Evaluation of Neutrophil-to-Lymphocyte Ratio, Monocyte-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio as Predictor Factors on Diabetic Retinopathy

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Abstract

Background: Diabetic retinopathy (DR) is a microvascular complication of type 2 diabetes mellitus. Inflammation is known to have a crucial role in the pathogenesis of DR. The neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) are biomarkers of the inflammatory response.

Aim: To determine the mean value of NLR, MLR and PLR as predictor factors in DR at the Universitas Sumatera Utara and Medan Baru Eye Hospital.

Methods: This study was an observational analytic study with a case-control design. A total of 26 DR subjects as cases and 26 subjects non-DR as controls. Examination of NLR, MLR and PLR with routine blood test.

Results: Mean values of NLR and PLR were higher in case group compared to controls (2.54(1.66), 131.62(78.87)) but the difference was not significant statistically (p=0.224, p=0.855). Mean MLR values were lower in case group compared to controls 0.25 (p=0.09). There were significant differences in visual acuity and blood sugar levels (p<0.001). NLR cannot be used as a predictor of DR (p=0.224 (95%CI=0.440-0.757).

Conclusion: There is a significant difference between visual acuity and blood sugar levels between DR subjects and non-DR subjects. NLR, MLR and PLR cannot be used as a predictor of DR.

Keywords: neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, diabetic retinopathy

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases that can threaten health and cause vascular complications, one of which is in the eye (American Diabetes Association, 2020; Yue et al., 2015; Retina, 2019). Diabetic retinopathy (DR) is a visual disorder caused by abnormalities in the retina, which is a progressive microangiopathy characterized by damage and blockage of blood vessels, resulting in an increase to DR prevalence worldwide (Yue et al., 2015; Retina, 2019; Sasongko, Widyaputri, & Agni, 2019; American Academy of Ophthalmology, 2020). DR can occur in DM patients aged 25-74 years. Amounted to 3.6% of younger-onset patients under 30 years old at diagnosis and 1.6% of older-onset patients over 30 years old at diagnosis are indicated to have visual acuity of 20/200 or worse (American Academy of Ophthalmology, 2020; Gayathri, Gopi, & Palanisamy, 2020).

In patients with type 2 DM (T2DM), prolonged exposure to hyperglycemia causes changes in biochemical and molecular pathways, including increased inflammatory oxidative stress, advanced glycation end products (AGEs), and the protein kinase C pathway, which will lead to endothelial damage and loss of pericytes (American Academy of Ophthalmology, 2020; Wang & Lo, 2018). Chronic low-grade inflammation contributes as major role in the initiation, progression, and accelerates the deterioration of microangiopathy and macrovascular disease in patients with DM. In further studies, inflammation acts as a significant factor in the early stages and progression of DR (Yue et al., 2015; Wang & Lo, 2018). Previous studies have

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shown that peripheral blood leukocytes and their subgroups are correlated to macrovascular and microvascular complications in patients with T2DM (Yue et al., 2015; Wang & Lo, 2018; Rubsam, Parikh, & Fort, 2018).

The white blood cell count and its subtype are representative indicators of inflammation (Yue et al., 2015). The neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) are potential markers of inflammation in various conditions, such as tumors, cardiovascular conditions and expected to predict a prognosis regarding systemic inflammatory conditions (Wang & Lo, 2018; Rubsam, Parikh, & Fort, 2018; Gunduz et al., 2015; Liu et al., 2015; Akyel et al., 2015; Oylumlu et al., 2015). NLR, MLR and PLR were assessed by full blood tests to calculate the leukocyte count and its subtypes (Yue et al., 2015; Wang, Chen, & Yang, 2020).

Based on the description, the study is expected to evaluate the potential of the NLR, MLR and PLR as predictors and early screening for DR in patients with T2DM.

Methods

Study Population

This study was an observational analytic study with a case-control design conducted at the Eye Polyclinic, Vitreo-Retina Division, Universitas Sumatera Utara and Medan Baru Eye Hospital from October to December 2020. The study sample consisted of 52 patients with a history of type 2 DM (26 patients with DR in the case group and 26 patients with T2DM without DR as the control group). With consecutive sampling techniques, this study took sample of T2DM patients aged ≥18 years and had clear refractive media. Meanwhile, patients with acute inflammation or infection, malignancy, chronic liver disease, heart disease, hypertension, microvascular complications of DM (except DR), elevated intraocular pressure and tumor were excluded from this study. This study was approved by the Research Ethical Committee from the Medical Faculty of University of North Sumatera, Medan, Indonesia.

