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Levels of YKL-40 in Cerebrospinal Fluid and Serum of Newly Diagnosed Patients with Relapsing-Remitting Multiple Sclerosis

Yeni Tanı Alan Atak ve Remisyonlarla Seyreden Multiple Sklerozlu Hastaların Beyin Omurilik Sıvısı ve Serumlarında YKL-40 Seviyeleri

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ÖZ

Amaç: Kitinaz-3 benzeri protein 1 (CHI3L1 yada YKL-40), reaktif astrositler tarafından ağırlıklı olarak üretilen yeni bir inflamatuar glikoproteindir. Bu çalışmanın amacı, yeni tanı alan atak ve remisyonlarla seyreden multipl skleroz (RRMS) hastalarının serum ve
beyin omurilik sıvısındaki (BOS) YKL-40 seviyelerini araştırmaktır.
Araçlar ve Yöntem: Bu çalışma Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi Eğitim ve Araştırma Hastanesi Nöroloji Kliniğinde 2012 Ekim ile 2016 Kasım ayları arasında yapıldı. Çalışmaya, 30 yeni tanı alan ve tedavi edilmemiş RRMS hastası (18 kadın, 12 erkek, yaş aralığı 18-40 yıl) ve 20 kontrol (12 kadın, 8 erkek, yaş aralığı 20-40 yıl) dahil olmak üzere toplam 50 katılımcı dahil edildi. RRMS hastalarının ve kontrollerin BOS ve serum YKL-40 düzeyleri ELISA testi ile ölçüldü.
Bulgular: RRMS hastalarında BOS YKL-40 seviyeleri (sırasıyla 168.8±28.3 ng/ml ve 70.1±14.3 ng/ml, p<0.001) kontrollere göre anlamlı olarak daha yüksekti. Ancak, serum YKL-40 seviyeleri açısından RRMS hastaları ve kontroller arasında anlamlı bir fark saptanmadı. RRMS hastalarında ve kontrollerde serum YKL-40 seviyeleri sırasıyla 51.2±12.1 ng/ml ve 46.8±8.2 ng/ml idi (p=0.102). RRMS hastalarında serum ve BOS YKL-40 aktivitesi arasında ilişki yoktu (r=0.134, p=0.308).
Sonuç: Çalışmamız, yeni tanı alan RRMS hastalarında tanısal lomber ponksiyon sırasında artmış BOS YKL-40 seviyeleri, hastalığın erken döneminde mikroglial aktivasyonun varlığını desteklemektedir. Bu sonuçlar daha spesifik glial inflamatuar biyobelirteçler ile yapılacak geniş ölçekli klinik çalışmalarla desteklenmelidir. Böylece gelecekte RRMS hastalarının erken tedavisinde glial inflamasyon üzerine etkili ilaçların da kullanımı ortaya çıkacaktır.

Anahtar Kelimeler: biyobelirteç; kitinaz-3 benzeri protein 1; multiple skleroz

ABSTRACT

Purpose: Chitinase-3-like protein 1 (CHI3L1 or YKL-40), novel inflammatory glycoprotein predominantly produced by reactive astrocytes. The aim of this study was to investigate YKL-40 levels in serum and cerebrospinal fluid (CSF) of newly diagnosed relapsing-remitting multiple sclerosis (RRMS) patients.
Materials and Methods: This study was conducted at the Neurology Clinic of Recep Tayyip Erdogan University Faculty of Medicine Training and Research Hospital between October 2012 and November 2016. A total of 50 participants, including 30 newly diagnosed and untreated RRMS patients (18 females, 12 males, age range 18-40 year) and 20 controls (12 females, 8 males, age range 20-40 year) were included in the study. CSF and serum YKL-40 levels of RRMS patients and controls were measured by ELISA.
Results: CSF YKL-40 levels of RRMS patients were significantly higher than in controls. (respectively,168.8±28.3 ng/ml and 70.1±14.3 ng/ml, p<0.001). However, no significant differences were detected in serum YKL-40 levels between RRMS patients and controls. Serum YKL-40 levels were 51.2±12.1 ng/ml in RRMS patients and 46.8±8.2 ng/ml in controls (p=0.102). There were no correlation between serum and CSF YKL-40 activity in patients with RRMS (r=0.134 p=0.308).
Conclusion: Our study supports the activation microglial in the early stages of newly diagnosed RRMS, as evidenced by elevated CSF YKL-40 levels. These findings should be corroborated by larger-scale clinical studies employing more specific glial inflammatory biomarkers. This may pave the way for the future use of drugs targeting glial inflammation in the early treatment of RRMS patients.

