

CHI3L1 and CXCL13 Levels of Serum and CSF in Treatment-Naïve Multiple Sclerosis: Associations with Disease Activity

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ABSTRACT

Introduction: Cytokines, intracellular polypeptides, and chemokines, which belong to a small protein superfamily, are crucial in regulating inflammation and cell migration. Profiling these molecules can offer significant insights into the inflammatory process and aid in predicting disease outcomes. This study seeks to quantify cytokine and chemokine levels in serum and cerebrospinal fluid (CSF) samples from treatment-naïve patients, both with and without poor prognostic indicators, to identify biomarkers indicative of disease activity.

Methods: A prospective, observational, cross-sectional study was carried out involving patients diagnosed with Multiple sclerosis (MS) or non-inflammatory neurological disorders at Marmara University Faculty of Medicine. Multiple sclerosis patients were further classified into two groups based on previously established clinical and radiological criteria: those with poor prognostic outcomes and those without. Levels of IL-8, IL-12/IL-23p40, IL-21, CHI3L1, and CXCL13 were measured in both CSF and serum samples using the ELISA method. Statistical analyses were conducted using Stata version 15.1.

Results: A total of 56 patients participated in the study. CHI3L1 ($p=0.003$) and CXCL13 ($p<0.001$) levels in CSF were significantly elevated in MS patients compared to the control group. Among the MS cohort, serum CXCL13 levels positively correlated with the EDSS score ($p=0.04$, $r=0.39$) and the number of spinal cord lesions ($p=0.009$, $r=0.48$). Additionally, a significant negative correlation was observed between CSF CXCL13 levels and patient age in the MS group ($p=0.03$, $r=-0.42$).

Conclusion: Increased CSF levels of CHI3L1 and CXCL13 may serve as potential diagnostic markers for Relapsing-Remitting MS (RRMS) patients. Given the ease of collecting serum samples, further research is necessary to explore the relationship between serum CXCL13 levels, EDSS scores, and spinal cord lesions in larger cohorts.

Keywords: Biomarker, cerebrospinal fluid, chemokines, cytokines, multiple sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is characterized by immune-mediated inflammation triggered by autoreactive lymphocytes (1,2). Although prognostic data are recommended for MS patients, our current understanding lacks definitive parameters to predict the disease's progression in individual patients (3).

Cytokines, intracellular polypeptides, play an active role in inflammation, while chemokines, a small protein superfamily, are crucial in directing various cells to inflammatory sites (4,5). Profiling these molecules can provide valuable insights into the underlying pathophysiological mechanisms of MS and potentially identify biomarkers that reflect disease activity.

This study aims to identify potential biomarkers indicative of disease activity by evaluating cytokine and chemokine levels in serum and cerebrospinal fluid (CSF) samples from treatment-naïve MS patients, categorized based on their prognostic expectations. We specifically targeted the following molecules, which are known to play significant roles in the pathophysiology of MS: IL-8, a proinflammatory cytokine

Highlights

- CSF levels of CHI3L1 and CXCL13 in the MS group are higher than the control group.
- Spinal cord lesions number was positively correlated with serum CXCL13 levels.
- A positive correlation was found between EDSS and serum CXCL13 levels.
- There is a negative correlation between CSF CXCL13 levels and age of patients.

and marker of oxidative stress; IL-12/IL-23p40, a chemoattractant for myeloid cells; IL-21, a regulator of B and CD4+ T cell proliferation and survival; CHI3L1, a marker of microglial activation; and CXCL13, a potent chemoattractant involved in recruiting B cells to the central nervous system (6–10).

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METHODS

Patients

Patients presenting to the neurology outpatient clinic at Marmara University Faculty of Medicine were selected for inclusion. Adult participants were eligible if they had been diagnosed with Multiple Sclerosis (MS) based on the McDonald 2017 criteria or with non-inflammatory neurological conditions where lumbar puncture was indicated for diagnostic or therapeutic purposes. All patients provided informed consent by signing the consent form.

