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ORIGINAL PAPER

The significance of neutrophil-to-lymphocyte ratio in idiopathic epiretinal membrane

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Abstract
Purpose To assess the levels of neutrophil-to-lymphocyte ratio (NLR) in patients with idiopathic epiretinal membrane (iERM) and to compare the NLR results of patients with iERM and healthy controls.
Methods This retrospective study enrolled 43 patients with iERM and 40 healthy subjects. Complete ophthalmologic examination and complete blood count measurements were performed of all subjects. Complete blood counts were performed within 2 h of blood collection.
Results There was a significant difference in NLR between iERM and control groups ($p < 0.01$). The receiver operating characteristics analysis revealed that the value of NLR to distinguish patients with iERM and controls was found to be 0.832. The best cutoff value was 1.90, with a sensitivity of 72% and specificity 70%.
Conclusions Our study for the first time provides evidence that subclinical systemic inflammation may cause or at least accompanies iERM using a novel biomarker NLR.

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Keywords Idiopathic epiretinal membrane · Inflammation · Neutrophil-to-lymphocyte ratio · Biomarker · Fibrocellular proliferation

Introduction

Idiopathic epiretinal membrane (iERM) is a nonvascular fibrocellular proliferation that develops on the surface of the internal limiting membrane. Spectrum of disease varies from fine cellophane-like transparent membrane without any visual disturbance to contractile membranes that result in macular distortion, metamorphopsia, and decreased visual acuity [1].

Histopathologic analysis of iERM has demonstrated that it includes glial cells, fibroblasts, and hyalocytes as a cellular component and collagen as an extracellular component [2]. Analysis of vitreous showed that cytokines like transforming growth factor (TGF), basic fibroblast growth factor (FGF), nerve growth factor (NGF), glial cell line-derived neurotrophic factor, and vascular endothelial growth factor may be involved in iERM formation [3–5].

Neutrophils have important role in the acute inflammatory response, and lymphopenia occurs related to cortisol production and physiologic stress, so that increased neutrophil-to-lymphocyte ratio (NLR) has been recently defined marker of systemic inflammation [6]. Moreover, NLR has widely been studied as rapid and low-cost diagnostic method in

various disorders complicated with acute and chronic inflammation.

In the literature, NLR has been shown as prognostic factor for some systemic diseases like cancers, cardiovascular diseases, inflammatory bowel disease, and pulmonary embolism [7–10]. Recently, NLR has also been studied in some ophthalmologic disease like age-related macular degeneration, keratoconus, glaucoma, and non-arteritic anterior ischemic optic neuropathy [11–15].

In the present study, we aimed to focus on the role of the inflammation in the pathogenesis of iERM by using NLR and to compare NLR levels between iERM patients and healthy controls.

Materials and methods

This study protocol has been approved by the Human Research and Ethics Committee of the Medipol University Hospital and adheres to the tenets of the Declaration of Helsinki.

The data were extracted from patients' medical records between February 2014 and May 2016. The study included 43 eyes of 43 patients with iERM and 40 eyes of 40 healthy age- and sex-matched subjects. Diagnosis of ERM was made clinically by fundus examination with slit-lamp examination, fundus photography, spectral-domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg Engineering, Germany), and fundus fluorescein angiography (FFA). For study group, any macular pathology (e.g., cystic lesion, macular hole) rather than epiretinal membrane was an exclusion criterion. Any anterior or posterior segment pathology (cell, haze, synechias, or reaction) was eliminated with slit-lamp examination in both groups. We excluded the possibility of glaucoma both in study and in control groups by intraocular pressure values and appearance of optic nerve in dilated fundus examination. Intraocular pressure values were under 21 mmHg, and there were no glaucomatous optic neuropathy findings in fundus examination. Additionally, study subjects underwent FFA in order to exclude inflammatory diseases such as uveitis or malignancies.

The control group consisted of randomly selected subjects from whom a complete blood count was obtained during routine preoperative laboratory examination for cataract surgery. They also underwent detailed fundus examination using SD-OCT. Subjects with any macular pathology were excluded.

Exclusion criteria for patients and controls were secondary epiretinal membrane (diabetic retinopathy, venous occlusion, retinal detachment, uveitis, age-related macular degeneration, trauma, and so forth), myopia of more than 6 diopters or a 26-mm greater axial length, macular or lamellar hole, opaque optical media that significantly affects vision or disturbs optical coherence tomography, a history of prior intraocular surgery, ocular trauma, inflammatory ocular diseases, retinal diseases, diabetes mellitus, systemic hypertension, acute infectious disease, systemic inflammatory diseases, cardiovascular diseases, malignancies, and any special drug use (e.g., iron preparations, chemotherapeutic agents, vitamins, corticosteroids).

