Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.06.354 2019;26(9):1880-4

Original Article

Monocyte / high-density lipoprotein ratio and neutrophil-to-lymphocyte ratio in age-related macular degeneration

Bekir Kucuk, Ender Sirakaya, Zeynep Duru, Necati Duru

Kayseri City Hospital, Department of Ophthalmology, Kayseri, Turkey

Abstract

Aim To evaluate the monocyte/high-density lipoprotein (HDL) ratio (MHR) and neutrophil-to-lymphocyte ratio (NLR) as biomarkers of systemic inflammation in age-related macular degeneration (AMD).

Materials and Methods: HDL, hematological profiles, erythrocyte sedimentation rates, C-reactive protein, MHRs, and NLRs were evaluated in 30 patients with neovascular AMD (Group 1), 30 with non-neovascular AMD (Group 2), and 30 controls (Group 3).

Results: MHRs and NLRs were significantly higher in Group 1 than in Group 3 (p = 0.002 and p < 0.001, respectively) and were independent predictors of neovascular AMD in multivariate analysis (odds ratio = 1.231 and 3.332; 95% confidence interval = 1.035–1.466 and 1.385–8.019; p = 0.019 and 0.007, respectively). The areas under the receiver operating characteristics curve for MHR and NLR were 0.669 and 0.769. The sensitivity and specificity of MHRs and NLRs in predicting neovascular AMD were 47% and 97% versus 63% and 80%, respectively.

Conclusions: By reflecting the balance of pro- and anti-inflammatory responses, the MHR can reliably indicate systemic inflammation in AMD.

Keywords: Age-related macular degeneration; monocyte-to-high-density lipoprotein ratio; neutrophil-to-lymphocyte ratio; systemic inflammation; neovascular AMD.

INTRODUCTION

Although age-related macular degeneration (AMD) is a leading cause of vision loss among elderly people, its pathophysiology remains unclear, and several factors have been attributed to its development (1). Among them, chronic inflammation and oxidative damage are taken into account in nearly all age-related neurodegenerative disorders, including AMD, Parkinson's disease, and Alzheimer's disease (2,3). Environmental factors have also been implicated in the condition's ethiopathogenesis due to findings that, along with aging, smoking and a high-fat diet increase macular oxidative stress, which is known to prompt the development of AMD (4,5). Regarding potential genetic factors, researchers have revealed that approximately 40 gene mutations are associated with the onset of AMD, largely because the proteins encoded by those genes relate to oxidative stress (6,7). To those factors, Rodrigues et al. have added some inflammatory cells and mediators, including immunoglobulins, complement components, cytokines, growth factors, and reactive oxygen radicals, that they identified as playing a role in the disease's pathogenesis (8). Altogether, findings to date suggest that as oxidative materials increasingly accumulate during aging, the resulting oxidative damage can initiate the development of AMD.

Extracellular accumulations in AMD indicate that chronic inflammation also influence the development of the disease (9). Biochemical parameters identified as potential indicators of inflammation in AMD include the neutrophil-to-lymphocyte ratio (NLR) and high-sensitivity C-reactive protein (10,11). Along with those parameters, higher monocyte counts and decreased levels of high-density lipoprotein (HDL) have additionally been identified as possible biomarkers of systemic inflammation. Drawing from those results, several researchers have also investigated the role of the monocyte/HDL ratio (MHR) in various systemic and ocular diseases associated with chronic inflammation, including branch retinal vein occlusion and pseudo exfoliation syndrome (12-15). Despite their efforts, however, the relationship of the MHR and AMD remains unknown (14,15).

Received: 27.07.2019 **Accepted:** 06.08.2019 **Available online:** 30.09.2019
Corresponding Author: Bekir Kucuk, Kayseri City Hospital, Department of Ophthalmology, Kayseri, Turkey
E-mail: bekirkucuk1983@hotmail.com

Considering that inflammation plays an important role in the etiopathogenesis of AMD, we evaluated whether an increased MHR could be a risk factor for the development of the disease by assessing MHRs and NLRs in patients with neovascular or non-neovascular AMD.