Clinical Examination and Biochemical Analysis

First, for exclusion of all study subjects, a thorough and specific record involving the symptoms and signs of the disease including a history of medication and related drug uses (steroids, antihypertensive agents, hepatoprotective agents, chemotherapy drugs, etc.). Then, several related examinations were carried out such as visual acuity examination using a Snellen chart, blood pressure examination (@ABN), examination of intraocular pressure using a transpalpebral tonometer (@Easytone EZTN-01), examination of the anterior segment using a slit lamp microscope (@Righton-1000), examination of the posterior segment using indirect ophthalmoscopy (@HEINE Ophthotecnic OMEGA500) with dilated pupils to assess the degree of DR. Furthermore, full blood examination by taking 3 ml of venous blood sample then analyzed with @SYSMEX XN-550. NLR, MLR, and PLR were calculated by comparing the respective values between absolute neutrophils, absolute monocytes, and platelets with absolute lymphocytes.

Statistic Analysis

The data obtained were analyzed using Chi-Square, Kruskal Wallis, and Anova with a significance level of 5%. Meanwhile, to determine the significant factors as predictors of DR, multiple logistic regression tests were used. Variables with a p-value <0.05 were considered statistically significant. All data were processed and analyzed using SPSS.

Results

This study consisted of 52 subjects, of which 26 subjects were the case group consisting of NPDR and PDR, and 26 subjects were the control group (DM non-DR). Table 1 shows the characteristics of the subjects based on the severity of DR. Most of the study subjects in each group were female (61.5% in the PDR group, 58.8% in the NPDR group, and 57.7% in the DM non-DR group). Most of the subjects were aged 46-65 years, of which 88.9% in the PDR group, 58.8% in the NPDR group, and 73.1% in the control group. Most subjects had DM for ≥5 years in the PDR group as many as 6 subjects (66.7%), in the NPDR group there were 12 subjects (70.6%), while in the DM non-DR group there were 13 subjects (50%). There were no differences in the characteristics of subjects in each study group on gender, age and duration of DM (p>0.05). Other characteristics can be seen in Table 1.

Table 1. Subject demographic characteristics

Demographic Characteristics	DR		DM Non-DR (n=26)	p
	PDR (n=9)	NPDR (n=17)		
Gender, n (%)				
Male	3 (33,3)	7 (41,2)	11 (42,3)	0,891[a]
Female	6 (66,7)	10 (58,8)	15 (57,7)	
Age, n (%)				
36 – 45 years	1 (11,1)	1 (5,9)	2 (7,7)	0,938[b]
46 – 65 years	8 (88,9)	10 (58,8)	19 (73,1)	
> 65 years	0	6 (35,3)	5 (19,2)	
Duration of DM				
≥ 5 years	6 (66,7)	12 (70,6)	13 (50)	0,362[a]
< 5 years	3 (33,3)	5 (29,4)	13 (50)	
Visual acuity, mean (SD)	0,92 (0,35)	0,73 (0,36)	0,21 (0,17)	<0,001[b]*
Random glucose test, mean (SD), mg/dL	240,78 (46,18)	241,18 (60,04)	107,54 (10,02)	<0,001[c]*
Neutrophil, mean (SD), cell/µL	6,03 (3,63)	5,23 (1,77)	4,90 (1,78)	0,701[b]
Lymphocyte, mean (SD), cell/µL	2,56 (1,14)	2,54 (1,08)	2,60 (0,70)	0,979[c]
Monocyte, mean (SD), cell/µL	0,61 (0,37)	0,59 (0,27)	0,66 (0,23)	0,335[b]
Thrombocyte, mean (SD), 10^3 cell/µL	249,78 (84,856)	301,29 (122,95)	290,50 (77,24)	0,305[b]

Note: [a]Chi Square, [b]Kruskal Wallis, [c]Anova, *significant p<0,05

Table 2 shows the differences in NLR, MLR, and PLR based on the severity of DR. The highest NLR value was in the NPDR group with a mean of 2.36 (SD = 1.40), the lowest was in the DM non-DR (control) group with a mean of 1.94 (SD=0.59). By using Kruskal Wallis test, no significant difference was found in NLR values based on the severity of DR (p=0.476). The mean value of MLR was the same in the PDR group and the control group, with a mean of 0.27 compared to the mean in the NPDR group that was lower (0.24). Using Anova test, MLR had no significant difference between these groups (p=0.963). The highest PLR was found in the NPDR group with a mean of 138.77 (SD=87.11) while the lowest was in the control group with a mean of 115.46 (SD=31.04). Using Kruskal Wallis test, there was no significant found in PLR between these groups (p=0.894).

