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Investigation Of Interleukin-38 in Patients With Primary Sjögren's Syndrome

Primer Sjögren Sendromlu Hastalarımızda İnterlökin-38 Düzeyinin Araştırılması

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ABSTRACT

Aim:
Interleukin-38 has been involved as an inflammatory mediator in rheumatic diseases. However, little is known about the role of IL-38 in the development of primary Sjögren's syndrome. The present study aimed to evaluate the role of IL-38 in primary Sjögren's syndrome and its clinical relevance.

Material and Method:
Between 2019 and 2020, 40 patients with primary Sjögren's syndrome and 39 healthy participants were included in the study. The serum IL-38 level was measured by ELISA in all participants. The serum levels of IL-38 were compared with clinical and laboratory features.

Results:
The serum IL-38 levels between the patients with primary Sjögren's syndrome and the controls were similar (58.0 pg/ml, min-max: 0-641.0 vs. 55.0 ng/ml, min-max: 0-338.0; p=0.511). No significant correlations were found between serum IL-38 level and SSDAI (r=-0.104, p=0.523). IL-38 level was mildly negatively correlated with RF (r=-0.364, p=0.021) and positively correlated with CRP (r=0.321, p= 0.044).

Conclusion:
The correlation of IL-38 with CRP and RF should be considered because it might be important clues for contribution to the disease process. IL-38 might be relevant to the heterogeneous nature of PSS and the future role of IL-38 might be a biomarker for specific clinical manifestations of pSS.

Keywords:
disease activity, interleukin-38, primary Sjögren's syndrome, Th-17 cells

ÖZET

Amaç:
İnterlökin (IL)-38, otoimmün hastalıklarda rol oynamaktadır. Bununla birlikte, birincil Sjögren sendromunun gelişiminde IL-38'in rolü hakkında yeterli bilgi bulunmamaktadır. Bu çalışma, IL-38'in primer Sjögren sendromundaki rolünü ve klinik ilişkisini değerlendirmeyi amaçladı.

Gereç ve Yöntem:
2019-2020 yılları arasında primer Sjögren sendromlu 40 hasta ve 39 sağlıklı katılımcı çalışmaya dahil edildi. IL-38'in serum seviyesi, tüm katılımcılarda ELISA ile ölçüldü. IL-38'in serum seviyeleri klinik ve laboratuvar özellikleri ile karşılaştırıldı.

Bulgular:
Primer Sjögren sendromu hastaları ve kontrol grubu, serum IL-38 düzeyleri açısından benzerdi (58.0 pg/ml, min-maks: 0-641.0 ve 55.0 ng/ml, min-maks: 0-338.0; p=0.511). Serum IL-38 düzeyi ile SSDAI arasında anlamlı bir ilişki bulunmadı (r=-0.104, p=0.523). IL-38 düzeyi RF ile orta derecede negatif (r=-0.364, p=0.021) ve CRP ile pozitif korelasyon (r=0.321, p= 0.044) bulundu.

Sonuç:
IL-38 Primer Sjögren sendromlu hastalarda CRP ve RF ile ilişkili bulunmuştur. IL-38'in gelecekteki rolü, pSS'nin spesifik klinik belirtileri için bir biyobelirteç olabilir.

Anahtar kelimeler:
Hastalık aktivitesi, interlökin-38, primer Sjögren sendromu, T yardımcı hücre-17

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INTRODUCTION

Interleukin-38 (IL-38) is the tenth member of the IL-1 family, the gene of which is in the family cluster on chromosome 2 (1). Interleukin-38 has an antagonistic function, as IL-1Ra, IL-36 Ra and IL-37 (2). The structure of IL-38 shows nearly fifty per cent similarity with IL-1 receptor antagonist (Ra) and IL-36Ra. Interleukin-38 is thought to exist in two forms, truncated and full-length. IL-38 regulates some inflammatory cytokines by binding to receptors, including the IL-36R, IL-1R and IL-1RAPL1 (3-5). Besides anti-inflammatory properties, it has been proposed that IL-38 might stimulate the pro-inflammatory process in some conditions according to the form of IL-38 or concentration (5, 6). The expression of IL-38 is detected in the lungs, heart, placenta, thymus, skin, proliferating B cells of the human tonsils, salivary gland, spleen, and fetal liver (1, 2, 4), and presents at low levels in inactive immune tissues (3). It has been demonstrated that IL-38 play a role in various diseases, especially in inflammatory and autoimmune diseases (7, 8).