MATERIALS and METHODS

Our cross-sectional, case–control study was conducted in the Department of Ophthalmology at Kayseri City Hospital in Turkey after being approved by the institutional review board and ethics committee. In compliance with the Declaration of Helsinki, all potential participants were examined to determine their eligibility for inclusion in the sample and, if eligible, provided their oral and written informed consent to participate. In age- and gender-matched groups, 30 patients with neovascular AMD (Group 1) and 30 patients with non-neovascular AMD (Group 2) were compared with 30 healthy controls without AMD (Group 3). In Groups 1 and 2, only patients newly diagnosed with AMD and without any prior intravitreal or laser treatment for macular edema were eligible to participate. By contrast, participants in Group 3 were selected from healthy individuals who attended the clinic in the Department of Ophthalmology.

Each participant received a complete ophthalmological examination involving a test for best-corrected visual acuity, slit lamp biomicroscopy, a stereoscopic fundus examination, and the measurement of intraocular pressure (IOP) with Goldmann applanation tonometry. AMD was diagnosed with reference to the results of the stereoscopic fundus examination, optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany), and fundus fluorescein angiography (FFA, VISUCAM NM/FA; Carl Zeiss Meditec AG, Dublin, CA, USA). Patients diagnosed with neovascular AMD exhibited choroidal neovascularization or retinal pigment epithelium detachments, whereas ones diagnosed with non-neovascular AMD exhibited macular drusen with or without geographic atrophy in at least one eye.

Exclusion Criteria

Patients with any history of significant ocular disease (e.g., uveitis, scleritis, and retinal disease other than AMD), ocular surgery, or ocular trauma were excluded from the sample. Patients were also excluded if they had any history of hematological disease, systemic inflammatory disease, acute or chronic infectious disorder, chronic obstructive pulmonary disorder, hyperlipidemia, malignancy, use of topical eye medication, steroid therapy, or steroid use 6 months prior to the study.

Blood Parameters

Blood lipid profiles, hematological profiles, erythrocyte sedimentation rates, and C-reactive protein (CRP) levels were evaluated in venous blood samples at the beginning of the study. MHR was calculated as the ratio of the monocyte count to the level of HDL, whereas NLR was calculated as the absolute neutrophil count divided by the absolute lymphocyte count. Each blood sample was drawn from the antecubital vein between 8:00 and 10:00 a.m. after participants had fasted for 10 h.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences version 25 (IBM, Armonk, NY, USA). A power analysis was performed to assess the sample size confirmed that 30 participants per group provided adequate power. Normality distribution for each continuous variable was checked with the Shapiro–Wilk test, and a one-way analysis of variance was conducted to compare variation across the three groups. A Bonferroni test and Tukey's test were also performed as a post hoc analysis for multiple comparisons among the groups, whereas an independent sample *t* test was used to compare variables in all three pairs of groups. Multiple logistic regression analyses were also performed, as was a receiver operating characteristic analysis to determine the specificity and sensitivity of biomarker and the discriminative value of intergroup differences for some variables. For all results, a *p* value of less than 0.05 indicated statistical significance.

RESULTS

The sample consisted of 30 patients with neovascular AMD aged 72.43±8.17 years (range: 56–87), 30 patients with non-neovascular AMD aged 72.07±7.52 years (range: 61–87), and 30 healthy controls aged 71.27±9.87 years (range: 56–87; p=0.865). The distribution of participants by sex did not differ significantly among the groups (p=0.730). The demographic characteristics of participants in each group appear in Table 1.