Table 2. Differences in NLR, MLR, and PLR values based on the severity of DR

	DR		DM Non-DR (n=26)	p
	PDR (n=9)	NPDR (n=17)		
Neutrophil-to-lymphocyte ratio, mean (SD)	2,87 (2,11)	2,36 (1,40)	1,94 (0,59)	0,476[a]
Monocyte-to-lymphocyte ratio, mean (SD)	0,27 (0,15)	0,24 (0,05)	0,27 (0,12)	0,678[b]
Platelet-to-lymphocyte ratio, mean (SD)	118,12 (62,84)	138,77 (87,11)	115,46 (31,04)	0,894[a]

Note: [a]Kruskal Wallis, [b]Anova

Table 3. Logistic regression analysis of predictor factors of diabetic retinopathy

Variable	Coef	p	OR	95% CI
Selection 1				
Gender	0,473	0,537	1,605	0,358 – 7,190
Age				
Age (1)	0,222	0,872	1,249	0,084 – 18,601
Age (2)	-0,115	0,929	0,891	0,071 – 11,165
Duration of DM	0,782	0,239	2,186	0,594 – 8,048
Neutrophil-to-lymphocyte ratio	1,017	0,040	2,765	1,045 – 7,315
Monocyte-to-lymphocyte ratio	-8,862	0,061	0,000	0,000 – 1,493
Platelet-to-lymphocyte ratio	0,000	0,724	1,000	1,000 – 1,000
Constant	-0,234	0,884	0,792	
Selection 2				
Gender	0,526	0,472	1,693	0,403 – 7,100
Duration of DM	0,801	0,228	2,228	0,606 – 8,194
Neutrophil-to-lymphocyte ratio	0,997	0,041	2,711	1,040 – 7,068
Monocyte-to-lymphocyte ratio	-8,935	0,052	0,000	0,000 – 1,101
Platelet-to-lymphocyte ratio	0,000	0,712	1,000	1,000 – 1,000
Constant	-0,237	0,822	0,789	
Selection 3				
Gender	0,523	0,477	1,688	0,400 – 7,130
Duration of DM	0,758	0,244	2,133	0,596 – 7,638
Neutrophil-to-lymphocyte ratio	0,940	0,041	2,561	1,040 – 6,306
Monocyte-to-lymphocyte ratio	-8,834	0,054	0,000	0,000 – 1,153
Constant	-0,439	0,625	0,645	
Selection 4				
Duration of DM	0,663	0,294	1,940	0,562 – 6,693
Neutrophil-to-lymphocyte ratio	0,927	0,052	2,527	0,990 – 6,466
Monocyte-to-lymphocyte ratio	-7,433	0,068	0,001	0,000 – 1,738
Constant	-0,493	0,584	0,611	
Selection 5				
Neutrophil-to-lymphocyte ratio	1,024	0,038	2,785	1,056 – 7,348
Monocyte-to-lymphocyte ratio	-7,563	0,059	0,001	0,000 – 1,347
Constant	-0,253	0,773	0,777	
Selection 6				
Neutrophil-to-lymphocyte ratio	0,491	0,126	1,634	0,872 – 3,065
Constant	-1,064	0,141	0,345	

Based on Table 3, a multiple logistic regression test was performed and it was found that no independent variable was found to be a predictor of DR in this study because until the last selection there was no independent variable that had a p-value <0.05.

Discussion

From this study, most of the subjects in the NPDR, PDR, and DM non-DR groups were female. This result was similar to a study conducted by Wang, Chen, and Yang (2020) that female are more likely to suffer from diabetes than male (133 cases out of 264 or 69%). It is known that estrogen is the dominant hormone in female which is thought to affect DM so that female with DM have higher tendency to develop DR than male (Mauvais, & Javis, 2017; Al-Amer et al., 2008).

In this study, it was found that most of the subjects were aged 46-65 years in all three groups. This was similar with a study by Yue et al. (2015) that T2DM patients are aged 48-63 years and DR are 47-61 years old. Wang, Chen, and Yang (2020) also reveal that most subjects were 56.48 ± 9.86 in DR group and 55.44 ± 11.27 in the non-DR group. The American Academy of Ophthalmology (2020) explains that DR can occur at the age of 25-74 years. Some studies found that patients with DR have an age range of 20-60 years, where 90% of T2DM often occurs at the age of 30 years and increase at the age of >45 years, which is caused by physiological degeneration of body cells with age. Age is a risk factor of DR as reduction in body function caused by cell apoptosis process started at age >40 years. In addition, retinal cell apoptosis induced by chronic hyperglycemia, inflammatory reactions and oxidative stress might result in DR (Shah, Gandhi, & Natarajan, 2018; Ahmed, Khalil, & Al-Qahtani, 2016).

