Leukocyte activity for the evaluation of inflammatory status in cases with neovascular age-related macular degeneration

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ABSTRACT

Purpose: To investigate systemic leukocyte activity in patients with neovascular age-related macular degeneration (nAMD).

Materials and Methods: Venous blood samples were obtained from 82 patients with nAMD (study group) and 86 age and sex-matched control individuals (control group). Demographics as well as complete blood count (CBC) parameters including white blood cell (WBC), neutrophil, lymphocyte, monocyte, and platelet counts were noted for each participant. Moreover, calculated results of neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/monocyte ratio (LMR) were recorded for further analysis.

Results: No statistically significant difference was observed between the study and control groups under consideration in terms of demographic features ($p>0.05$). Among all studied CBC parameters, statistically significant difference was found in LMR, WBC, and monocyte counts ($p=0.039$, $p=0.041$, and $p<0.001$, respectively). These results were found as 3.54±1.20, 7.54±1.59 $\times 10^3$ µL, and 0.61±0.16 $\times 10^3$ µL, in the study group and 3.92±1.20, 7.04±1.55 $\times 10^3$ µL, and 0.53±0.15 $\times 10^3$ µL, in the control group, respectively.

Conclusion: Patients with nAMD have increased systemic leukocyte activity, which may be indicative of systemic inflammation in these patients.

Keywords: Age-related macular degeneration, inflammation, lymphocyte/monocyte ratio, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio.

INTRODUCTON

Age-related macular degeneration (AMD) is a progressive degenerative disease of the macula, which leads to visual field loss and decreased visual acuity.[1] Inflammation plays a key role in the pathogenesis of various retinal diseases, including AMD.[2]

Dysregulation of the innate immune system is among the factors which trigger the onset of AMD. Inflammatory cells like microglia, monocytes/macrophages, neutrophils, and tissue-resident T cells also contribute to pathophysiology.[3] Histopathological analyzes demonstrated that retinal pigment epithelial cell (RPE) degeneration and photoreceptor cell death are among the mechanisms in the pathogenesis of the disease.[4] Macrophages and microglia may be closely associated with degeneration of the RPE. Pigment epithelia are located in close proximity to the vascular choroid that supplies the outer retina, and migration of macrophages from the choroid to the retina in order to produce proinflammatory chemokines is a possible cause of RPE injury. Recently, it has been suggested that infiltration of M1 macrophages into the subretinal space

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between the RPE and photoreceptor outer segments causes dry-type AMD-like changes.[5] Also, studies have found an increased infiltration of lipocalin-2 (LCN-2) positive neutrophils into the choroid and retina of patients with early, dry AMD compared to age-matched controls, and this is suggestive of neutrophil activity.[6] Moreover, it has been demonstrated that inflammatory cells located at the Bruch's membrane (BM), alongside specific proinflammatory molecules like C3a and C5a identified in drusen, possess the capability to trigger vascular endothelial growth factor (VEGF), consequently playing a role in the progression of wet AMD.[7]

Various blood parameters have been used as an indicator of inflammation and associated with several inflammatory conditions including cardiovascular and endocrinological diseases, malignancies, ulcerative colitis, and end-stage renal disease; as well as AMD.[8,9] Previous studies investigated the neutrophil/lymphocyte ratio (NLR),[10] platelet/lymphocyte ratio (PLR),[11] and monocyte/lymphocyte ratio[12] as an indicator of systemic inflammation in patients with AMD. In this study, we aimed to determine whether complete blood count (CBC) parameters including white blood cell (WBC), neutrophil, lymphocyte, monocyte, and platelet counts as well as other inflammatory biomarkers that are calculated scores of NLR, PLR, and lymphocyte/monocyte ratio (LMR) show any significant differences in neovascular AMD (nAMD).

MATERIALS AND METHODS

Patients

In this retrospective study, 82 patients diagnosed with nAMD and a control group comprising 60 individuals matched in age and sex; who underwent routine blood sampling as part of preoperative preparation for cataract surgery between January and November 2020, were included. All participants underwent examination by an anesthesiologist, confirming the absence of any systemic diseases that could affect the blood parameters. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. Patients with any active infectious diseases, and those with the history of diabetes mellitus, cancer, any autoimmune diseases, chronic obstructive pulmonary disease, myeloproliferative diseases, cases with a recent history of steroid use, and smokers were excluded from the study.

