ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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The Predictive Role of CXCL12 (SDF-1 alpha) in Multiple Sclerosis

Multipl Sklerozda CXCL12'nin (SDF-1 alfa) Prediktif Rolü

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ABSTRACT Objective: Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease characterized by multifocal inflammation, progressive myelin loss and consequent axonal demyelination. Pathogenic mechanisms of MS are oligodendrocyte damage as a result of leukocyte chemotaxis to central nervous system and production of inflammatory mediators, demyelination and neuronal injury. CXCL12 (SDF-1 alpha) is a basic chemokine which is chemoattractant for resting and activated T cells. We aimed to evaluate whether CXCL12 levels may be a predictive marker of neuroinflammation in MS patients. **Material and Methods:** We designed a prospective case control study of fifty-four MS patients and twenty-eight healthy controls were enrolled to the study. Plasma CXCL12 levels of the groups were measured in blood samples. The groups were compared in terms of plasma CXCL12 levels. Lymphocyte count, which is a marker of inflammation, was also compared. In the study group subsequent analysis was demonstrated the association between clinical parameters and plasma CXCL12 levels. **Results:** CXCL12 levels were statistically higher than the control group. Mean CXCL12 level of MS patients was 2026,5±398,7 pg/ml and the mean CXCL12 level of the controls was 1840,6±256,0 pg/ml. Lymphocyte count of the patients was also statistically higher in the study group. According to the receiver operator characteristic (ROC) curve analysis mean CXCL12 levels were discriminative factors in patients in the study group. There was no statistically significant association between plasma CXCL12 levels and clinical parameters. **Conclusion:** We conclude that elevations in CXCL12 levels might be a promising marker pointing out the pathogenic role of inflammation in multiple sclerosis.

Keywords: Multiple sclerosis; CXCL12; lymphocyte count; inflammation

ÖZET Amaç: Multipl Skleroz (MS) multifokal inflamasyon, ilerleyici miyelin yıkımı ve sonunda akson demiyelinizasyonu ile karakterize inflamatuar, otoimmun, demiyelinizan bir hastalıktır. MS'in patogenetik mekanizmaları, merkezi sinir sistemine (MSS) lökosit kemotaksisi ve inflamatuar mediatörlerin üretimi sonucu oluşan oligodentrosit hasarı, demiyelinizasyon ve nöronal yaralanmadır. CXCL12 (SDF-1 alfa), aktive edilmiş olan ve olmayan T hücreleri için kemoatraktan olan bir temel kemokindir. Çalışmamızda CXCL12 düzeylerinin MS hastalarında nöroinflamasyonun prediktif belirleyicisi olup olmayacağını değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** Elli dört MS hastası ve 28 sağlıklı kontrol ile prospektif vaka kontrol çalışması yaptık. CXCL 12 düzeyleri, kan örneklerinde ölçülerek gruplar arasında karşılaştırıldı. Lenfosit sayısı inflamasyonun bir belirteci olarak gruplar arasında karşılaştırıldı. Çalışma grubunda klinik parametreler ile plazma CXCL12 seviyeleri arasındaki ilişki istatistiksel analizlerle gösterildi. **Bulgular:** MS hastalarının CXCL12 düzeyleri kontrol grubuna göre istatistiksel olarak daha yüksekti. MS hastalarının ortalama CXCL12 seviyesi 2026,5±398,7 pg/ml idi ve kontrollerin ortalama CXCL12 düzeyi 1840,6±256,0 pg/ml idi. Hastaların lenfosit sayıları da çalışma grubunda istatistiksel olarak daha yüksekti. ROC eğrisi analizine göre, CXCL12 seviyeleri çalışma grubundaki hastalarda ayırtedici faktörlerdi. Plazma CXCL12 düzeyleri ile klinik parametreler arasında istatistiksel olarak anlamlı bir ilişki yoktu. **Sonuç:** CXCL12 düzeylerindeki yükselmeler, multipl sklerozda inflamasyonun patojenik rolünü belirtmede umut vericidir.