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease. T-helper (Th) 17 pathway and its regulator cytokines have been considered one of the chief mediators in the pSS pathogenesis (9, 10). IL-36 cytokines potently induce the Th-17 response (11). IL-38 might be a control point by inhibiting IL-36/IL-17 mediated inflammation (10, 11). There are little-known data concerning the potential role of IL-38 in pSS. The importance of IL-38 in the pathogenesis of pSS and its association with clinical characteristics is still unclear. This study aimed to investigate serum IL-38 in patients with pSS and its clinical relevance.

MATERIAL AND METHOD

Participants:

This cross-sectional study was conducted at the Rheumatology outpatient clinic between August 2019 and August 2020. Forty women with pSS, meeting the 2016 American College of Rheumatology (ACR/European League Against Rheumatism (EULAR)) classification criteria (12) were included in the study. Thirty-nine sex- and age-matched healthy individuals were selected for the control group. The exclusion criteria were being younger than eighteen years old, having a concomitant disease, and being pregnant for both groups. All the patients and control subjects had no history of inflammatory and autoimmune disease including, secondary Sjögren's syndrome, malignancy, viral hepatitis, recent infection, or renal and hematological disease. The study protocol complied with the Declaration of Helsinki, and ethical permission was obtained from the ethical review board of the University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital (Approval number: 69/03, Approval date: 05.08.2019). All participants signed informed consent forms before inclusion in the study.

All patients were examined by the same rheumatologist. Patients' demographic data and medical history, along with disease-related clinical and laboratory data, were recorded. The disease activity was evaluated by The EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (13).

Laboratory

Laboratory findings of all groups, including complete blood parameters, fasting plasma glucose level, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and liver and kidney function tests, and laboratory features of the pSS patients including rheumatoid factor (RF), anti-nuclear antibody (ANA), anti-Sjögren's syndrome-related antigen-A (SS-A), anti-SS-B, complement 3 (C3) and C4 were recorded. Blood samples were collected for the IL-38 measurements from all subjects. Serum was separated by centrifugation at 1500 rpm for 10 minutes and stored at -80 C until analysis.

IL-38 analysis

IL-38 concentrations of pSS patients and controls were measured by enzyme-linked immunosorbent assay (ELISA) using the human bioactive kit produced by Boster Biological Technology, USA.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows version 11.5 software (SPSS Inc., Chicago, IL, USA). The data normality was assessed by using visual and analytical methods. Continuous variables were presented using mean±standard deviation (SD) or median (minimum-maximum (min-max)), while categorical variables were expressed as number (n) and percentage (%). The independent samples t-test or the Mann-Whitney U-test was used for continuous variables, and Chi-square tests were conducted for categorical variables to compare parameters. Association between IL-38 and laboratory data was evaluated using the Spearman correlation coefficient. A two-sided p-value of <0.05 was considered statistically significant.

RESULTS:

Both study groups consist of female participants. There was no significant difference in the mean age between the groups (The mean age of patients was 46.1±10.9 years (range, 23.0-63.0); the mean age of the control group was 42.9±5.4 years (range, 34.0-54.0); p>0.05). The median disease duration was 19.0 months (range, 1.0-120.0). The demographic and clinic characteristics of the patients and control group are shown in Table 1.