The results of the visual acuity obtained a mean of 0.80 (SD=0.36) in the case group and 0.21 (SD = 0.17) in the control group. This was similar with the study of Nursalim and Sumual (2016), which found that the mean visual acuity in the mild, moderate and severe NPDR groups was 0.45, 0.40 and 0.38. However, these results were not statistically significant. Sasongko et al in 2017 reported the prevalence of visual impairment and blindness with the incidence of DR where visual acuity 20/20-20/60=43.6 (95%CI=39.7-47.4), visual acuity 20/60-20/200=43.8 (95%CI=39.8-47.6), visual acuity 20/200-20/400=5.60 (95%CI=3.83-7.41) and visual acuity <20/400=7.03 (95%CI=5.04–9.01) with p=0.001 (Nauli et al., 2018). Some literature reported that DM can cause microvascular complications in the form of DR, where retinopathy can worsen visual acuity (Nursalim & Sumual, 2016; Nauli et al., 2018).

Meanwhile, the mean random glucose level was 263.78 (SD=54.68) in the case group and 107.54 (SD=10.02) in the control group. With Mann Whitney and T-independent tests, there were significant differences in visual acuity and random glucose level between cases and controls (p<0.001). The results of the study of Nauli et al. (2018) found that postprandial glucose levels <200 mg/dl in the severe NPDR group of 33.3% and PDR of 38.2%. Samaiporn et al. (2004) found that 45% of DR patients had blood sugar at <140 mg/dl. Blood sugar levels play a crucial role in the incidence and progression of DR. Uncontrolled blood sugar will cause DR to occur more quickly in DM patients. The Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) show that controlled blood sugar levels will reduce the risk of DR. In addition, DCCT also reports that intensive glycemic control will reduce the progression DR towards severe NPDR, PDR and the incidence of macular edema and reduce DR by 54%. Glycemic control is much more effective in preventing or delaying the onset of DR in diabetic patients than in inhibiting the severity of pre-existing DR (American Academy of Ophthalmology, 2020; Wang & Lo, 2018). Wong in study of Nauli et al. (2018) had a meta-analysis of three large population-based studies that found a stratified association between the level of glycemia and the frequency of signs of retinopathy.

In this study, the highest degree of DR was NPDR, amounting to 17 subjects (65.4%). This was similar with the study of Al-Amer et al. (2008), from 341 subjects with DR, 245 subjects were in the NPDR group and 96 subjects in the PDR group. The study of Samaiporn et al. (2004) in Thailand found that the prevalence of DR correlated with the duration of DM. In that study, patients who had diabetes <5 years (15.26%) were more likely to develop complications of NPDR. While the duration of DM was 15-20 years, the prevalence of DR increased to 53.06% and developed into PDR by 10%. Pintary, Kartasasmita, and Juliati (2016) found that the PDR group was found with a percentage of 60%.

Furthermore, the highest NLR value was found in the NPDR group, while the lowest NLR value was in the DM non-DR group. The result is similar with a meta-analysis study by Liu et al. (2018) in China, which showed that NLR levels were significantly higher in patients with DR (SMD=0.77; 95%CI: 0.49–1.05) compared to patients without DR. Ulu et al. (2013) also found that NLR values were considerably higher in patients with DR compared to non-DR. It is known that neutrophils contribute in the progression and development of microangiopathy and inflammation when adhered to endothelial cell walls. NLR is the ratio of peripheral blood neutrophils to lymphocytes, which integrate different but complementing immune pathways in circulating blood. Elevated NLR can be a sign of an increased number of neutrophils that adhere to endothelial cells and causing vascular endothelial damage leading to extensive chronic inflammation. Therefore NLR is believed to exhibit increased microvascular inflammation in patients with DR. Then lymphocytes function as a key feature of the immune response that have the ability to regulate and involved in the inflammatory response (Yue et al., 2015; Liu et al., 2018; Wang et al., 2015; Kuang & Wang, 2015).