Exclusion criteria include the use or prior use of disease-modifying treatments for MS, experiencing an acute MS relapse or receiving treatment for one in the past 30 days, presence of a Gadolinium-enhancing lesion on MRI, any inflammatory condition other than MS, nonspecific white matter lesions on MRI, or any condition that precluded lumbar puncture. Treatment-naïve MS patients were divided into two groups: those with poor prognostic expectations (PE) and those without (NPE). Patients were assigned to the PE group if they met any of the following criteria:

- Late disease onset (11,12),
- Multifocal relapses involving motor, cerebellar, or sphincter functions (11,13),
- Reaching an EDSS score of 4.0 within 5 years of disease onset (14),
- Presence of new or expanding lesions on T2-weighted images, or an active lesion with Gadolinium enhancement on T1-weighted images (15),
- Presence of black holes or atrophy on T1-weighted images (15),
- Presence of spinal cord or brainstem lesions on MRI (16).

Both clinical and radiological evaluations were performed. Patients were asked to provide demographic information, background and family medical history, the date of MS diagnosis, the systems affected during past demyelinating relapses, the treatments they received, and whether any poor prognostic indicators were present. Neurological examinations were repeated, and disability was assessed using the EDSS. Magnetic resonance imaging (MRI) scans taken within one year prior to the study or within six months after the lumbar puncture (LP) were reviewed. The number of supratentorial, periventricular, juxtacortical, infratentorial, and spinal lesions on MRI were recorded for each participant.

Serum and Cerebrospinal Fluid Collection and Analysis

Serum samples and atraumatic lumbar punctures (LP) were performed simultaneously after obtaining informed consent from patients. Paired serum and CSF samples were analyzed for cell count, total protein, albumin levels, CSF/serum albumin ratio (QAIB), CSF/serum IgG ratio, and intrathecal IgG synthesis (IgG Index). Albumin levels in both serum and CSF were measured automatically using the Beckman Coulter AU5800 system. Oligoclonal bands (OCBs) were identified by isoelectric focusing. If the QAIB exceeded the normal value for the patient's age, blood-brain barrier dysfunction was suspected, and such patients were excluded from the study (17). The collection, storage, and processing of serum and CSF samples followed the biobank standardization recommendations by Teunissen et al. (18).

Levels of IL-8 (Thermo Fisher, Cat. No. BMS204-3), IL-21 (Thermo Fisher, Cat. No. BMS2043), IL-12/IL-23p40 (Thermo Fisher, Cat. No. BMS2013), CHI3L1 (R&D Systems, Cat. No. DC3L10), and CXCL13 (Thermo Fisher, Cat. No. EHCXCL13) were measured using the ELISA method, following the manufacturers' instructions. IL-8, IL-21, and IL-12/IL-23p40 were measured in 1:2 diluted serum and native CSF, CHI3L1 was measured in 1:50 diluted serum and CSF, and CXCL13 was measured in 1:2 diluted serum and CSF.

Statistical Analysis

Statistical analyses were conducted using Stata version 15.1 (StataCorp, College Station, TX). Normality of the data was assessed using the Kolmogorov-Smirnov test. For descriptive analysis of numeric variables, median values and interquartile ranges (IQR) were reported, while categorical variables were expressed as percentages. Categorical data were analyzed using the Fisher's Exact Probability Test. For continuous variables, the Mann-Whitney U test was employed for comparisons between two independent groups, while the Kruskal-Wallis test was used for more than two independent groups. The Wilcoxon signed-rank test was applied to evaluate continuous data in dependent groups. Pearson and Spearman's rank correlation coefficients were calculated to assess relationships between two continuous variables. A p-value of less than 0.05 was considered statistically significant.

The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee on 07.12.2018 under protocol code 09.2018.819.

