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Evaluation of the relationship between lymphoma predictors, disease activity, and netrin-1 in primary Sjögren's syndrome

Primer Sjögren sendromunda hastalık aktivitesi, lenfoma prediktörleri ve netrin-1 arasındaki ilişkinin değerlendirilmesi

Ahmet Kor[1], Hatice Ecem Konak[2], Esra Fırat Oğuz[3], Yüksel Maraş[4], Ebru Atalar[2], Kevser Orhan[2], Özcan Erel[5], Şükran Erten[6]

[1]*Aksaray Training and Research Hospital, Clinic of Rheumatology, Aksaray, Türkiye*
[2]*Ankara City Hospital, Clinic of Rheumatology, Ankara, Turkey*
[3]*Ankara City Hospital, Clinic of Medical Biochemistry, Ankara, Turkey*
[4]*Ankara City Hospital, University of Health Sciences Turkey Faculty of Medicine, Department of Rheumatology, Ankara, Turkey*
[5]*Ankara City Hospital, Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey*
[6]*Ankara City Hospital, Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Rheumatology, Ankara, Turkey*

Abstract

Objective: One-third of patients with primary Sjögren's syndrome (PSS) do not have anti-SSA or -SSB antibodies. The risk of developing B-cell non-Hodgkin lymphoma (NHL) in PSS is significantly increased, and its prevalence is approximately 5%. There is a continuing need for new markers that can have diagnostic value in PSS and predict lymphoma development. In this study, we aimed to investigate the usability of netrin-1 as a diagnostic marker in PSS and its relationship with disease activity and predictors of lymphoma.

Methods: Fifty-two PSS patients and 62 healthy volunteers were included in the study. The European Alliance of Associations for Rheumatology Sjögren's syndrome disease activity index (ESSDAI) was used to evaluate systemic disease activity in PSS patients. Netrin-1 values were calculated by the quantitative sandwich enzyme immunoassay method using an ELISA kit (catalog number: E-EL-H2328; Elabscience, lot number: GZWTKZ5SWK, Texas, USA).

Results: Serum netrin-1 levels were similar in PSS [90.3 (43.87-166.41)] and healthy controls [111.6 (63.39-171.36)] (p=0.190). Netrin-1 serum levels were associated only with lymphopenia (p=0.014), one of the predictive markers of lymphoma in PSS, but not with other markers (p>0.05) and ESSDAI score (r=0.637, p=0.067).

Conclusion: Serum netrin-1 levels are not high in PSS and there is no significant correlation between netrin-1 and lymphoma predictive values, except lymphopenia, and the ESSDAI score, which is an indicator of disease activity.

Keywords: Primary Sjögren's syndrome, netrin-1, lymphoma

Öz

Amaç: Primer Sjögren sendromlu (PSS) hastaların üçte birinde anti-SSA veya -SSB antikorları negatiftir. PSS'de B-hücreli non-Hodgkin lenfoma (NHL) gelişme riski önemli ölçüde artar ve prevalansı yaklaşık %5'tir. PSS'de tanısal değere sahip olabilecek ve lenfoma gelişimini öngörebilecek yeni belirteçlere yönelik ihtiyaç devam etmektedir. Bu çalışmada, netrin-1'in PSS'de tanısal bir belirteç olarak kullanılabilirliğini ve onun hastalık aktivitesi ve lenfoma prediktörleri ile ilişkisini araştırmayı amaçladık.

Yöntem: Çalışmaya 52 PSS hastası ve 62 sağlıklı gönüllü dahil edildi. PSS hastalarında sistemik hastalık aktivitesini değerlendirmek için Avrupa Romatoloji Dernekleri Birliği Sjögren sendromu hastalık aktivite indeksi (ESSDAI) kullanıldı. Netrin-1 değerleri ELISA kiti (Elabscience, Texas, ABD; katalog numarası: E-EL-H2328; lot numarası: GZWTKZ5SWK) kullanılarak kantitatif sandviç enzim immunoassay yöntemi ile hesaplandı.

Bulgular: PSS [90,3 (43,87-166,41)] ve sağlıklı kontrollerde [111,6 (63,39-171,36)] serum netrin-1 düzeyleri benzerdi (p=0,190). Netrin-1 serum düzeyleri PSS'de lenfomanın prediktif belirteçlerinden sadece lenfopeni (p=0,014) ile ilişkili iken, diğer belirteçlerle (p>0,05) ve ESSDAI skoru (r=0,637, p=0,067) ile ilişkili değildi.

Sonuç: Serum netrin-1 seviyeleri PSS'de yüksek değildir ve netrin-1 ile lenfoma prediktif değerlerden lenfopeni hariç diğerlerinin ve hastalık aktivite göstergelesi olan ESSDAI skorunun anlamlı korelasyon ilişkisi yoktur.