Keywords: biomarker; chitinase-3-like protein 1; multiple sclerosis
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INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated, inflammatory, and neurodegenerative disease of the central nervous system (CNS) characterized by increasing incidence and prevalence.[1] While the precise etiology of MS remains elusive, it is hypothesized to arise from the interplay of genetic predisposition (e.g., HLA-DRB1*15:01) and environmental factors (e.g., Epstein-Barr virus infection, cigarette smoking, vitamin D3 deficiency, and early-life obesity) impacting immune system function.[2] MS commonly affects young adult women, typically between the ages of 20 and 40, and represents a leading cause of non-traumatic disability in this population globally.[1,2] A great majority of patients (%85) present with relapsing-remitting form in early disease course (RRMS).

A key pathological feature of RRMS is demyelination, characterized by oligodendrocyte loss. Longitudinal studies indicate that demyelinating neuroinflammation and axonal neurodegeneration are critical, sequential processes in MS pathogenesis.[3] Two primary immune cell types contribute to demyelination: autoreactive CD4+ T cells and proinflammatory CD20+ B cells. Evidence suggests that axonal damage occurs concurrently with demyelination, even in early disease stages. Histopathological analyses reveal that reactive astrocytes within acute lesions secrete chemokines, activating microglia and increasing blood-brain barrier permeability.[4] Activated microglia, alongside inflammation and proinflammatory cytokines, exacerbate demyelination and promote tissue damage.[3,4]

Microglia play multifaceted roles in RRMS, and are implicated in both the initiation and perpetuation of demyelination through interactions with infiltrating peripheral immune cells. Conversely, microglia are also likely crucial for the recruitment and differentiation of cells involved in remyelination, the process of myelin restoration.[5] While microglial activation is most prominent at the margins of active demyelinating lesions, it is also observed in periplaque regions and even within seemingly normal-appearing white matter.[4,5]

YKL-40 (chitinase 3-like protein 1, CHI3L1) is a novel inflammatory glycoprotein that non-enzymatically binds chitin and heparin.[6] It has been described as a cerebrospinal fluid (CSF) biomarker of neuroinflammation in humans.[7] Primarily an astrocytic protein, YKL-40 is principally synthesized and secreted by activated resident macrophages, such as microglia. While widely studied as a CSF biomarker that is elevated in early RRMS,[8] its precise physiological and biological roles remain unclear, leading to some controversy regarding its utility. Nevertheless, studies have reported its involvement in cell proliferation and differentiation, angiogenesis, oxidative stress, inflammation, and tissue remodeling.[9] Investigations of YKL-40 in various inflammatory diseases have yielded accumulating evidence of microglial/macrophage activation in RRMS patients. In MS brain tissue, YKL-40 expression has been observed in reactive astrocytes and microglia at the periphery of chronically active lesions.[3,4] However, clinical studies examining glial activity in early RRMS remain limited. Therefore, this study aimed to investigate YKL-40 levels in the CSF and serum of patients with newly diagnosed RRMS.

MATERIALS and METHODS

This study has been approved by the Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (dated September 7, 2012, and numbered 2012/126). All patients and controls were informed about the study and written informed consent was obtained. The procedures were in accordance with the revised form of the 2008 Declaration of Helsinki.

