Original Article

Relation Between the Novel Marker Monocyte to High-Density Lipoprotein Cholesterol Ratio and Severity in Multiple Sclerosis

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Abstract

Introduction: This study aimed to establish whether there is a relationship between the Monocyte to High-Density Lipoprotein Cholesterol (HDL-C) ratio (MHR) and severity of disease, and whether it can be used as a new marker for predicting disability in Multiple Sclerosis (MS), a chronic disease, which is usually contracted in early adolescence. **Methods:** 184 patient subjects who had been definitively diagnosed with MS, based on the McDonald criteria, and 105 healthy subjects with a similar age and gender profile were included in the study. The patients' Expanded Disability Status Scale (EDSS) scores, MS subtypes, length of time with the disease and demographics were captured. Blood samples were collected for hematologic and biochemical testing. The MHR values were calculated and statistically compared with those of the control group. **Results:** The average age of the MS patients was 38.3 ± 8.6 years and their average EDSS score was 2.5 [0-7.5]. The patient arm consisted of 118 (64.1%) females and 66 (35.9%) males. In the patients with MS, the MHR was 15.01 ± 0.63 compared to 9.61 ± 0.25 in the controls. This difference was statistically significant ($P < 0.001$). In the MS patients, the MHR cut-off value was 12.95 compared to controls, which was statistically significant ($P < 0.001$). Also, a statistically-significant (r: 0.297, $P < 0.001$) positive correlation was found between the MHR and EDSS score. **Conclusion:** The Monocyte to High-Density Lipoprotein Cholesterol ratio is associated with disease severity and disability in MS patients, and may be used as an independent marker for predicting disability. However, broader-scale studies are needed for more conclusive results.

Keywords: Disability, high-density lipoprotein, inflammation, monocyte, multiple sclerosis

Introduction

Multiple Sclerosis (MS) is an autoimmune, inflammatory, demyelinating and neurodegenerative disease, marked by episodes of relapsing neurological dysfunction, usually affecting females. MS is the leading cause of neurologic disability affecting the younger age groups in developed countries.[1]

MS is characterized by disruption of the blood-brain barrier (BBB) due to lesions occurring in the white matter, and migration of immune reactive cells to the disturbed region, which target and damage oligodendrocytes and myelin sheaths. The etiology of the disease is thought to principally involve the peripheral activation of Th1 lymphocytes which traffic to the central nervous system and react with myelin autoantigens.[2,3] However, it has been recently shown that monocytes and macrophages originating from myeloid cells play a crucial role in the secretion of proinflammatory and prooxidant cytokines which exacerbate immune response in MS.[4,5]

The blood-brain barrier is a selective and dynamic barrier that has a major role in maintaining brain homeostasis. It is composed by specialized brain endothelial cells which are firmly connected via adherent and tight junctions.[6] Previous studies have shown that oxidative and inflammatory events contribute to endothelial dysfunction in MS patients. It has been shown that high-density lipoprotein cholesterol (HDL-C) protects endothelium against the adverse effects of low-density lipoprotein (LDL-C) and the oxidation caused by LDL-C molecules.[7,8] For this reason, it was believed that HDL-C exhibits anti-inflammatory and antioxidant properties. It has been shown that increased monocyte count and decreased HDL-C levels may be related to inflammation and oxidative stress.[9,10] Recently, the monocyte count to HDL-C ratio (MHR) is increasingly recognized as a novel clinically-relevant biomarker of pathological inflammation and a new predictor and prognostic factor.[11,12] Moreover they are inexpensive and readily available components of the standard complete blood count (CBC). However, to the best our knowledge, no studies have thus far investigated MHR in MS patients. Our study was the first of its kind in this respect.

This study aimed to establish whether there is a relationship between the MHR and severity of disease, and whether it can be used as a new marker for predicting disability in Multiple

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Sclerosis (MS), a chronic disease, which is usually contracted in early adolescence.

Methods

Study population and design

Patients who had been definitively diagnosed with MS based on the McDonald criteria between September 2018 and March 2019 and who were not having an episode of relapse were randomized and prospectively included in the study. The diagnosis of MS, identification of the clinical form of the disease, and its outpatient monitoring were made at the MS Outpatient Clinic, Neurology Ward, Kayseri City Hospital. The study was approved by the local ethics committee and conducted according to the Declaration of Helsinki. Consent was received from all subjects.