In contrast to NLR, MLR values were higher in the control and PDR groups than NPDR group. However, using Mann-Whitney test, there was no significant difference in MLR between these groups (p=0.678). This is similar with study by Wang et al. (2020) in China, which found that the MLR in the DR group was slightly lower than that in the non-DR group (2.28±1.03 and 2.34±1.28, p=0.628), but there were no significance between the groups. A previous study showed that monocytes are acting as a marker of inflammation because monocyte activation leads to the synthesis of inflammatory cytokines and monocytes can influence the angiogenic process in atherosclerosis. However, the mechanisms underlying the association between MLR and DR need be investigated in future studies. Several studies have shown that retinal neovascularization in PDR occurs due to monocyte aggregation in retinal vascular. In addition, the traction and entry of monocytes into the retina by attaching to the outer surface of the retinal vasculature and damaging the blood-retinal barrier can reduce monocyte levels in the peripheral blood. These theories are thought to be why the MLR value is higher in the control and PDR groups compared to the NPDR (Yue et al., 2015; Purnamasari & Rachmawati, 2018; Jaipersad et al., 2014; Benhar et al., 2016).

The highest PLR value was in the NPDR group, the lowest was in the control group. This is not in line with study by Yue et al. (2015) that PLR had the highest mean value in the PDR group (115.73 [145.97–87.98]) and the lowest in the DM group (94.04 [120.19–70.73]) with p<0.01. An elevated in the PLR ratio may indicate an increase in the platelet count, while a decrease in the lymphocyte count may indicate to inflammatory state. Moreover, increased oxidative damage of lymphocytes in hyperglycemic leads to lymphocyte apoptosis and increased PLR. Platelets are thought capable to release various immune regulatory, cytokines, chemokines and other mediators, thereby regulating the inflammatory response in blood vessels in an autocrine or paracrine manner. While platelets can also directly regulate neutrophils, endothelial cells, and lymph, it allows platelet aggregation into injured tissues. Thus, based on platelet function, increased PLR indicates a relatively active platelet inflammatory response in DR patients. Another study describing retinal capillary occlusion due to microvascular thrombus in which platelets and leukocytes play a role, has been considered

a pathological condition in the early stages of DR. This could be the basis that the NPDR stage has a higher PLR ratio than the PDR (Yue et al., 2015; Chatterjee & Geisler, 2016; Akdogan, Ustundag-Budak & Huysal, 2016; Wei et al., 2017; Atak et al., 2019).

Table 3 shows that using the multiple logistics regression test, there are no independent variables that are predictors of the occurrence of DR in this study. A meta-analysis study by Liu et al. (2018) showed that NLR levels were considerably higher in patients with DR (SMD=0.77; 95% CI: 0.49–1.05) than in patients non-DR, but no significant correlation between NLR and the degree of DR was observed in that study (SMD = 0.57; 95% CI: -0.34-1.47). This was similar with Akdogan in 2016 that NLR cannot predict DR with SMD (95% CI)=0.19(-0.05, 0.42) (Ulu et al., 2013). However, this is not in line with study by Wang et al. (2020), that univariate analysis found the NLR was able to assess the risk of DM for DR (OR=0.46, 95%CI=1.19-1.79 with p = 0.000). Kuang and Wang (2015) also found that NLR significantly predicted DR with SMD(95% CI)=1.10(0.54, 1.66) with p=0.000. Yue et al. (2015) found that logistic regression analysis revealed that MLR was an independent risk factor for DR (OR=54,574, 95%CI: 2,708-1099,907). Using the Receiver Operating Characteristics (ROC) curve, the use of MLR as a parameter for DR diagnosis is projected at 2.25 and produces a sensitivity and specificity of 47.1% and 69.6%, sequentially with an area under the curve of 0.581 (95%CI: 0.510-0.653). In the study of Wang, Chen, and Yang (2020), univariate analysis found NLR and PLR increased the risk of DR. However, PLR was not independently associated with DR as a continuous variable (OR(95%CI)=1.05(0.99, 1.11) p=0.135), the highest number of PLR indicating a twofold increased risk of developing DR (OR (95%)CI=2.20(1.05-4.59) p=0.037).

This study found some limitations such as the number of samples in this study is relatively small. Therefore, further study may be needed with other variables and a larger sample. Subsequently, additional laboratory tests may also be required which may help to exclude the study sample to reduce bias.

Conclusion

In the distribution of the subject characteristics, there was a significant difference in visual acuity and blood sugar levels between DR and non-DR. However, NLR, MLR, and PLR cannot predict the occurrence of a DR, although clinically there is an increase in the value between these ratios.

Conflict of Interest

The authors declare that there is no conflict of interest.

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