Participants

This study enrolled 30 newly diagnosed and treatment-naïve patients with relapsing-remitting multiple sclerosis (RRMS) (18 females, 12 males; age range 18-40) and 20 controls (12 females, 8 males; age range 20-40), recruited from the Neurology Clinics of Recep Tayyip Erdogan University Faculty of Medicine Training and Research Hospital between October 2012 and November 2016. A definit RRMS diagnosis was established based on the 2017 revisions of the McDonald criteria,[10] incorporating patient history, neurological examination, visual evoked potentials, oligoclonal bands, IgG index, and brain and spinal cord Magnetic Resonance Imaging (MRI) findings. All RRMS

patients presented with Expanded Disability Status Scale (EDSS) scores between 0.5 and 1.

The control group comprised individuals presenting to the neurology outpatient clinic with headache, subsequently diagnosed with benign intracranial hypertension following diagnostic investigations. Control subjects had no history of chronic medication use or infectious-autoimmune diseases. The control group was selected based on this specific diagnosis as obtaining CSF from healthy individuals was deemed ethically inappropriate. Body mass index (BMI) for each individual was calculated.

Biochemical Analysis

From both RRMS patients and controls, 3 ml of peripheral venous blood and CSF samples were drawn within 48 hours of their hospital admission. The blood samples were centrifuged (3000 x g for 10 min), and serum was collected, transferred to Eppendorf tubes and stored at -80°C until analysis. CSF samples were obtained by lumbar puncture following a standardized protocol and then collected in 2 mL polypropylene tubes, and frozen at - 80°C until use. The time between collection and freezing of both CSF and serum samples was <30 min.

Measuremet of YKL-40

Serum and CSF YKL-40 levels of all participants were performed using commercial ELISA kit (Cat No:ab355786, Abcam Biological Reagents, England) according to the manufacturer's instructions. The mean intra-assay and inter-assay coefficients of variation were 6.7 % and 6.9 %, respectively. The sensitivity was calculated to be 15.6ng/ml.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables exhibiting a normal distribution are presented as mean ± standard deviation (SD), whereas categorical variables are expressed as percentages. Non-parametric variables were compared using the Mann-Whitney U test. Baseline characteristics of the two groups were compared using Student's t-test for continuous variables and the Chi-square test for categorical variables. The normality of variable distributions was assessed using the Kolmogorov-Smirnov test. Subsequently, Pearson's correlation analysis was employed to evaluate the association between serum and CSF YKL-40 levels. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline Characteristic

The demographic data, clinical characteristics and study parameters in with all participants were summarized in Table. No statistically significat differences were observed among the between patients with RRMS and controls including sex, age and body mass index (Table 1).

Table 1. Demographic data, clinic characteristics and study parametres of all the participants.

Variables	RRMS (n=30)	Controls (n=20)	p
Age (years)	29.2±5.5	30.7±3.6	0.416
Male, n	12	8	0.745
Female, n	18	12	0.673
BMI (kg/m²)	24.9±4.1	26.1±3.6	0.482
Clinical presentation			
Sensory	13	none	-
Optic neuritis	7	none	-
Brainstem	3	none	-
Spinal	3	none	-
Others	4	none	-
Serum YKL-40(ng/ml)	51.2±12.1	46.8±8.2	0.102
CSF YKL-40 (ng/ml)	168.8±28.3	70.1±14.3	p<0.001

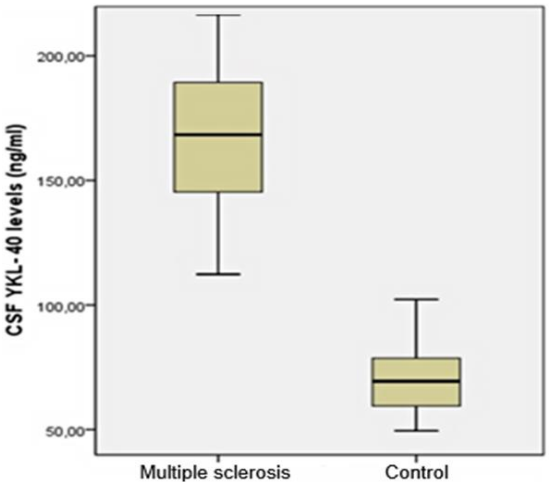

Fig 1. CSF YKL-40 levels in patients with multiple sclerosis and controls (p<0.001).