Patients' age, gender, length of time with the disease, medications being used, neurological systems affected in the clinical course, neurological examination findings, duration of follow-up, and clinical form were captured. The Expanded Disability Status Scale (EDSS) scores were used to evaluate the functional status of MS patients.[13] Subjects with an EDSS score between 0 and 3.5 were classified as having mild-moderate MS, and those having an EDSS score between 4 and 10 were classified as having severe MS.

Healthy subjects were selected among healthy individuals with a similar age and gender profile who visited the check-up service at our hospital and presented with no sign of a neuropsychiatric, systemic, metabolic, infective or inflammatory condition.

Exclusion criteria

The exclusion criteria included heart failure, renal or hepatic failure, active hepatobiliary disease, active infectious disease, chronic inflammatory or immunologic conditions, hematological diseases, malignancy, and immunological disease, smoking or alcohol use, immunosuppressive drug use, receiving an antiinflammatory drug over the past 10 days, known adrenal or metabolic conditions, individuals younger than 18 years or older than 50 years of age, or receiving treatment with a systemic medication that could affect hematological parameters, such as antihyperlipidemic therapy.

Laboratory assessment

In our hospital, blood samples were routinely drawn from the antecubital vein at 08.00 to 10.00 am after an overnight fasting period. After performing the comprehensive neurologic examinations, we measured the blood lipid profiles, hematology profiles, and C-reactive protein (CRP) levels of participants. The other biochemical analyses were determined by standard methods. We calculated MHR as the ratio of the monocyte count to the level of HDL and the neutrophil-to-lymphocyte ratio (NLR) as the ratio of the neutrophil count to the lymphocyte count.

Statistical analysis

The software package IBM SPSS for Windows Version 22.0 was used for statistical analysis. Numerical variables were summarized by mean ± standard deviation or median [min-max] values, and categorical variables by numbers and percentages. Parametric test assumptions (normality and homogeneity of variance) were checked before comparing the groups for numerical variables. Normal distribution of the numerical variables was verified by the Shapiro Wilks test. The homogeneity of variance for the compared groups was examined using the Levene test. The presence of difference between 2 independent groups in terms of the numerical variables was examined in independent groups using the t test, if parametric test conditions were met, or using the Mann Whitney U test, if the conditions were unmet. Welch ANOVA was used for comparing more than 2 independent groups. The difference between the groups in terms of categorical variables was examined using the Chi-square test. The relationship between the numerical variables was given with the Pearson or Spearman correlation coefficients. The MHO value separating the study group and the controls was determined using ROC curve analysis. The level of significance was taken as $P < 0.05$.

Results

A total of 184 MS patients and 105 healthy subjects were included in the study. Baseline clinical characteristics, disease properties, and laboratory parameters of subjects are shown in

Table 1: Clinical and laboratory parameters of MS patients vs controls

Parameters	Patients with MS (n=184)	Control (n=105)	P
Gender (F/M)	118/66 (64.1%/35.9%)	72/33 (68.6%/31.4%)	0.444
Age	38.3±8.6	36.3±9.0	0.51
Neutrophil (×10³/L)	4.6±2.15	4.35±1.18	0.198
Lymphocyte (×10³/L)	2.31±0,57	2.22±0.63	0.415
Monocyte count (×10³/L)	0.62±0.21	0.46±0.11	<0.001
HDL-C (mg/dl)	44.13±10.9	49.79±6.93	<0.001
LDL-C (mg/dl)	110.96±33.56	103.83±29.38	0.071
Hemoglobin (g/dl)	13.50±2.22	13.94±1.38	0.038
Total platelet count (×10³/L)	260.54±65.67	262.35±50.87	0.794
Creatinine (mg/dl)	0.68±0.15	0.68±0.14	0.935
WBC (×10³/L)	7.20±1.47	7.16±2.39	0.849
Triglyceride	109 [45-574]	100 [40-272]	0.074
Glucose (mg/dl)	94 [73-285]	93 [73-132]	0.077
hsCRP (mm/h)	2 [0,3-11,1]	2 [0,5-16]	0.571
ESR (mm/h)	9.39±4.58	8.93±4.52	0.456
Age of disease onset Median [Min - Max]	31 [15-49]	-	
Disease Duration/year Median [Min - Max]	7 [0-29]	-	
EDSS Median [Min - Max]	2,5 [0-7.5]	-	