Table 1. Patient characteristics and baseline laboratory measurements between the groups

	Group I	Group II	Group III	p
Gender, Male/Female	12/18	15/15	13/17	0.730[a]
Age, years	72.43±8.17	72.07±7.52	71.27±9.87	0.865[b]
Neutrophil count, 10^3 /lL	4.70±1.45	4.52±1.05	3.26±0.75	**<0.001**[b]
Lymphocyte count, 10^3 /lL	2.05±0.55	2.59±1.19	2.14±0.63	**0.034**[b]
Monocyte count, 10 /lL	576.0 ± 105.4	546.0 ± 112.1	421.3 ± 69.8	**0.002**[b]
HDL, (mg/dL)	47.43±13.77	53.03±10.76	49.33±9.66	0.167[b]
C-reactive protein, (mg/dL)	3.83±1.89	2.19±1.24	2.67±1.49	**0.028**[b]
ESR, (mm/h)	9.00±5.54	7.90±3.90	8.90±4.46	0.607[b]
Neutrophil-to-lymphocyte ratio	2.37±0.73	1.92±0.55	1.68±0.73	**0.001**[b]
Monocyte-to-HDL ratio	13.47±6.99	10.89±4.71	8.90±2.43	**0.003**[b]

HDL; high-density lipoprotein, LDL; low-density lipoprotein ESR; erythrocyte sedimentation rate. Values are expressed as mean ± standard deviation, [a] Chi-Square test, [b] One-way ANOVA test

Table 1 also presents the baseline laboratory measurements of participants by group. Mean MHR was 13.47±6.99 in Group 1, 10.89±4.71 in Group 2, and 8.90±2.434 in Group 3 (p=0.003), while mean NLR was 2.37±0.73 in Group 1, 1.92±0.55 in Group 2, and 1.68±0.73 in Group 3 (p = 0.001). Mean MHR and NLR were significantly higher in Group 1 than in Group 3 according to the results of post hoc analysis (p=0.002 and p < 0.001, respectively). However, mean MHR and NLR did not differ significantly between Groups 2 and 3 (p =0.285 and p=0.357, respectively). In multivariate analysis, NLR and MHR surfaced as independent predictors of neovascular AMD (OR: 1.231 and 3.332; 95% confidence interval=1.035–1.466 and 1.385–8.019; p=0.019 and 0.007, respectively), as shown in Table 2. The areas under the receiver operating characteristic curve for MHR and NLR were 0.669 and 0.769. Sensitivity and specificity of MHRs and NLRs in predicting neovascular AMD were 47% and 97% versus 63% and 80%, respectively (Figure 1).

Table 2. Predictors of neovascular age-related macular degeneration in multivariate regression analysis

Variable	Odds ratio (95% confidence interval)	p - value
CRP	0.952 (0.727 - 1.248)	0.722
ESR	1.030 (0.908 – 1.168)	0.645
MHR	1.231 (1.035 – 1.466)	**0.019**
NLR	3.332 (1.385 – 8.019)	**0.007**

CRP; C-reactive protein, ESR; erythrocyte sedimentation rate, MHR; Monocyte-to- high-density lipoprotein ratio, NLR; Neutrophil-to-lymphocyte ratio.

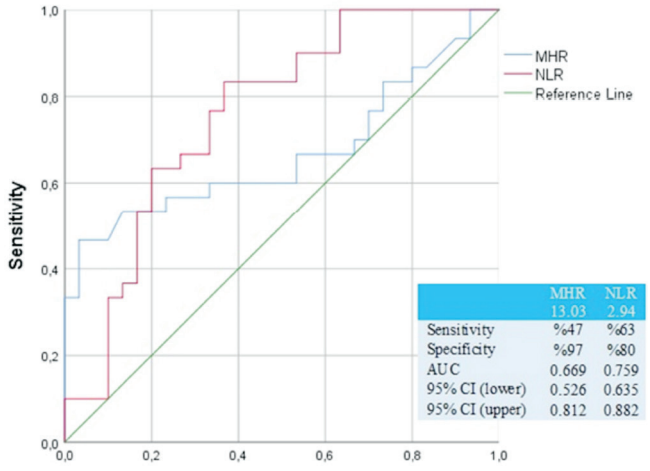

Figure 1. The receiver operating characteristics analysis for monocyte-to- high-density lipoprotein ratio (MHR) and neutrophil-to-lymphocyte ratio (NLR) in predicting neovascular age-related macular degeneration. AUC; area under the curve, CI; confidence interval.