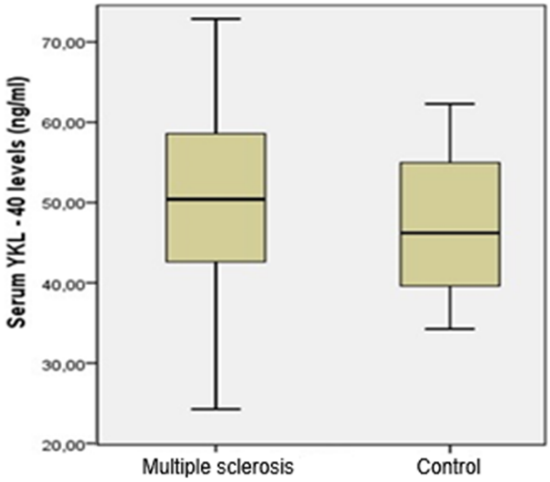

Fig 2. Serum YKL-40 levels in patients with multiple sclerosis and controls (p=0.102).

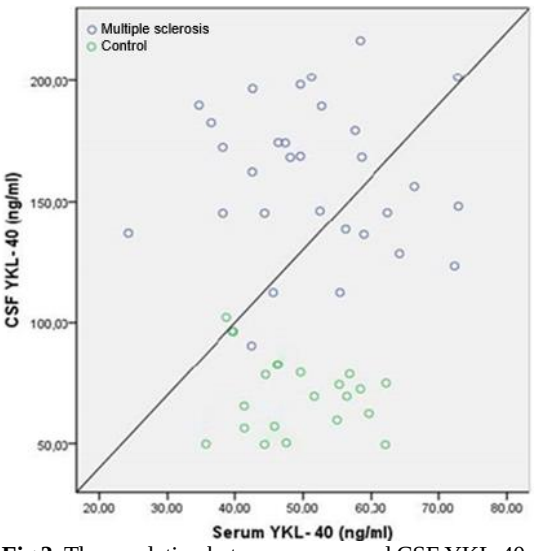

Fig 3. The corelation between serum and CSF YKL-40 activity in patients with multiple sclerosis (r=0.134 p=0.308).

CSF and Serum YKL-40 Levels in All Participants

Cerebrospinal fluid (CSF) YKL-40 levels were significantly elevated in RRMS patients compared to controls. Specifically, CSF YKL-40 levels measured 168.8±28.3 ng/mL in the MS group and 70.1±14.3 ng/mL in the control group (p<0.001) (Figure 1).

Conversely, no statistically significant difference in serum YKL-40 levels was observed between the two groups. Serum YKL-40 concentrations were 51.2±12.1 ng/mL in RRMS patients and 46.8±8.2 ng/mL in controls (p=0.102) (Figure 2). Furthermore, no correlation was found between serum and CSF YKL-40 activity within the RRMS patient cohort (r=0.134, p=0.308) (Figure 3).

DISCUSSION

Results from the present investigation have shown that CSF levels of the glial marker YKL-40 are elevated in patients with newly diagnosed RRMS patients. This observed elevation in CSF YKL-40 concentrations among RRMS patients serves as evidence for intense inflammatory processes initiating even during the early stages of the disease. However, no significant differences in serum YKL-40 levels were detected between RRMS patients and controls.