Table 1. Clinical features and laboratory parameters of pSS patients and control group

	pSS (n=40)	Controls (n=39)	p value
Age (mean±SD), years	46.1±10.9	42.9±5.4	0.113
Gender (Female): n (%)	40 (100)	39 (100)	
Duration time of pSS from diagnosis, (median (min-max)), months	19 (1-120)	-	
Current Treatment	28 (70)		
Hydroxychloroquine	8 (20)		
Cyclophosphamide	2 (5)		
Hydroxychloroquine+steroid*	1 (2.5)		
Azathioprine	1 (2.5)		
ANA, n (%)			
Negative	4 (10)		
Positive	36 (90)		
Anti SS-A, n (%)			
Negative	4 (10)		
Positive	36 (90)		
Anti SS-B, n (%)			
Negative	25 (62.5)		
Positive	15 (37.5)		
RF, median (min-max) (IU/mL)	19.2 (0-2514)		
Negative, n (%)	20 (50)		
Positive, n (%)	20 (50)		
ESDAII, n (%)	35 (87.5)		
Low disease activity	5 (12.5)		
Moderate disease activity	-		
High disease activity			
CRP (mg/L)	3 (0.4-12.9)	2 (0.3-15.0)	0.220**
IL-38 (median (min-max)) (pg/mL)	58 (0-641.0)	55 (0-338.0)	0.511**

ANA: antinuclear antibody, CRP: C Reactive Protein, ESSDAI: The EULAR Sjögren's Syndrome Disease Activity Index, pSS: primary Sjögren's syndrome, SD: standard deviation, IL: interleukin, min: minimum, max: maximum

*≤7.5 mg corticosteroid

**Statistically significance was analyzed with the Mann -Whitney U test

There were no statistically significant differences between the patients and control subjects in terms of the serum IL-38 levels (58.0 pg/ml, min-max: 0-641.0 vs. 55.0 ng/ml, min-max: 0-338.0; p=0.511). No significant correlations were found between the serum IL-38 levels and the clinical manifestations in pSS patients (Table 2)

Table 2. Associations of serum IL-38 level with clinical manifestations

Clinical manifestations	Number of patients n (%)	Median IL-38 level (pg/ml) (min-max)	p-value
Dry mouth			
Positive, n (%)	21 (52.5)	59 (0-641)	0.635
Negative, n (%)	19 (47.5)	55 (1-274)	
Dry eyes			
Positive, n (%)	26 (65)	61 (0-641)	0.228
Negative, n (%)	14 (35)	52.5 (1-119)	
Joint involvement			
Positive, n (%)	29 (72.5)	57(0-641)	0.495
Negative, n (%)	11 (27.5)	63 (1-134)	
Raynaud's phenomenon			
Positive, n (%)	. . . . .	262	0.129
Negative, n (%)	39 (97.5)	57 (0-641)	
Pulmonary involvement			
Positive, n (%)	. . . .	158.5 (55-262)	0.352
Negative, n (%)	38 (99.5)	58 (0-641)	
Hematologic involvement			
Positive, n (%)	.	78.0 (21-641)	0.600
Negative, n (%)	37	57 0-274	
Skin involvement			
Positive, n (%)	. . . . .	46.0	0.603
Negative, n (%)	39 (97.5)	59 (0-641)	

Serum IL-38 level was compared between patients' each clinical manifestation whether did or did not have

Dry mouth and dry eyes were defined according to patients' symptoms and the Schirmer test, respectively

and ESSDAI (r=-0.104, p=0.523). In terms of laboratory parameters, IL-38 level was mildly negatively correlated with RF (r=-0.364, p=0.021) and positively correlated with CRP (r=0.321, p= 0.044).

DISCUSSION

In the present study, serum IL-38 levels were similar in the patients and controls. We did not find any association between IL-38 level and disease activity. The level of serum IL-38 was correlated with RF and CRP. To our knowledge, this is the first study to evaluate the association of IL-38 with clinical manifestations.