DISCUSSION

Although the etiopathogenesis of AMD remains unclear, systemic inflammation, parainflammation, and oxidative stress are thought to contribute to its development or progression (3,9,16). The accumulation of amyloid-β and other inflammation-associated plasma proteins in the retina known as *drusen* also suggest the importance of systemic inflammation in the pathogenesis of AMD. In the same direction, some anti-inflammatory treatments have been shown to decrease the risk of developing AMD (17).

There are no biomarkers used to diagnose and monitor AMD reflect the inflammatory status of the disease. Although some researchers have designed studies using urine samples to detect such biomarkers, most candidates have been analyzed in peripheral blood, serum, or plasma (18). Among those possible biomarkers, the levels of complement components were found to be higher in patients with AMD than in controls (19,20). At the same time, Cao et al. reported that the central proinflammatory cytokines interleukin-6 and tumor necrosis factor-α had higher systemic concentrations in patients with neovascular AMD than in healthy controls, and both of those cytokines have been examined as biomarkers of choroidal neovascularization as well (21-23). The acute phase reactant CRP has also frequently been investigated as a candidate blood-based biomarker of AMD. In particular, Hong et al. found in their meta-analysis that individuals with CRP levels higher than 3 mg/L had a 2-fold greater risk of developing neovascular AMD than ones with CRP levels lower than 1 mg/L (24). Despite numerous studies geared toward pinpointing specific, sensitive biomarkers for AMD, no such biomarker has been identified for the early diagnosis or monitoring of the disease.

In other recent studies, the NLR and MHR have been used to examine systemic inflammation in various disorders (13,25-27). Among the results, NLR emerged as a significant biomarker of systemic inflammation in end-stage renal disease, familial Mediterranean fever, systemic lupus erythematosus, and several ocular disorders such as dry eye disease, idiopathic acute anterior uveitis, diabetic retinopathy, retinal vein occlusion, and AMD (26-33). Kurtul et al. observed that an elevated NLR is independently associated with neovascular AMD, and Ilhan et al. detected a significant correlation between the NLR and severity of AMD (10,30). Similarly, we found that mean NLR was significantly higher in patients with neovascular AMD than in controls.

Beside the NLR, the MHR is a newly discovered inflammatory biomarker that is superior to subtypes of white blood cells (13,25). In inflammatory reactions, monocytes are important because they secrete proinflammatory and pro-oxidant cytokines. Conversely, as an antioxidant and anti-inflammatory molecule, HDL reduces the transmigration of monocytes, mitigates the accumulation of mononuclear cells, and protects endothelial cells by increasing the expression of nitric oxide synthase. Considering that dynamic, researchers have recently hypothesized that the MHR could indicate inflammation (13,25,34).

To the best of our knowledge, however, our study was the first to involve investigating MHRs in patients with AMD with and without neovascularization. Among our results, MHRs

were significantly higher in patients with neovascular AMD than in controls. Furthermore, in multivariate analysis, the MHR and NLR emerged as independent predictors of neovascular AMD. In non-neovascular AMD, monocytes, macrophages, and dendritic cells were found to factor into the creation of drusen (9). In other research on the topic, Grossniklaus et al. reported that monocytes are numerous in choroidal neovascularization and increase the secretion of inflammatory and angiogenic cytokines (35).

Of course, HDL has long been known to play an important role in the pathogenesis of AMD (36). In particular, researchers have demonstrated that increased HDL levels are associated with a decreased risk of developing AMD (37,38). In our study, despite no significant difference in HDL levels, monocyte counts and MHRs were significantly higher in patients with neovascular AMD. Taken together, those and our results suggest that the MHR can be a reliable biomarker of systemic inflammation in AMD, precisely because it reflects the balance of pro- and anti-inflammatory responses.

Among the limitations of our study, the sample was relatively small to generalize the findings to the whole population. Furthermore, our study was cross-sectional in nature, whereas more convincing results could likely have been obtained by performing follow-up measurements of the parameters.