Abbreviations: WBC=white blood cell, LDL-C=low- density lipoprotein cholesterol, HDL-C=high-density lipoprotein cholesterol, hsCRP=high-sensitivity C-reactive protein, ESR=sedimentation

Tables 1 and 2. There was no statistically significant difference between the study groups in terms of age, sex, neutrophils, lymhocycte, LDL-C, total platelet count, creatinine, white blood cell count, triglyceride, glucose, hsCRP, ESR, and NLR levels. MHR and monocyte levels were significantly higher, and HDL-C and hemoglobin levels significantly lower in patients with MS than in controls.

The MHR levels was statistically significantly high in the MS patients compared to controls, whereas the NLR ratio was higher in the patient subjects compared to controls, although not to a statistically significant degree ($P < 0.001$ and $P = 0.802$, respectively) [Table 2].

The MHR ratio was statistically significantly higher in patients who were classified as having severe disease, as determined based on the EDSS scores, compared to those with mild or moderate disease ($P = 0.005$). The MHR ratio was also statistically significantly higher in patients with SPMS or PPMS with highly active disease, compared to other disease subtypes ($P < 0.001$) [Table 3].

A statistically significant correlation was detected between the MHR levels and the neutrophil and triglyceride levels, whereas no such correlation could be found with other laboratory levels. Based on the clinical parameters, the MHR levels statistically significantly increased with longer time with disease and higher EDSS score ($P = 0.027$ and $P \leq 0.001$, respectively) [Table 4].

The ROC analysis was performed to determine the MHR cutoff value (>12.95) for predicting significance in MS patients. The sensitivity value was taken as 63.6.%, the specificity value as 92.4%, and the area under the ROC curve value as 0.76 [Figure 1].

Discussion

This prospective study investigated whether any correlation existed between the MHR level, which is considered as an inflammation marker in MS patients, and the disability score, prognosis, demographics and clinical variables. We found that the MHR levels were statistically higher than in the healthy controls. Moreover, this difference increased with higher EDSS scores, gaining statistical significance in the progressive forms of the disease. To the best of our knowledge, this study is the first of its kind, demonstrating the relationship between MHR and disease severity in patients with MS.

The basic pathological characteristics of the central nervous system (CNS) tissues in MS patients provide a clue for the pathogenesis, although the etiology of MS has not yet been completely understood.[14] Clinicians have been investigating markers that can serve as a direct or indirect predictor of inflammation and oxidative stress, which play a major role in MS pathology and in predicting disease prognosis. Several studies have reported elevated levels of oxidative stress and inflammation markers such as platelet/lymphocyte

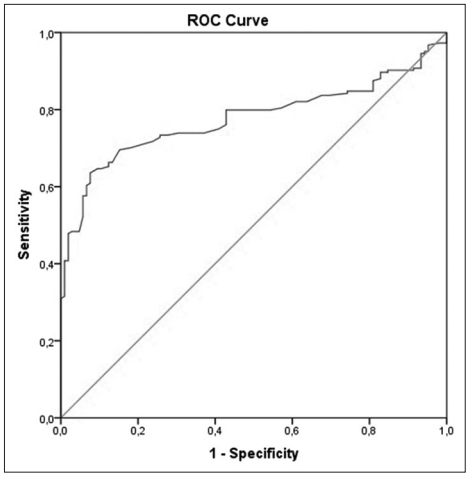

Figure 1: ROC curve

Table 2: Monocyte-to-high density lipoprotein ratio (MHR) and neutrophil-to-lymphocyte ratio (NLR) in patients with MS

Variables	Patients with MS Mean±SD	Controls Mean±SD	*P*
MHR	15.01±0.63	9.61±0.25	<0.001
NLR	2.08±0.64	2.02±0.85	0.802