RESULTS

Demographic Data, Clinical and Radiological Characteristics

A total of 67 patients were screened and assessed for the study. Among them, 27 patients were included in the control group, which consisted of individuals with non-inflammatory neurological conditions, all of whom experienced headaches. In total, the study comprised 27 control patients and 29 MS patients, of whom 21 were classified as having poor prognostic expectations (PE) and 8 without poor prognostic expectations (NPE).

Females accounted for 62.1% (n=18) of the MS group and 92.6% (n=25) of the control group. A summary of demographic, clinical, and radiological characteristics is presented in Table 1. Across the 29 MS patients, a total of 36 previous demyelinating relapses were documented. There was no significant difference in the number of prior relapses between the PE and NPE groups (p=0.22). However, EDSS scores were significantly higher in the PE group compared to the NPE group (p=0.04). No notable differences were observed in functional system scores between the groups. In both groups, pyramidal system scores ranged from 0 to 1 (p=0.27). While all other functional system scores were 0 in the NPE group, cerebellar and brainstem scores in the PE group ranged from 0 to 3 (p=0.14, p=0.27 respectively).

MRI data were available for 28 MS patients, with a median interval of 1.5 months between the lumbar puncture (LP) and MRI. One patient's MRI was missing, so they were excluded from the MRI-related analysis. More than three periventricular lesions were identified in 93% of MS patients, while 50% had over nine lesions in the supratentorial region. There was no significant difference between the PE and NPE groups regarding lesion burden in the supratentorial, periventricular, juxtacortical, and infratentorial regions.

Serum and CSF Analysis

No significant differences were observed between the groups in terms of CSF total protein, albumin levels, or cell count (Table 2). The QAIB values calculated for each participant remained within the age-adjusted reference range. The median oligoclonal band (OCB) type for both PE and NPE groups was Type 2, while the median IgG index values were 0.71 (Range: 0.50–2.04) for the PE group and 0.61 (Range: 0.47–1.75) for the NPE group.

When comparing serum and CSF levels of all the cytokines and chemokines measured across participants, no significant differences were found between serum and CSF levels.

Table 1. Demographic data, clinical and radiological characteristics

	Control	PE	NPE	p
Female, n (%)	25 (93)	13 (62)	5 (63)	0.02
Male, n (%)	2 (7)	8 (38)	3 (37)	
Age, median (range)	37.2 (21.9 – 53.1)	36.4 (22.9 – 63.1)	33 (22–47.3)	0.60
Number of previous relapses, median (range)	NA	1 (0–4)	1 (0–4)	0.22
EDSS, median (range)	NA	1 (0–3.5)	0 (0–1)	0.04
Supratentorial 3–8 lesions, n (%)	NA	9 (45)	5 (62)	0.23
Supratentorial 9+ lesions, n (%)	NA	11 (55)	3 (38)	0.4
Spinal cord lesion number, median (range)	NA	1 (1–6)	0 (0–0)	<0.001

n: number; NA: not applicable; NPE: no poor prognostic expectation; PE: poor prognostic expectation.

Table 2. Initial CSF analysis

	Control, median (IQR)	PE, median (IQR)	NPE, median (IQR)	p
CSF protein (mg/dL)	34 (10.8)	38.8 (15.6)	36.5 (7.4)	0.05
CSF albumin (mg/L)	121.6 (64.8)	145.7 (59.4)	145.6 (50)	0.07
CSF cell count	0 (2)	0 (8)	0 (0)	0.09
OCB	-	2	2	0.22
OCB Tip 2, n (%)	-	15 (71)	5 (63)	-
IgG Index >0.6, n (%)	-	17 (80)	4 (50)	-

CSF: cerebrospinal fluid; IQR: interquartile range; OCB: oligoclonal bands; NPE: no poor prognostic expectation; PE: poor prognostic expectation.