Anahtar Kelimeler: Multipl skleroz; CXCL12; lenfosit sayısı; inflamasyon

Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease characterized by multifocal inflammation, progressive myelin loss and consequent axonal demyelination.[1,2] Pathogenic mechanisms of MS are oligodendrocyte damage as a result of leukocyte chemotaxis to central nervous system and production of inflammatory mediators, demyelination and neuronal injury.[2] Not only T lymphocytes, but also activated macrophages, reactive astrocytes and microglial infiltration involve in pathogenesis of MS lesions. Infiltration of MS lesions, T lymphocyte and macrophage accumulation occur under the control of cytokines and chemokines.[3,4] CXCL12 (SDF-1 alpha) is a basic chemokine which is chemoattractant for resting and activated T cells.[5] It is mainly produced by bone marrow supernatant but is expressed by most of the cells.[6] CXCL12 is expressed in the central nervous system (CNS) and is thought to play an important role in T cell trafficking. Although MS is accepted as a T-cell mediated otoimmune disease, the role of CXCL12 on lesion formation and neuroinflammation could not be determined.[1]

In current study we aimed to investigate whether CXCL12 levels can be a predictor of MS activity at early stages.

MATERIAL AND METHODS

Fifty-four (12 male, 42 female; study group) MS patients who admitted to Ankara Numune Training and Research Hospital MS outpatient clinic between March 2012 and July 2012 were involved in this study and compared with twenty eight non MS healthy controls (7 male, 21 female; control group). Ethical approval for the entire study was obtained from the Ethics committee of Ankara Numune Training and Research Hospital. This is a tertiary referral research and training hospital in Ankara, Turkey. Where mandated, written consent was obtained from all participants.

MS diagnosis was made according to revised Mc Donald's criteria.[7] Patients with autoimmune diseases, acute disseminated encephalomyelitis, chronic inflammatory diseases such as brucellosis, stroke were excluded from the study.

Clinical data recorded and evaluated were age and gender of the patients, hemoglobin and hematocrit levels, platelet count, fasting blood glucose levels, urea and creatinine levels, serum CRP levels, basofile, eosinofile and lymphocyte count. The MS type was defined as type 1 including Relapsing-Remitting Multiple Sclerosis (RRMS), Radiologic Isolated Syndrome (RIS) and Clinically Isolated Syndrome (KIS) and type 2 Primary Progressive Multiple Sclerosis, Secondary Progressive Multiple Sclerosis. The plaque localization was also recorded as periventricular, supratentorial, infratentorial and spinal.

In all cases, after an 8 hour fasting, blood samples were obtained into EDTA tubes and centrifuged to obtain plasma and stored at -80°C. Plasma CXCL12 levels were measured using CXCL12 ELISA kit (ELX808, Biotek Instruments, USA).

STATISTICAL ANALYSIS

SPSS for Windows, Version 15.0 was used. Numerical variables were reported as mean+standart deviation and median (minimum-maximum). Categorical variables were reported as numbers and percents. The normality of numerical variables were calculated by Shapiro Wilks Test. Differences between patient and control groups were calculated using T test in case of providing assumptions of parametric test and using Mann Whitney U test in case of not providing these assumptions. In means of categorical variables, chi square test was used to determine whether there is difference or not. In means of numerical variables, Kruskal Wallis test was used to determine whether there is difference or not between MS patient subgroups. Two-way comparisons were made with Mann Whitney U test with Bonferroni corrections. Correlation between numerical variables was determined with Spearman correlation coefficient. The receiver operator characteristic (ROC) curve analysis was used to establish the cut off values for serum CXCL 12 levels. $P<0.05$ was accepted as significant.

RESULTS

The demographic characteristics and laboratory results of the patients and control group are shown in (Table 1). There was no statistically significant difference for age and gender in patient group and healthy subjects. Mean age of the patient group was 35.8±10.0 years old and 39.1±9.2 years old in the control group.

The difference in CXCL12 levels was statistically significant between the study and control groups (mean CXCL12 level was 2026.5±398.7 pg/ml in the study group and 1840.6±256.0pg/ml in the control group, p=0.013). Lymphocyte count was higher in the patient group and this difference was statistically significant (p=0.002). C-reactive protein levels showed also statistically significant difference between the groups (p=0.031). When RRMS and secondary progressive MS were considered in terms of lymphocyte count, the difference was significant (0.039).