Interleukin-38 has recently become an intriguing cytokine for its ability to suppress proinflammatory cytokines and promising therapeutic potential (3). IL-38 is closely related to the IL-36 axis and Th17 pathway, which play crucial roles in the pathogenesis of chronic inflammation as in pSS (9-11). So far, the contribution of IL-38 to pSS pathogenesis is largely unknown. In this regard, Ciccia et al.'s (10) study results demonstrated that over-expressing IL-38 messenger ribonucleic acid (mRNA) and increasing IL-38 protein expression in the salivary glands of the patients with pSS. On the other hand, Luo et al. (11) recently reported decreased expression of IL-38 in the serum and saliva of pSS patients. Our study results pointed out similar serum IL-38 levels between the groups. The in vivo process of IL-38 or the interaction between resident tissue cells and the cytokine milieu, including IL-38, may be a reason for discordant outcomes. Although the results did not reach statistical significance, we observed high IL-38 levels in some pSS patients and some clinical manifestations such as hematological and pulmonary involvement. Non-significances may be explained by the small number of patients. Luo et al. (11) suggested that there might be IL-38 expression equilibrium among different tissues and systems, which may also be the case for our study. Likewise, it has been reported that IL-38 can regulate the release of different kinds of cytokines from different cell types (14). The interesting aspect is IL-38 has three different defined receptors and two known forms for the present (5, 6). We believe that the biological function of IL-38 and its impact on the clinical manifestations might be shaped according to the receptor pathway or form of IL-38.

IL-38 was mildly correlated with CRP positively and RF negatively in our results. Consistently, it has been declared that IL-38 is associated with CRP levels through immunological pathways (15). RF has been suggested as a possible prognostic factor in the pSS patients and is associated with more severe disease (16). Considering these associations, it can be interpreted that IL-38 is implicated in controlling inflammation. These results support its anti-inflammatory effect and proposed the potential role of IL-38 in the pathogenesis of pSS. We couldn't find any association with ESSDAI levels. We thought that it may be related to unequal group distribution in terms of ESSDAI. Also, it can be suggested that IL-38 might be related to clinical manifestations rather than disease activity.

Primary Sjögren's syndrome is a heterogeneous disease, and it couldn't be identified exactly which pathways directly related to the appearance of clinical findings (17, 18). The innate and adaptive immune systems have been implicated in the disease process. One of the pivotal pathways responsible for pSS is the IL23/IL-17/IL-22 axis (10, 19). Interleukin-38 inhibits Th17 mediated inflammation (11). It has been stated that IL-38 may play a role in the pathogenesis of pSS via the Th17 pathway (11, 18). Three signalling pathways concerned with IL-38 were defined as IL-38/IL-36R axis, IL-38/IL-1RAPL1 axis, and SIRT1/HIF1 . After binding to the 1RAPL1 receptor, IL-38 affects Th17 inflammation via activating protein-1/ cJun N-terminal kinase (AP-1/JNK) signalling pathway. The interesting point is that IL-38 has a dual effect depending on the binding form to the 1RAPL1receptor; truncated form leads to anti-inflammatory effects and full-length form leads to pro-inflammatory effects. In the light of such information, this axis may be relevant to pSS. Moreover, IL-38 might be responsible for specific manifestations of the disease with the supporting results of further studies.

The main limitation of the present study is its small sample size. The study was a hospital-based cross-sectional study, and so patients were unrepresentative of the general population. Albeit in small percentages, immunosuppressive drug usage was another limitation of the present study.

CONCLUSION

As a result, IL-38 is might be a potential executive cytokine in pSS. The clues regarding the correlation of IL-38 with CRP and RF should be considered. IL-38 might be relevant to the heterogeneous nature of pSS and the future role of IL-38 might be a biomarker for specific clinical manifestations of pSS.

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Authorship Contributions

Concept-SG, SCS; Design-SG, SCS; Supervision-SG; Materials-SG, FNA; Data collection and/or processing-SG, SCS; Analysis and/or interpretation- SG, SCS, FNA; Literature search: SG; Writing-SG; Critical review: SG, SCS

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