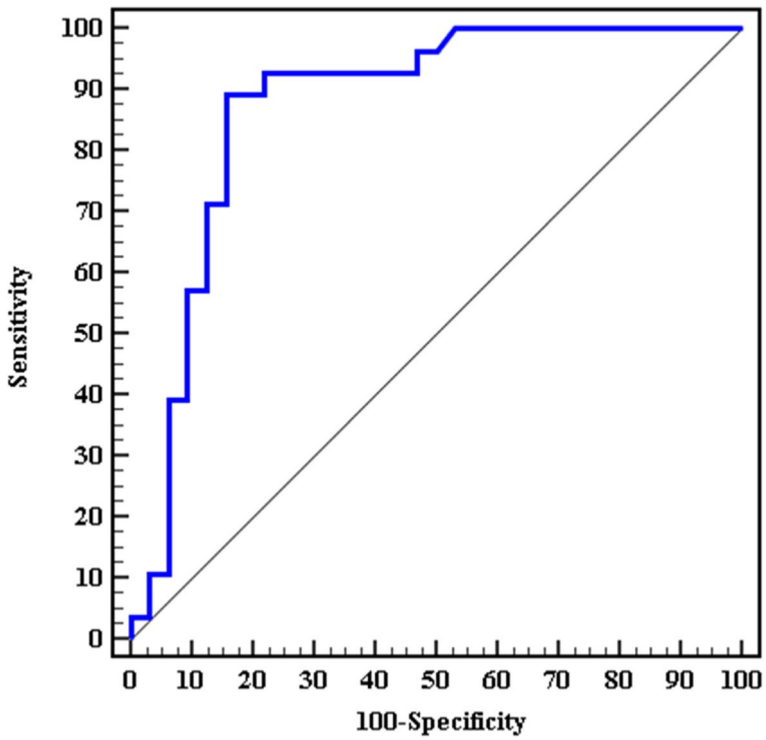

Fig. 2 ROC curve for prediction of P-DR based on the serum level of neutrophils to lymphocytes ratio. Neutrophils/lymphocytes ratio had an area under ROC curve of 0.874 (95% CI: 0.779–0.97; $P = < 0.001$)

Table 4 Univariate and multivariate logistic regression analysis for the parameters predict P-DR in the studied 60 diabetic patients

	Univariate		[a]Multivariate	
	p	OR (LL–UL 95%C.I)	*p*	OR (LL–UL 95%C.I)
Neutrophils to lymphocytes ratio	0.001*	4.347 (1.781–10.608)	0.015*	3.312 (1.262–8.696)
Male	0.516	1.424 (0.491–4.129)		
Female	0.516	0.702 (0.242–2.038)		
Age (years)	0.014*	1.097 (1.019–1.180)	0.102	1.098 (0.982–1.227)
Smoking	0.019*	7.105 (1.383–36.497)	0.315	2.644 (0.396–17.639)
BMI (kg/m^2)	0.123	1.076 (0.980–1.180)		
Duration (years)	0.020*	1.108 (1.016–1.209)	0.696	0.971 (0.837–1.127)
PN	0.003*	8.333 (2.089–33.243)	0.890	0.864 (0.109–6.867)
HbA1C blood level	0.006*	1.746 (1.175–2.594)	0.121	1.612 (0.881–2.950)

OR Odds ratio, *C.I* Confidence interval, *LL* Lower limit, *UL* Upper limit

[a] All variables with $p < 0.05$ was included in the multivariate

* Statistically significant at $p \leq 0.05$

populations. Also, the presence of other confounding factors in different samples could be a cause [36].

Patients with P-DR were older than those without P-DR, similar findings were reported by other researchers [36, 41] which could be explained by other risk factors associated with aging [36]. Smoking was directly associated with the P-DR group in this study. It is well documented that the deleterious changes in the retinal microvasculature are caused by smoking [42]. In a trial to figure out the role of different diabetic medications and P-DR. insulin seems to have a protective effect on the retina as the incidence of P-DR was lower among insulin users. Recent studies concluded that the use of an insulin pump reduces glycemic variability which will in place reduce the development of DR [43, 44]. Moreover, an experimental rat study showed that subconjunctival

injection of insulin-loaded particles alleviates retinal changes and reduces retinal cell apoptosis [45].

The Toronto clinical neuropathy scoring system showed higher scores and more severity of PN in patients with P-DR. These expected results are explained by the fact that retinal neurodegeneration runs parallel with peripheral neuropathy and has the same pathogenesis mechanisms [46, 47].