Table 3. Serum and CSF cytokine and chemokine levels

	Control, median (IQR)	MS, median (IQR)	p	PE, median (IQR)	NPE, median (IQR)	p
Serum CHI3L1 (ng/ml)	44.4 (41.2)	40.1 (26.8)	0.11	38.1 (24.3)	52.4 (36.1)	0.27
Serum CXCL13 (pg/ml)	93.5 (80.5)	48.5 (92.8)	0.13	75.2 (108.4)	36.4 (49.2)	0.07
CSF CHI3L1 (ng/ml)	73.1 (62.1)	107.7 (78.0)	0.003	120.1 (94)	106.0 (62.5)	0.009
CSF CXCL13 (pg/ml)	4.6 (2.5)	26 (63.8)	<0.001	26 (79.8)	27.8 (47.9)	<0.001

CHI3L1: protein chitinase 3-like 1; CSF: cerebrospinal fluid; CXCL13: C-X-C motif ligand 13; IQR: interquartile range; NPE: no poor prognostic expectation; PE: poor prognostic expectation.

In the majority of serum samples, interleukin levels were below the minimum detection limit. Specifically, IL-8 was undetectable in 94.7% (n=54) of cases, IL-12/IL-23p40 in 38.6% (n=22), and IL-21 in 84.2% (n=48) of all serum samples. For CSF samples, IL-8 was below the detection threshold in 8.8% (n=5), IL-12/IL-23p40 in 87.8% (n=50), and IL-21 in 22.8% (n=13) of cases. The opposite was found for CHI3L1, where levels exceeded the measurable limit in 1 serum sample (1.8%) and 6 CSF samples (10.5%). No values outside measurable limits were detected for CXCL13 in either serum or CSF samples.

Analyses were performed for both the MS and control groups. Cerebrospinal fluid levels of CHI3L1 and CXCL13 were significantly higher in the MS group compared to the control group, whereas no significant differences were observed in serum levels (Table 3). Cytokine and chemokine levels in serum and CSF were also compared among the three groups (Table 3). Cerebrospinal fluid levels of CHI3L1 and CXCL13 were significantly elevated in both the PE and NPE groups compared to controls (p=0.009, p<0.001).

In subgroup analyses, the CSF CHI3L1 level was significantly lower in the control group compared to the PE group (p=0.001). Although the median CSF CHI3L1 level in control patients appeared lower than that of the NPE group, this difference was not statistically significant (p=0.3). The CSF CXCL13 level was significantly lower in the control group compared to the NPE group (p=0.007) (Figure 1). Additionally, the CSF CXCL13 level in

the PE group was significantly higher than in the control group (p<0.001) (Figure 1). No statistically significant differences were found in any serum or CSF parameters between MS patients with and without poor prognostic expectations.

Correlation Analysis

First, the correlation between demographic data and serum and CSF parameters was analyzed. In MS patients, a statistically significant but weak negative correlation was observed between CSF CXCL13 levels and age (p=0.03, r=-0.42). No correlations were found between age and serum CHI3L1 levels (p=0.13), CSF CHI3L1 levels (p=0.45), or serum CXCL13 levels (p=0.36).

A weak positive correlation was identified between serum CXCL13 levels and disability scores for all MS patients (p=0.04, r=0.39) (Figure 2). However, no similar correlation was found between EDSS and CSF CXCL13 levels (p=0.98).

No significant results were observed when comparing serum and CSF chemokine levels with individual poor prognostic indicators or the presence of multiple poor prognostic factors. Among the MS patients, 21 (75%) were identified as having spinal cord lesions, and a moderate positive correlation was found between the number of spinal cord lesions and serum CXCL13 levels (p=0.009, r=0.48) (Figure 3). No correlation was found between the number of spinal cord lesions and serum