There was no statistically significant correlation between clinical parameters and plasma CXCL12 levels (Table 2). CXCL12 levels and attack duration and number of attack were analyzed using Spearman correlation test but the difference between groups was not statistically significant (0.0975). CXCL12 levels were not correlated with total attack number (p= 0.651). No difference was observed for CXCL12 levels between patients during attack period and patients outside attack period (p=0.959). CXCL12 levels were not correlated with

TABLE 1:. The demographic characteristics and laboratory results of the patients and control group.

	Patients with MS (n=54)	Patients without MS (n=28)	p
Age x	35.8±10.0	39.1±9.2	0.054
Gender			
Male	12	7	0.137
Female	42	21	
Hemoglobine (g⁄dl)	13.0±1.5	13.6±1.4	0.074
Hematocrite (%)	39.1±3.6	41.2±3.4	0.074
MCV (fl)	83.4±5.6	84.5±4.0	0.356
Platelet (10^3⁄µl)	248.1±43.0	268.8±59.9	0.076
WBC (10^3⁄µl)	7.0±2.7	7.9±1.8	0.089
Urea (mg⁄dl)	26.8±7.9	28.7±7.1	0.296
Creatinine (mg⁄dl)	0.72±0.18	0.75±0.18	0.551
CRP (mg⁄l)	1.59±2.30	2.67±2.98	0.031
Vit B12 (pg⁄ml)	398.8±305.2	321.2±125.0	0.945
Folate (ng⁄ml)	8.5±3.8	8.6±2.06	0.361
Basofile (10^3⁄µl)	2.12±0.87	4.88±3.71	<0.001
Eosinofile (10^3⁄µl)	0.30±0.80	0.12±0.07	0.534
Lymphocyte (10^3⁄µl)	2.16±3.10	2.42±0.76	0.002
Plasma CXCL12 (pg⁄ml)	2026.5±398.7	1840.6±256.0	0.029

MS: Multiple sclerosis; **CRP:** C-reactive protein; **Vit B12:** Vitamine B12.

TABLE 2: Correlation between plasma CXCL12 levels and demographic and clinical characteristics of the patients.

	Age	Gender	NOA	Attack duration	EDSS	MS type	CRP	Lymphocyte count
CXCL12	CC=0.014	CC=0.258	CC=0.082	CC=-0.201	CC=-0.330	CC=-0.072	CC=-0.126	CC=-0.133
	P=0.905	P<0.001	P=0.564	P=0.105	P=0.681	P=0.610	P=0.269	P=0.241

NOA: Number of Attack; **EDSS:** Expanded Disability Status Scale; **CRP:** C reactive protein.

EDSS scores (p=0.681). A ROC area under the curves (AUC) of serum CXCL12 levels and lymphocyte count is shown in Figure 1. The AUCs (95 % CI) for these parameter was shown in Table 3.

DISCUSSION

In current study, we evaluated the plasma CXCL12 levels in patients with MS and analyzed it's association between demographic and clinical parameters. The mean plasma CXCL12 levels and lymphocyte count was statistically significantly higher in MS patients. The correlation analysis showed no statistically significant association between plasma CXCL12 levels and demographic and clinical parameters. To best of our knowledge, this is the first study determining the predictive role and cut off values of plasma CXCL12 levels in MS patients. According to the ROC analysis, plasma CXCL12 levels at a cut off value of 1534.5 pg/ml was found to be promising marker in MS patients.

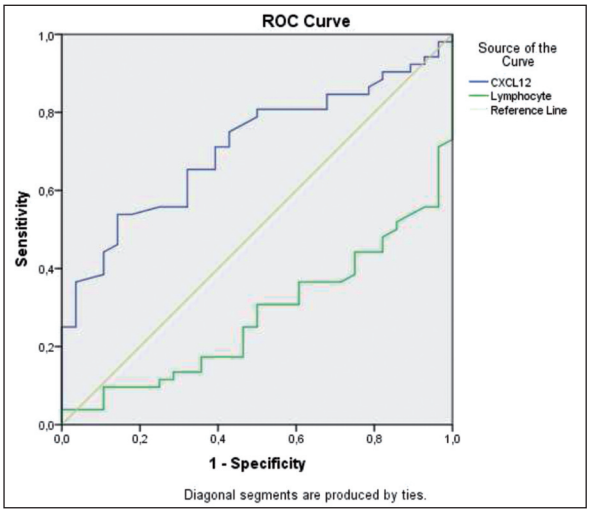

FIGURE 1: ROC analysis demonstrating the predictive power of plasma CXCL12 levels and lymphocyte count in MS patients.