Laboratory

Venous blood samples were collected in dipotassium ethylenediaminetetraacetic acid (EDTA) tubes and biochemistry tubes. An automatic blood counter Beckman Coulter DxH 900 (Beckman Coulter, Miami, Florida, USA) was used for CBC analysis; and WBC, neutrophil, lymphocyte, monocyte, platelet levels as well as NLR, PLR, and LMR scores were evaluated through these samples.

Statistical Analyses

The data were stored on a computerized database and analyzed using SPSS version 24.0 statistical software (IBM, Armonk, NY). Quantitative variables were expressed as mean ± standard deviation. In addition to the descriptive analysis tests, the gender compatibility of the patient and control groups were evaluated with the chi-square test. Continuous variables were analyzed for uniformity of distribution using the Shapiro-Wilk test and a logarithmic transformation was applied to the studied parameters to normalize the data. Independent samples t-test was then performed to compare the mean age as well as all CBC parameters including NLR, PLR, and LMR scores between the study group and the controls. A probability value of below 0.05 was considered as significant.

RESULTS

A total of 168 individuals including 77 females (45.8%) and 91 males (54.2%) composed the entire study population. Female to male ratio was 35 / 47 and 42 / 44 in the study and control groups, respectively (p=0.424). The mean age was 74.96 ± 6.61 (range, 65 – 93) years in the study group and 72.47 ± 5.70 (range, 65 – 90) years in the control group (p=0.380).

Scores of all studied CBC parameters including NLR, PLR, and LMR are shown in Table 1. In the study group, the mean WBC and monocyte counts were significantly higher than the controls (p=0.041 and p<0.001, respectively). However, there was no statistically significant difference in terms of neutrophil, lymphocyte, and platelet counts between the two groups (p=0.175, p=0.390, and p=0.625, respectively).

Calculated scores of NLR, PLR, and LMR were found as 2.51±1.40, 136.55±62.40, and 3.54±1.20 in the study group, respectively; whereas, they were found as 2.35±0.93, 135.21±49.90, and 3.92±1.20 in the control group, respectively (p=0.654, p=0.736, and p=0.039, respectively). Significantly lower LMR was found in the study group when compared to the controls.

CONCLUSION

There are several risk factors for AMD, such as aging, smoking, exposure to sunlight, specific gene mutations,

Table 1: *Studied complete blood count parameters in both groups*

	Normal range	Study group (n=82)	Control group (n=86)	p-value*
WBC ($\times10^3$ µL)	4.00 - 10.30	7.54 ± 1.59	7.04 ± 1.55	**0.041**
Neutrophil ($\times10^3$ µL)	2.10 - 6.10	4.60 ± 1.30	4.32 ± 1.21	0.175
Lymphocyte ($\times10^3$ µL)	1.30 - 3.50	2.11 ± 0.72	1.99 ± 0.62	0.390
Monocyte ($\times10^3$ µL)	0.30 - 0.90	0.61 ± 0.16	0.53 ± 0.15	**< 0.001**
Platelet ($\times10^3$ µL)	156.00 - 373.00	255.54 ± 71.84	248.97 ± 62.60	0.625
NLR	-	2.51 ± 1.40	2.35 ± 0.93	0.654
PLR	-	136.55 ± 62.40	135.21 ± 49.90	0.736
LMR	-	3.54 ± 1.20	3.92 ± 1.20	**0.039**

WBC: white blood cell; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; LMR: lymphocyte/monocyte ratio.
*Independent samples T-test

and inflammation.[10] The roles of inflammatory mediators and cells, such as immunoglobulins, complement proteins, cytokines, growth factors, and oxidative stress, in the pathogenesis of AMD have been shown.[13] Neutrophils are part of the innate immunity and able to release various cytokines, chemokines, matrix metalloproteinases, and VEGF. Activated neutrophils produce proteases such as matrix metalloproteinase-9 (MMP-9), which degrade and remodel the extracellular matrix in the angiogenesis process.[14] Inflammatory mediators and especially VEGF are increased in the vitreous of patients with AMD, generally associated with an altered cytokine system.[15,16]