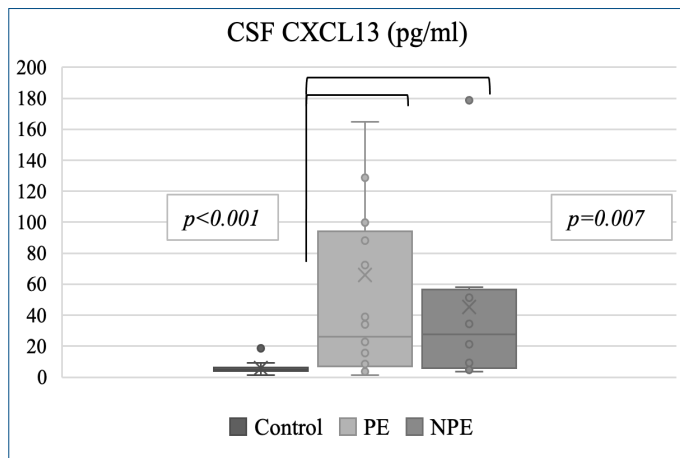


Figure 1. Comparison of CSF CXCL13 levels of control, PE and NPE groups (CSF: cerebrospinal fluid; CXCL13: C-X-C motif ligand 13; NPE: no poor prognostic expectation; PE: poor prognostic expectation).

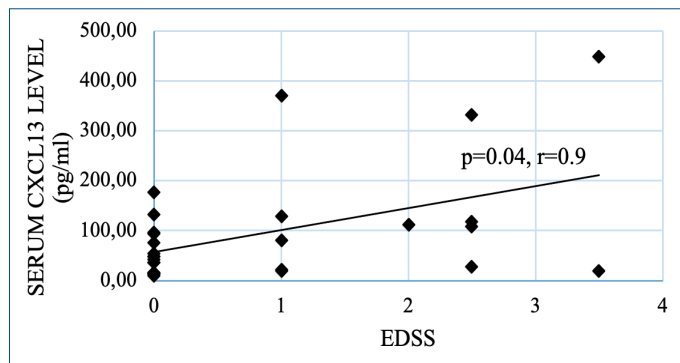


Figure 2. The correlation between EDSS scores of MS patients and serum CXCL13 levels (CXCL13: C-X-C motif ligand 13; EDSS: Expanded disability status scale).

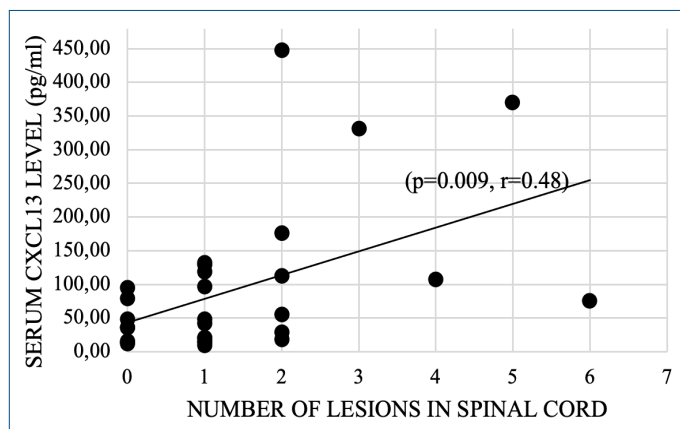


Figure 3. Correlation between the number of spinal cord lesions and serum CXCL13 in MS patients (CXCL13: C-X-C motif ligand 13).

CHI3L1 ($p=0.97$), CSF CHI3L1 ($p=0.14$), or CSF CXCL13 levels ($p=0.67$). Additionally, no significant relationships were detected between any cranial MRI parameters and serum or CSF cytokine and chemokine levels.

Lastly, routine serum and CSF parameters were evaluated in relation to serum and CSF cytokine and chemokine levels. A moderate positive correlation was found between the IgG index and CSF CXCL13 levels in MS patients ($p=0.02$, $r=0.48$).

DISCUSSION

CHI3L1 (Chitinase-3-like protein 1, also known as YKL-40) is secreted by microglia, macrophages, epithelial cells, and astrocytes, and is involved in the pathogenesis of chronic autoimmune diseases (6). It is a glycosidase molecule and a marker of glial activation, released by microglia, monocytes, activated astrocytes, and vascular smooth muscle cells (6), (19). While its specific role in the CNS remains unclear, it is believed to contribute to the regulation of astrocyte responses in controlling CNS inflammation (20).