Although the role of PRVEPs in the detection of P-DR is established and recommended, [17] finding a cheap, available screening tool is demanding. In recent years, attention was drawn toward the NLR as a marker of low-grade inflammation in various diseases [48–51]. NLR is considered a reliable predictive marker to diagnose the severity of clinical diabetic retinopathy with obvious changes by fundus examination [25, 52].

In the state of low-grade inflammation, the neutrophils infiltrate the vessel wall and secrete various substances and proteolytic enzymes resulting in endothelial damage. However, the lymphocytes are capable to modulate the neutrophils' effect and also have an anti-atherosclerotic role. High NLR represents endothelial damage and dysfunction due to the higher neutrophilic activity [53]. Endothelial damage, in turn, caused significant chronic inflammation which exacerbated microvascular complications with further progression of retinal injury in DR [54]. These data support the role of chronic low-grade inflammation in the pathogenesis of diabetic retinopathy [53].

The stability of NLR compared to other leucocyte parameters makes it more useful as it combines two independent markers. Other leucocytes may be changed by various pathological conditions [23].

The only way for improving retinal health and function among T2DM patients is the early detection of DR in the pre-clinical phase. NLR was statistically higher among the group of patients with P-DR. This result allows us to assume that NLR could be of help in the detection of P-DR.

Previous research tackled NLR in DR [23, 55]. Their results showed high NLR among patients with DR as compared either with diabetics without DR or with healthy control. Moreover, Cagri et al. [56] determined NLR optimal cut-off value of 2.11 or more to predict proliferative or severe non-proliferative DR with relatively low sensitivity and specificity (76%, 80%). Moreover, Wang J-R et al. [55] determined NLR cut-off value to predict DR = 1.84, with a lower sensitivity and specificity of 56% and 64%, respectively.

To the best of our knowledge, no studies have discussed NLR in P-DR detection. ROC curve analysis in this study determined an NLR cut-off value of 1.97 with reasonable sensitivity and specificity (89.29%, 84.37%) to predict P-DR. It means that when NLR is ≥ 1.975 to predict P-DR we expect that 89.29% of the positive results will be correctly diagnosed as P-DR (true positive) and 84.37% of the negative results are truly not retinopathy (true negative). The area under the curve (AUC) that measures the quality of the model's predictions was 0.874 which is considered good according to the interpretation published by Nahm FS et al. [57].

A univariate linear regression model was applied to study other factors that could predict P-DR. It revealed that NLR, age, smoking, diabetes duration, PN, and HbA1c are predictors of P-DR. A multivariate logistic regression model to determine the independent factors for P-DR prediction. It revealed that NLR is the only independent factor to predict P-DR. This finding further emphasized the role of NLR in the prediction of P-DR.

This result may help the internist with a simple available laboratory test (NLR) to find out the cases that need early ophthalmic consultation and intervention to ensure the best outcome.

Conclusions

Visual evoked potentials have an important role in evaluating the function of the visual pathway in diabetics and to diagnose pre-clinical diabetic retinopathy before the occurrence of structural damage. Neutrophil–lymphocyte ratio is a reliable marker to detect DR in the pre-clinical phase since it has proved to be of good sensitivity (89.29) and specificity (84.37%). It should be measured routinely in patients with type 2 diabetes in order to select the suspected cases of diabetic retinopathy in its pre-clinical phase before irreversible structural and functional deterioration of vision. Cases with abnormal neutrophil–lymphocyte ratio should be confirmed with pattern reversal visual evoked potentials and referred to an ophthalmologist to prevent serious ocular complications.

Abbreviations

BMI	Body mass index
DM	Diabetes mellitus
DR	Diabetic retinopathy
HbA1C	Glycated hemoglobin
IOLD	Interocular P100 latency difference
NLR	Neutrophil-lymphocyte ratio
PRVEPs	Pattern reversal visual evoked potentials
PN	Peripheral neuropathy
P-DR	Pre-clinical diabetic retinopathy
ROC	Receiver operating characteristic
T2DM	Type 2 diabetes mellitus
TCNS	Toronto clinical neuropathy scoring system
VEPs	Visual evoked potentials

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Authors' contributions
SS: collection of data, interpretation of data, writing of the paper. IK: idea of the research, collection of data. MM: referring the cases, clinical assessment. RA: collection of data, analysis of data, writing of the paper. WS: making the study design, analysis of data, writing of the paper. All authors have read and approved the manuscript.

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Availability of data and materials
All data and materials are presented in the main paper.

Declarations

Ethics approval and consent to participate
The Ethics Committee formally approved this study of the Faculty of Medicine, Alexandria University (FWA 00018699/0201111, date: 21/6/2018). The study was explained to the participants and a written informed consent was given by each participant.

Consent for publication
A written informed consent was given by each participant regarding the publication of their information.

Competing interests
The authors declare that they have no competing interests.

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