Drusen, the accumulation of extracellular deposits between the RPE and BM consisting of various lipid, carbohydrate, and protein debris, is generally the earliest optical coherence tomographic finding of AMD; however, later stages develop geographic atrophy of the neuroretina as well as retinal pigment epithelium, and macular neovascularization (MNV) that protrude through the BM into the retina leading to exudative or hemorrhagic changes.[17] Local inflammation leads to the development of drusen formation, RPE/photoreceptor degeneration, BM disruption, and MNV. Activation of choroidal dendritic cells (antigen -presenting cells) plays an important role in local RPE cell damage. Dendritic cells contribute to the formation of drusen, trigger inflammatory and immune-mediated processes, and cause progressive forms of AMD.[18] When activated T lymphocytes are injected intravenously into mice with early uveitis, an inflammatory condition of the retina and choroid, early infiltration of T lymphocytes around the optic disc and retinal periphery has been shown to stimulate dendritic cells.[19]

Age-related macular degeneration is an inflammatory disease of the retina, the systemic and local retinal immune responses are connected with disease.[20] Inflammatory cells may migrate from surrounding tissues including the neuronal retina and choroid to the macula or be recruited from circulating immune cells.[21] Immune functions are mainly mediated by mononuclear phagocytes, such as microglia, monocytes, and macrophages in the retina. The distribution of mononuclear phagocytes throughout the retina alters with age and genetics. Increased concentration of these cells within the normally immune-privileged subretinal space with age affects the integrity and function of RPE and photoreceptors.[22] The significant difference in monocyte levels between the study group and control group in our study may be a supporting manifestation of this pathophysiological process.

Studies have evaluated NLR as an indicator of systemic inflammatory conditions associated with the prognosis and severity of diseases. In a study by Ilhan et al.[10] including 81 patients with non-nAMD, 84 patients with nAMD, and 80 age and sex-matched control group, a significant difference was found in NLR values between non-nAMD and nAMD groups; non-nAMD and control groups; nAMD and control groups. Kurtul and Ozer reported that increased NLR is independently associated with neovascular

AMD, with sensitivity and specificity of 73% and 60%, respectively.[23] More recently, Sengul et al.[11] have published higher NLR and PLR; which are inversely proportional to best corrected visual acuity, and directly proportional to central macular thickness in patients with nAMD. In a systematic review and meta-analysis including 777 AMD patients and 401 healthy controls, patients with AMD had a higher NLR (weighted mean difference: 0.37, CI 95% 0.08 to 0.66; p=0.013) when compared to healthy controls.[24] In our study, the difference in NLR levels are found to be insignificant between the two groups; although LMR levels revealed a statistically significant difference.

The role of systemic inflammation in cancer and cardiovascular disease has been reported by performing a complete blood count.[25,26] Complete blood count parameters are also used to show inflammation in several diseases such as uveitis, retinopathy of prematurity, and retinal vein occlusion.[27-29] Furthermore, increased systemic inflammation may be one of the major causes of treatment resistance in cases with recalcitrant nAMD. Scores of CBC including NLR, PLR, and LMR could be used in both ophthalmological and systemic diseases and appraised as simple, inexpensive, reliable indicators of the inflammatory status. According to their CBC parameters, personal adjustment of both the screening and treatment frequency may simply be performed in nAMD cases.

Our study has several limitations which must be disclosed; mainly the retrospective nature and small sample size, alongside the absence of longitudinal information of the patients, such as the duration of nAMD, number of previous intravitreal injections, whether they were newly diagnosed or not. Further studies with large cohorts are needed to investigate the relation of the inflammatory status with nAMD activity.

An extensive dysregulation of different arms of the immune system is associated with the pathophysiology of AMD. The role of inflammation in nAMD patients can be determined from a CBC and prolonged inflammation leads to increased neutrophil and monocyte counts. LMR may also be a good indicator of the prognosis of AMD alongside NLR.

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