CONCLUSIONS

In sum, we demonstrated in our study that inflammation plays a pivotal role in the development of AMD and that the MHR and NLR can function as simple, inexpensive, reliable biomarkers of inflammatory activity to predict the risk of developing neovascular AMD. Nevertheless, researchers should assess the role of the MHR and NLR in the prognosis of AMD in large populations and in patients' responses to treatments for the disease.

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

Financial Disclosure: There are no financial supports

Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

Bekir Kucuk ORCID:0000-0002-4185-0656
Ender Sirakaya ORCID:0000-0003-2013-6338
Zeynep Duru ORCID:0000-0003-3803-3046
Necati Duru ORCID:0000-0002-4424-6964

REFERENCES

1. 1. Congdon NB. O'Colmain C. Klaver R. et al, Causes and prevalence of visual impairment among adults in the United Sta-tes. Arch Ophthalmol 2004;122:477-85.
2. McGEER, PL. McGEER EG. Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 2004;1035:104-16.
3. Ambati J, Atkinson JP, Gelfand BD. Immunology of agerelated macular degeneration. Nat Rev Immunol 2013;13:438-51.
4. Chiu CJ, Chang ML, Zhang FF et al. The relationship of major American dietary patterns to age-related macular degenera-tion. Am J Ophthalmol 2014;158:118-27.
5. Rangasamy T, Cho CY, Thimmulappa RK, et al. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke–induced emphysema in mice. J Clin Invest 2004;114:1248-59.
6. Canter JA, Olson LM, Spencer K, et al. Mitochondrial DNA polymorphism A4917G is independently associated with age-related macular degeneration. PLoS One 2008;3:e2091.
7. SanGiovanni JP, Arking DE, Iyengar SK, et al. Mitochondrial DNA variants of respiratory complex I that uniquely
8. characterize haplogroup T2 are associated with increased risk of age-related macular degeneration. PLoS One, 2009;4:e5508.
9. Rodrigues EB. Inflammation in dry age-related macular degeneration. Ophthalmologica 2007;221:143-52.
10. Anderson DH, Mullins RF, Hageman GS, et al, A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 2002;134:411-31.
11. Kurtul BE, Ozer PA. The relationship between neutrophil-tolymphocyte ratio and age-related macular degeneration. Korean J Ophthalmol 2016;30:377-81.
12. Boekhoorn SS, Vingerling JR, Witteman JC, et al. C-reactive protein level and risk of aging macula disorder: The Rotterdam Study. Arch Ophthalmol 2007;125:1396-401.
13. Bolayir A, Gokce SF, Cigdem B et al. Monocyte/high-density lipoprotein ratio predicts the mortality in ischemic stroke pa-tients. Neurol Neurochir Pol 2018;52:150-5.
14. Korkmaz A, Demir M, Unal S, et al. Monocyte-to-high density lipoprotein ratio (MHR) can predict the significance of an-giographically intermediate coronary lesions. Int J Cardiovasc Acad 2017;3:16-20.
15. Mirza E, Oltulu R FEBO, Katipoğlu Z, et al. Monocyte/HDL ratio and lymphocyte/Monocyte ratio in patients with pseudoexfoliation syndrome. Ocul Immunol Inflamm 2018:1-5.
16. Şatırtav GE, Mirza R, Oltulu GD, et al. Assessment of Monocyte/HDL Ratio in branch retinal vein occlusion. Ocul Immu-nol Inflamm 2019:1-5.
17. Donoso LA, Kim AD, Frost A. et al. The role of inflammation in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2006;51:137-52.
18. Swanson MW, McGwin G Jr. Anti-inflammatory drug use and age-related macular degeneration. Optom Vis Sc 2008;85:947-50.
19. Guymer RH, Tao LW, Goh JK, et al. Identification of urinary biomarkers for age-related macular degeneration. Invest Ophthalmol Vis Sci. 2011;52:4639-44.
20. Reynolds R, Hartnett ME, Atkinson JP, et al. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. Invest Ophthalmol Vis Sci 2009;50:5818-27.
21. Hecker LA, Edwards AO, Ryu E, et al. Genetic control of the alternative pathway of complement in humans and agerelated macular degeneration. Hum Mol Genet 2009;19:209-15.
22. Cao S, Ko A, Partanen M, et al. Relationship between systemic cytokines and complement factor H Y402H polymorphism in patients with dry age-related macular degeneration. Am J Ophthalmol 2013;156:1176-83.
23. Cousins SW, Espinosa-Heidmann DG, Csaky KG. Monocyte activation in patients with age-related macular degeneration: a biomarker of risk for choroidal neovascularization? Arch Ophthalmol 2004;122:1013-8.
24. Oh H, Takagi H, Takagi C, et al. The potential angiogenic role of macrophages in the formation of choroidal neovascular