In our study, CSF CHI3L1 levels were significantly lower in the control group compared to the MS group, which is consistent with previous findings. A study involving 59 RRMS patients and 39 healthy controls also found that CSF CHI3L1 levels were elevated in MS patients, regardless of whether they were experiencing a relapse (21). Additionally, that study reported a correlation between CHI3L1 levels, clinical relapse, and EDSS scores (21). Another follow-up study examining the short-term course of MS noted a slight increase in CSF CHI3L1 levels with age and increasing disability but found no correlation between CHI3L1 levels and the number of relapses either in the past year or over the following two years. Moreover, no association was observed with progression to the secondary progressive phase at the end of the follow-up period (22). In our study, although CSF CHI3L1 levels were significantly higher in MS patients compared to the control group, no significant correlation was found between CHI3L1 levels and disability scores. This lack of association may be due to the fact that all MS patients in our study were newly diagnosed and had relatively low levels of disability.

In a multicenter study by Canto et al., it was found that patients who progressed to clinically definite MS had higher CSF CHI3L1 levels compared to those who remained with CIS and those with non-inflammatory neurological diseases (19). Additionally, patients with CSF CHI3L1 levels above 170 ng/ml reached an EDSS score of 3.0 more quickly than those with lower levels (19). However, a study investigating the role of CSF CHI3L1 levels in the conversion of RIS to RRMS yielded conflicting results (23). In that study, when regression analysis was conducted with variables such as spinal cord lesions, CSF CHI3L1 levels, and the presence of four Barkhof criteria, only spinal cord lesions were identified as a risk factor for the conversion from RIS to RRMS, while CSF CHI3L1 levels were not found to be an independent risk factor (23). In our study, although there appeared to be differences in CSF CHI3L1 levels between the PE and NPE groups, this difference was not statistically significant. Key poor prognostic factors in our study included late onset, multifocal relapses, and lesions in the spinal cord or brainstem—factors that are also associated with the conversion from CIS to RRMS and RIS to RRMS. Based on these, we expected to observe differences in CSF CHI3L1 levels between the PE and NPE groups. However, the lack of significant findings may be due to the small sample size. Furthermore, as noted by Thouvenot et al., CHI3L1 may not serve as a standalone prognostic marker and could become more meaningful when evaluated in conjunction with stronger prognostic indicators, such as spinal cord lesions.

In the Canto et al. study, the number of lesions on T2-weighted images and contrast-enhancing lesions on T1-weighted images were associated with CSF CHI3L1 levels (19). In our study, no MS patients with clinical or radiological relapses were included, and no correlation was found between CSF CHI3L1 levels and lesion location or number. We believe that this lack of correlation may be attributed to both the small sample size and the high supratentorial lesion burden present in most of our MS patients.

C-X-C Motif Ligand 13 (CXCL13) is a potent chemoattractant that signals the recruitment of B cells to the CNS, facilitating the formation of B cell follicles and secondary lymphoid tissues by interacting with the CXCR5 receptor on B lymphocytes (7). CXCL13 originates from ectopic

lymphoid follicles and is believed to play a key role in the development and maintenance of these ectopic lymphoid tissues (24,25). Studies have demonstrated CXCL13 production in MS lesions with active demyelination, where it has been detected in perivascular infiltrates and cells within the parenchymal lesion areas. It has also been reported that CSF CXCL13 levels are closely correlated with intrathecal immunoglobulin production (26).

In our study, we observed significantly higher CSF CXCL13 levels in the MS group, including both PE and NPE groups, compared to controls. These findings are consistent with previous studies. One study indicated that MS patients had elevated CSF CXCL13 levels but found no correlation between these levels and disease duration, EDSS, or differences between RRMS, PPMS, and control groups in serum CXCL13 levels (27). Ferraro et al. further found that higher baseline CSF CXCL13 levels in CIS patients were associated with a greater likelihood of conversion to RRMS, with a positive correlation noted between CXCL13 and the IgG index (28). However, no correlation was observed between CSF CXCL13 levels and MRI data or disability scores (28). Our findings also confirm higher CSF CXCL13 levels in MS patients compared to controls and a positive correlation with the IgG index, but we found no association between CSF CXCL13 levels and lesion localization or burden. Similar to Ferraro et al., we observed no significant correlation between clinical disability scores and CSF CXCL13 levels.