MS is an inflammatory demyelinating disease where autoimmune mechanisms are considered to play role at the background and progresses with focal myelin destruction and to some extent with axonal loss in the central nervous system.[1] It is characterized by infiltration of autoreactive T cells to brain and spinal cord leading to demyelinization and axonal loss.[5] The major component causing the formation and dissemination of MS active plaques is transendothelial leukocyte migration caused by blood brain barrier dysfunction.

Blood brain barrier is a layer which surrounds the brain microvascular endothelial cells to isolate the central nervous system and to provide immunity.[3,8] As it is a semi-permeable membrane, it regulates the passage of ions, polar organic substances proteins and cells. Under certain circumstances, as a result of chemokine accumulation blood brain barrier is disrupted.[3]

CXCL 12, is a member of CXC chemokine family and is first described in bone marrow connective tissue as chemoattractant and C cell precursor. Via CXCR4 receptors, it regulates the passage of mononuclear cells to the lymphoid tissue and at the same time provides a support for the B and T cells to develop an effective immune response. It is expressed by many parenchymal tissues including brain; it is expressed in neurons and microglia and astrocytes.[9]

It is chemoattractant for monocytes, T lymphocytes, pre-B cells, hematopoietic precursor cells, endothelial cells and astrocytes.[10,11]

CXCL12 is expressed at lower levels in CNS, but it can take a role in MS pathogenesis by stimulating the traffic of autoreactive T cells towards the CNS. At this early stage, minority of the autoreactive T cells are considered to begin neuroinflam-

TABLE 3: The area under curve (AUC), cut of values and sensitivity and specificity for plasma CXCL12 levels and lymphocyte count

	AUC	SE	95 % CI	Cut of value	Sensitivity (%)-specificity (%)
CRP	.709	.057	.597-.822	1534.5	90.4-82.1
Lymphocyte count	.278	.056	.168-.367	-	-

AUC: Area Under Curve; **SE:** Standart Error; **CI:** Confidence Interval of Population Mean, **CRP:** C-reactive protein.

mation by secreting proinflammatory mediators including IL-1β. Besides this, CXCL 12 is known to be secreted by the astrocytes in MS lesions.[12]

In this study, serum CXCL 12 levels together with lymphocyte count were increased in the study group as compared to control group. Although flow cytometric study could not be performed, increased levels of CXCL 12 and lymphocyte count are significant in terms of showing the pathogenic role of systemic inflammation in MS patients.

Blood samples were taken both during the acute MS attack and remission period but the CXCL 12 levels were not significantly different which means inflammation continues in both periods. In literature, levels of CXCL 12 if cellular origin which is expressed in MS lesions can differ in normal and inflammated blood brain barrier.[13,14]

In conclusion, we think that plasma CXCL12 levels may lead to inflammation and have a role in MS pathogenesis. The limitation of our study is our low number of patients and control group. However, further studies with more participants and with randomized and controlled nature will strength this hypothesis.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Semra Mungan;* ***Design:*** *Semra Mungan, Neşe Öztekin;* ***Supervision/Consultancy:*** *Semra Mungan;* ***Data Collection and/or Processing:*** *Semra Mungan, Işıl Güzel;* ***Analysis and/or Interpretation:*** *Semra Mungan, Neşe Öztekin;* ***Source:*** *Semra Mungan, Neşe Öztekin;* ***Article Writing:*** *Semra Mungan, Neşe Öztekin, Işıl Güzel;* ***Critical Review:*** *Neşe Öztekin, Semra Mungan;* ***Resources and Funding:*** *Neşe Öztekin;* ***Ingredients:*** *Sema Uysal.*

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