membranes. Invest Ophthalmol Vis Sci 1999;40:1891-8.
25. Abdulaeva E. The role of complement system and other inflammatory factors in the development of age-related
26. macular degeneration. Kazan Med J 2018;99:657-64.
27. Ucar FM. A potential marker of bare metal stent restenosis: monocyte count-to-HDL cholesterol ratio. BMC Cardiovasc Disord 2016;16:186.
28. Ahsen A, Ulu MS, Yuksel S, et al. As a new inflammatory marker for familial Mediterranean fever: neutrophil-tolymphocyte ratio. Inflammation 2013;36:1357-62.
29. Turkmen K, Ozcicek F, Ozcicek Ozcicek, et al. The relationship between neutrophilto-lymphocyte ratio and vascular
30. calcification in endstage renal disease patients. Hemodial Int 2014;18:47-53.
31. Kim HA, Jung JY, Suh CH, et al. Usefulness of neutrophil-tolymphocyte ratio as a biomarker for diagnosing infections in patients with systemic lupus erythematosus. Clin Rheumatol 2017;36:2479-85.
32. Ozgonul C, Sertoglu E, Ayyildiz O, et al. Novel biomarkers for patients with idiopathic acute anterior uveitis: neutrophil to lymphocyte ratio and platelet to lymphocyte ratio. Int J Ophthalmol 2017;10:262.
33. Ilhan N, Daglioglu MC, Ilhan MC, et al. Assessment of neutrophil/lymphocyte ratio in patients with age-related macular degeneration. Ocul Immunol Inflamm 2015;23:287-90.
34. Sekeryapan B, Uzun F, Buyuktarakci S, et al. Neutrophil-tolymphocyte ratio increases in patients with dry eye. Cornea 2016;35:983-6.
35. Dursun A, Ozturk S, Yucel H, et al. Association of neutrophil/lymphocyte ratio and retinal vein occlusion. Eur J Ophthalmol 2015;25:343-6.
36. Ulu SM, Dogan M, Ahsen A, et al. Neutrophil-to-lymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. Diabetes Technol Ther 2013;15:942-7.
37. Yue S, Zhang J, Wu J, et al. Use of the monocyte-tolymphocyte ratio to predict diabetic retinopathy. Int J Environ Res Public Health 2015;12:10009-19.
38. Grossniklaus HE, Ling JX, Wallace TM, et al. Macrophage and retinal pigment epithelium expression of angiogenic
39. cytokines in choroidal neovascularization. Mol Vis 2002;8:119-26.
40. Peter I, Huggins GS, Ordovas JM, et al. Evaluation of new and established age-related macular degeneration susceptibility genes in the Women's Health Initiative Sight Exam (WHI-SE) Study. Am J Ophthalmol 2011;152:1005-13.
41. Cho BJ, Heo JW, Kim TW, et al. Prevalence and risk factors of age-related macular degeneration in Korea: the Korea
42. National Health and Nutrition Examination Survey 2010–2011. Invest Ophthalmol Vis Sci 2014;55:1101-8.
43. Reynolds R, Rosner B, Seddon JM. Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration. Ophthalmology 2010;117:1989-95.