Khademi et al. recorded the highest CSF CXCL13 levels in patients with viral and bacterial CNS infections, and they found that CXCL13 levels were elevated in all MS groups during relapses compared to remission periods (29). In RRMS patients, those with the highest CSF CXCL13 levels had more frequent relapses and a greater number of brain lesions (29). Additionally, CXCL13 levels were significantly higher in patients who converted from CIS to RRMS, making it a potential prognostic marker for conversion (29). In our study, we grouped clinically definite MS patients based on their prognostic expectations but found no significant difference between the PE and NPE groups. This could be attributed to a reduced prognostic value of CSF CXCL13 in established disease or the fact that CXCL13 is more predictive during periods of active inflammation. Alternatively, the cross-sectional design of our study and the relatively low levels of chronic inflammation in our cohort may have influenced these results.

The most intriguing findings of our study relate to serum CXCL13 levels. When comparing the control and MS groups, no statistically significant difference was observed. This aligns with other studies, none of which reported significant differences in serum CXCL13 levels between MS patients and controls. Since CSF is in closer proximity to the CNS, previous research has primarily focused on evaluating biomarkers in CSF for demyelinating diseases. However, obtaining serum is a simpler and less invasive procedure. In our study, serum CXCL13 levels in MS patients were detectable and comparable to those in CSF ($p=0.84$). Although previous studies have reported correlations between CSF CXCL13 levels and EDSS scores, no data on serum levels were available (30). In our research, we found that EDSS scores increased in parallel with rising serum CXCL13 levels in MS patients.

Earlier studies emphasized brain lesion counts but did not consider spinal cord lesion numbers. In our study, a positive correlation was found between the number of spinal cord lesions and serum CXCL13 levels. Given the well-documented negative impact of spinal cord lesions on disability, the correlation between serum CXCL13 levels, EDSS scores, and spinal cord lesions does not appear to be coincidental. It will be important to validate these findings in larger sample groups (31). If confirmed in larger studies involving more patients and healthy controls, serum CXCL13 could emerge as a biomarker with the potential to reflect disease activity.

Our study had a prospective design with a cross-sectional examination, focusing on naive and newly diagnosed MS patients to avoid interference from immunomodulatory treatments. Previous studies on MS prognosis have primarily examined CIS or RIS conversion to RRMS, suggesting that different results might emerge when compared to cohort studies. Our study aimed to provide descriptive results by analyzing demographic, clinical, and radiological data, along with serum and CSF cytokine and chemokine levels, which is a key strength of our research as it allows for comprehensive insights. However, the small number of participants, a limitation caused by the Covid-19 pandemic, should be noted.

As a result, cerebrospinal fluid levels of CHI3L1 and CXCL13 were significantly elevated in MS patients, highlighting their potential as biomarkers for MS. Moreover, the observed correlation between serum CXCL13 levels, clinical disability scores, and spinal cord lesions, combined with the ease of serum sampling, underscores the importance of further evaluating these associations in larger patient and control populations.

Ethics Committee Approval: This study was approved by Marmara University, School of Medicine's Clinical Research Ethics Committee on 07.12.2018 with the protocol code of 09.2018.819. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- EV, KA; Design- EV, AY, GS, GH; Supervision- GS, KA, DG, GH; Resource- KA, GS, GH, DG; Materials- EV, AY; Data Collection and/or Processing- EV, AY, CI; Analysis and/or Interpretation- EV, CI; Literature Search- EV; Writing- EV; Critical Reviews- GS, KA.

Conflict of Interest: The authors declared that there is no conflict of interest.

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