The sedimentation rates (<20 mm/h for woman and <15 mm/h for man) and C-reactive protein levels (0–5 mg/dl) were within the normal range in both study and control groups.

Laboratory assessment

Complete blood count values measured from the blood obtained via antecubital venipuncture using an automatic blood counter (Beckman Coulter Inc, Miami, FL). Levels of neutrophils, lymphocytes, platelets, red blood cells, and white blood cells were measured as part of the complete blood count. The NLR was calculated as the ratio of the neutrophils to lymphocytes.

Statistical analysis

The Statistical Package for the Social Science version of 22.0 software (SPSS, Chicago, IL). Comparisons between the groups were made using the Student's *t* test. Categorical variables were compared using the Chi-square test. Receiver operating characteristic (ROC) analysis was performed to determine the cutoff threshold using Youden's index and to quantify the accuracy of NLR. Sensitivity, specificity, and the area under the ROC (AUROC) curve were used for an overall estimation of the accuracy of the classifier.

Results

The mean subject age was 69.4 ± 8 years (range: 50–87 years) in the study group (24 females, 19

males) and 70.4 ± 7 years (range: 50–86 years) in the control group (20 females and 20 males). There were no significant differences between the groups with respect to age or sex ($p = 0.586$ and $p = 0.596$, respectively) (Fig. 1). Summary statistics of the laboratory data are shown in Table 1. The mean neutrophil count was significantly higher in the study group compared with the control group ($p < 0.01$). The lymphocyte values for the study group were significantly lower compared with those for the control group ($p < 0.01$). The mean NLR was significantly higher in the iERM group compared with control group ($p < 0.01$). The ROC analyses of the studied variables are shown in Fig. 2. According to this, the AUROC value of the NLR to distinguish patients with iERM and healthy control was found to be 0.832. The best cutoff value was 1.90, with a sensitivity of 72% and specificity 70%.

Discussion

In this study, we analyzed the levels of NLR in patients with iERM and control subjects. To our knowledge, this is the first study investigating a relationship between systemic inflammation and iERM. The present study showed that patients with iERM have higher NLR than healthy subjects. The result of this study suggests that subclinical systemic inflammation may cause or at least accompanies iERM.

Idiopathic ERM is the most common type of fibrocellular proliferation over the internal limiting membrane. Aging is one of the shown risk factors, so prevalence of ERM peaks between the ages of 70–79 [16]. Recently anomalous PVD is the most widely accepted theory to explain ERM pathogenesis. During anomalous PVD, posterior cortical vitreous splits and does not separate totally from internal limiting membrane. Hyalocytes within the cortical vitreous remnants are potentially responsible for the ERM formation [17]. Cellular component of ERM includes glial cells (Müller cells, fibrous astrocytes, and microglia), hyalocytes, fibroblasts, and myofibroblasts [18]. Extracellular component includes fibronectin and type I, II, III, IV, V, VI collagens [19].

Analysis of vitreous of patients with iERM showed increased amount of cytokines which are mediators for inflammation. Harada et al. [4] showed basic FGF m RNA expression which mediates maturation of glial cells in the 67% of patients with iERM. Minchiotti et al. [20] detected TGF β 1 and nerve growth factor mRNA that have role in fibroblast activities in the vitreous of iERM patients. In another study TGF β2 and NGF levels were found higher in the vitreous of patients with iERM, and it was supposed that TGF β2 might be responsible for differentiation of glial cells into myofibroblasts [3]. Myofibroblasts have critical role in ERM development as they produce collagenous extracellular matrix causing ERM contraction. Bu et al. [21] showed that TGF β1 is a stimulant factor for differentiation of Müller cells to myofibroblasts in their in vitro study. These studies suggest the probable effect of inflammation in iERM formation.

Recently, NLR have been accepted as a valuable marker of inflammation. It is a simple and inexpensive parameter which can be easily calculated from complete blood count results. The relation between higher levels of NLR and systemic inflammation is not clear yet.

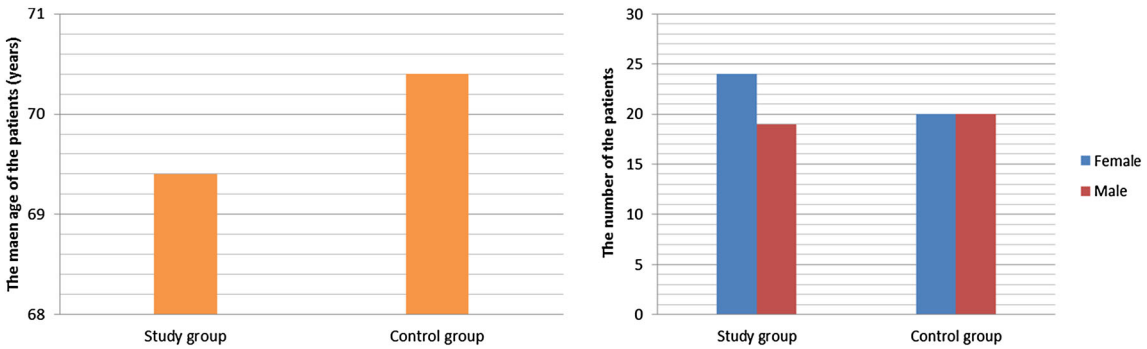

Fig. 1 Graphics of the distributions of age and gender of the patients

Table 1 Comparison of the mean values of neutrophil, lymphocyte and NLR of the subjects