The growing importance of biomarkers for the early diagnosis of MS estimation of disease activity, and monitoring of treatment response in MS patients is increasingly recognized. Numerous studies have measured YKL-40 concentrations in both cerebrospinal fluid (CSF) and serum of patients with MS. Although no significant difference has been observed among MS subtypes, it has been concluded that YKL-40 may serve as a biomarker for the early diagnosis of MS and the prediction of disability progression. For example, Comabella et al. and Lopez-Gomez et al.[11,12] determined that the YKL-40 levels were high in CSF in the first period of MS. These authors also suggested that YKL-40 could be used as a prognostic marker for disability among patients who would progress to clinically definite MS. Tamam et al.[13] reported that measurements of CSF, HoxB3 (Homeobox protein Hox-B3), and YKL-40 exhibited high sensitivity in predicting CIS to MS conversion. However, a universally accepted clinical, radiological, or

biomarker capable of predicting transitions between distinct MS clinical forms (e.g., RRMS to SPMS) remains to be established. A recent literature review by Andre Bastos et al.[14] suggested that glial markers, such as glial fibrillary acidic protein (GFAP) and CHI3L1, are more effective than neurofilament light chain (NFL) in identifying progression to secondary progressive multiple sclerosis (SPMS). A meta-analysis conducted by Floro et al.[15] between January 2010 and December 2020, encompassing 673 MS patients and 336 healthy controls, revealed significantly higher CSF YKL-40 levels in the MS group. The authors concluded a strong correlation between CSF YKL-40 levels and MS disease progression. While evidence supports a robust association between early diagnosis and disease activity in MS patients, as well as CSF glial activation markers, this relationship remains unclear with respect to serum glial activation markers. For example, a single-center, prospective study by Kusnievora et al.,[16] evaluating the relationship between serum NFL and YKL-40 with treatment response and disease activity in 57 MS patients, concluded that serum NFL, but not YKL-40, may serve as a potential biomarker for monitoring treatment response and confirming clinical recurrence in MS. Consistent with these findings, our study also demonstrated no significant difference in serum YKL-40 levels between MS patients and controls.

The present study focused on newly diagnosed, treatment-naïve, young adult MS patients. This selection criterion was important for two reasons. First, the exclusion of patients with late-stage or advanced-age RRMS mitigated potential confounding effects. It was hypothesized that older patients and those with more advanced disease might exhibit baseline elevations in serum YKL-40 due to age-related or disease-related inflammation. Second, pre-treatment YKL-40 measurement eliminated the influence of corticosteroids, immunomodulators, and monoclonal antibodies. Prior studies have demonstrated that corticosteroids and immunomodulatory therapies (e.g., glatiramer acetate, interferon beta-1a and 1b) commonly used in RRMS treatment can modulate the inflammatory response.[17] In addition, changes were observed in serum and CSF biomarker levels after natalizumab and cladribine treatments.[18,19]

This study is subject to several limitations. First, the relatively small sample size and the ethically motivated use of a non-healthy control group should be acknowledged. Second, the study's focus on CSF YKL-40 levels without comparison to other established neuroinflammatory glial markers, such as NFL and GFAP, limits the ability to clarify its role in early diagnosis. Third, serum and CSF YKL-40 levels were assessed only at admission, precluding an analysis of longitudinal changes and their correlation with MS disease progression.

Finally, In this study, elevated CSF YKL-40 levels suggest the presence of heightened glial activity (astrocyte-microglia interactions) in early MS. Future studies incorporating larger, more diverse patient cohorts across various racial and ethnic backgrounds are warranted to further elucidate the role of YKL-40 in MS pathogenesis.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

Ethics Committee Permission

This study has been approved by the Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (dated September 7, 2012, and numbered 2012/126).

Authors' Contributions

Concept/Design: SK, AK, MCC. Data Collection and/or Processing: SK, AK, MCC. Data analysis and interpretation: SK, MCC, AK. Literature Search: SK, AK, MCC. Drafting manuscript: SK, AK. Critical revision of manuscript: SK, AK, MCC.

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