Table 3: Clinical attributes affecting MHR in MS patients

Variables		MHR Mean±SD	*P*
Gender	Female	15.72±0.60	0.051
	Male	17.33±0.60	
EDSS range	0-3.5(mild-moderate) (*n*=129)	14.27±0.64	0.005
	4-10 (severe) (*n*=55)	17.08±0.54	
Types of MS	RRMS (*n*=146)	14.82±0.63	<0.001
	SPMS (*n*=24)	18.79±0.41	
	PPMS (*n*=4)	19.25±0.33	
	CİS (*n*=10)	11.12±0.21	

Table 4: Correlation analysis between MHR and other laboratory and clinic data among patients with MS

Variables	r	*P*
Neutrophil	0.213	0.004
Lymphocyte	0.135	0.473
Platelet	0.126	0.088
Hemoglobin	0.057	0.446
Hematocrit	0.266	0.146
Total cholesterol	−0.047	0.804
LDL cholesterol	−0.006	0.940
Triglycerides	0.298	<0.001
C-reactive protein	0.038	0.610
ESR	0.306	0,100
NLR	0.67	0.764
Age at disease onset	−0.115	0,120
Length of time with disease	0.163	0.027
EDSS	0.297	<0.001

ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), HLA genotype, chitinase 3-like 1, Vascular Endothelial Growth Factor Receptor 2, and monocyte/lymphocyte ratio (MLR) in patients with MS.[15-17] Nevertheless, a definitive biomarker indicating diagnosis and activity of MS is yet to be discovered.[18]

Monocytes are considered to be a subtype of leukocytes, which play an important role in inflammation. Activated monocytes interact with damaged or activated brain endothelium. This leads to overexpression of some proinflammatory mediator molecules including interleukin (IL)-1, and IL-6, tumor necrosis factor -α, transforming growth factor -α and -β, platelet-derived endothelial cell growth factor macrophage colony-stimulating factor, and insulin-like growth factor.[19,20] After this, monocytes differentiate into the macrophages that eventually ingest oxidized LDL-C and form the initial foamy cells.[21-23] Contrarily, HDL-C molecules counteract macrophage migration and remove cholesterol debris from those cells. Recent studies also indicate the role of HDL-C in modulating monocyte activation, adhesion, and also in controlling the proliferation of progenitor cells that differentiate to monocytes. Besides its anti-inflammatory and antioxidative effects, HDL-C molecules also increase endothelial nitric oxide synthase expression.[24-26] Therefore, monocytes exert a proinflammatory and prooxidant effects, but HDL-C functions as a reversal factor during these processes.[12] Given those findings and our results, we suggest that MHR is a better indicator of systemic inflammation than other hematological parameters in inflammatory disorders, largely because MHR indicates the balance of proinflammatory and anti-inflammatory reactions.

Despite all advances, predicting disease prognosis in MS remains difficult, as different neuro-inflammatory mechanisms act in individual patients.[27] Açıkgöz *et al.* in patients with Behçet's Disease and Köseoğlu *et al.* in patients with OSAS have found the MHR values to be high, and concluded that it might be associated with systemic inflammation and endothelial dysfunction.[10,28] However, in none of the previous studies had MHR, a recently-recognized marker of chronic inflammation, been associated with the disability score, an indicator of inflammation severity in MS patients. In this study, the contribution of the MHR level to the change in disability was significant, and patients with higher baseline MHR experienced greater worsening of EDSS scores. A similar relationship also existed between the length of time with disease and the MHR level. Higher MHR levels were strongly correlated with the progressive forms of MS.

Our study had several limitations. Firstly, it was carried out at a single center, and the number of patients included was relatively small. Secondly, the serum HDL-C levels and complete blood monocyte count were variable over time, and measurement at a single time point in our study may not be fully representative of the serum levels. Thirdly, all of the patients were using disease-modifying treatments, which may affect blood leukocyte levels and thus the results.

Conclusion

Altogether, inflammation seems to play an important role in MS. Our results have shown that MHR levels, an indicator of the degree of inflammation, is related with the disease severity and disability in MS, and an independent marker for predicting disability. We suggest that the MHR levels are a useful, practical, cheap and easily-calculable marker for predicting disability in MS. Our study is the first of its kind to demonstrate this relationship. Nevertheless, broader-scale, randomized studies are needed to establish clearer results and elucidate the pathophysiology.

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Conflicts of interest

There are no conflicts of interest.

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