Variables	Patients with iERM ($n = 43$) mean ± SD	Controls ($n = 46$) mean ± SD	P* value
Neutrophil 10^3/L	4.91 ± 1.2	3.72 ± 1.2	<0.01
Lymphocyte 10^3/L	1.75 ± 0.4	2.21 ± 0.5	<0.01
NLR	3.03 ± 1.2	1.77 ± 0.7	<0.01

NLR neutrophil lymphocyte ratio, *SD* standard deviation, *iERM* idiopathic epiretinal membrane

* Independent *t* test

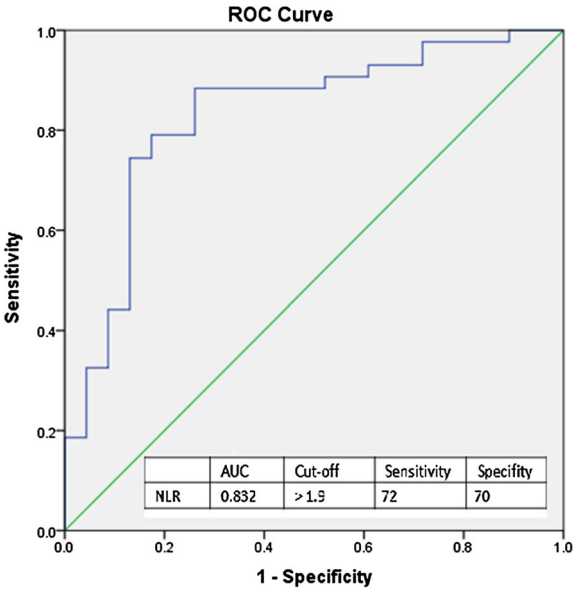

Fig. 2 Best cutoff neutrophil-to-lymphocyte ratio value; area under the receiver operating characteristic curve

Predictive value of NLR as an indicator of inflammation has been studied in some ophthalmologic diseases. Ilhan et al. [11] reported higher level of NLR value in patients with age-related macular degeneration when compared with controls and found correlation with severity of disease. In another study NLR was found higher in patients with non-arteritic anterior ischemic optic neuropathy compared with control group. There was a negative correlation with visual acuity at the first and third months examination [15]. Ozgonul et al. [14] evaluated NLR in patients with pseudoexfoliation syndrome (PEX) and pseudoexfoliation glaucoma (PXG) and reported elevated NLR compared to controls in both PEX and PXG patients. They claimed NLR was more sensitive indicator for predicting PEX syndrome in earlier stage. Karaca et al. [12] found higher NLR in patients with progressive keratoconus than in the non-progressive group and controls. In another study evaluating NLR in POAG, it was reported that patients with POAG had higher level of NLR than control group, and there was a correlation between pattern standard deviation and NLR.

Our study showed that the patients with iERM had higher NLR values than the patients with cataract had. The NLR value may be associated with the severity of cataract. We may assume that the patients with cataract have higher NLR levels compared to those without cataract. As far as we know, there has been no studies supporting this hypothesis in literature. Even if we assume this hypothesis is true, as the patients with ERM in our study had higher NLR levels compared to controls, the conclusion would not be changed.

Limitations of our study include relatively small number of subjects. Also we did not evaluate whether there is a relation between NLR levels and grade of iERM or visual prognosis. Another major limitation is the detailed present inflammatory status of patients and controls. As our study was designed as a retrospective study, we could only had CRP and sedimentation rates from the records. Other inflammatory factors such as TGF β1, TGF β2, interleukin (IL)-1 beta, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, interferon necrosis factor alfa could not be evaluated. These issues might be subjects of further studies with larger number of patients. Additionally, further studies comparing the NLR before and after vitrectomy with membrane peeling would be helpful to see how the ratios are of importance.

In conclusion, this is the first study which evaluates NLR in patients with iERM in the literature. We found higher NLR in patients with iERM compared with controls. According to this result, it might be speculated that subclinical systemic inflammation may

cause or at least accompanies iERM. Further studies are needed to investigate the role of systemic inflammation in iERM pathogenesis.

Compliance with ethical standards

Conflict of interest None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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