Anahtar Kelimeler: Primer Sjögren sendromu, netrin-1, lenfoma

Correspondence / İletişim:
Ahmet Kor MD, Aksaray Training and Research Hospital, Clinic of Rheumatology, Aksaray, Turkey
Phone: +90 532 646 86 61 **E-mail:** ahmetkor_61@hotmail.com **ORCID ID:** orcid.org/0000-0002-5794-6951
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Introduction

Primary Sjögren's syndrome (PSS) is a chronic autoimmune disease not related to other autoimmune diseases, characterized by lymphocytic infiltration of exocrine glands, mainly with glandular dysfunction of salivary and lacrimal glands. Keratoconjunctiva sicca (dry eyes) and xerostomia (dry mouth) are the main symptoms.[1] Since two-thirds of PSS patients are positive for anti-SSA/Ro or -SSB/La autoantibodies, these antibodies are considered one of the main criteria in the 2016 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) PSS classification.[2] Since anti-SSA or SSB antibodies are negative in one-third of PSS patients, many studies have been conducted to detect new autoantibodies that can be used in diagnosing PSS. However, the need for diagnostic biomarkers continues. These novel autoantibodies include autoantibodies against serum salivary gland protein-1, parotid secretion protein, muscarinic-3 receptor, and carbonic anhydrase-6.[3-5]

B-cell non-Hodgkin lymphoma occurs in approximately 5% of patients with PSS and is considered to be the main source of mortality.[6] Studies have been ongoing for a long time to determine the markers (laboratory, pathological and clinical) that may have predictive value in developing lymphoma in PSS. Among the best markers with prognostic value in the development of lymphoma are mixed cryoglobulinemia, cryoglobulinemic vasculitis, and persistent salivary gland swelling.[7-17] Other lymphoma predictive values include skin purpura which may be associated with cryoglobulinemia, low C4 level and organ involvement associated with cryoglobulinemic vasculitis (glomerulonephritis, peripheral neuropathy);[18,19] high MALT involvement in salivary gland histopathology; the presence of monoclonal gammopathy; RF positivity;[20-22] lymphopenia; neutropenia; increased serum beta-2 microglobulin; elevated free immunoglobulin light chains; splenomegaly; lymphadenopathy; cytokines; chemokines; growth factors; monoclonal B lymphocyte expansion in metachronous tissue histopathology; genetic abnormalities and EULAR Sjögren's syndrome disease activity index (ESSDAI).[23]

The need for new markers associated with diagnosis, disease activity indicators, and lymphoma development in PSS continues. The relationship of netrin-1 with PSS has yet to be evaluated. In this study, we investigated the value of netrin-1 molecule as a diagnostic marker and disease activity indicator in PSS and the relationship between serum levels and predictors of lymphoma. Netrin-1 is a laminin-like matrix protein from the axonal guide protein family. Netrin-1 acts as a chemorepulsan and inhibits the migration of neutrophils, monocytes, and lymphocytes through Unc5b and adenosine A2B receptors.[24-26] Netrin-1 plays a pathogenic role in obesity, the development of atherosclerosis,[27,28] the initiation of sepsis and inflammation,[24] osteoporosis by stimulating osteoclast differentiation,[29] and the development of inflammatory arthritis.[30] In our previous study, we showed that netrin-1 was found to be higher in the serum of patients with systemic sclerosis (SSc) compared to healthy controls ($p<0.0001$) and that it may be associated with the pathogenesis of systemic sclerosis.[31]. Deleted in Colorectal Carcinoma (DCC) is a transmembrane netrin-1 receptor that actively induces cell death when dissociated from the netrin-1 ligand. When netrin-1 binds to the DCC receptor, it causes inhibition of DCC-induced apoptosis. It contributes to the increase in the population of diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) tumor cells.[32] In addition, netrin-1 causes progression in plasma cell malignancies.[33] These associations of netrin-1 with lymphoid malignancies prompted us to investigate the relationship of netrin-1 with markers that can predict lymphoma in PSS.

Materials and Methods

Study Design

This study was designed as an analytical case-control study. Fifty-two patients (47 women, five men) followed in the rheumatology department of Ankara City Hospital and classified according to the 2016 ACR/EULAR criteria[34] for PSS were included in the PSS group. In the control group, 62 healthy volunteers (55 females, seven males) of similar age and gender to the PSS group were selected. Active infection, pregnancy, malignant disease, and the presence of rheumatological disease other than PSS were the exclusion criteria. Superficial and abdominal ultrasonography (USG) for lymphadenopathy and splenomegaly; pulmonary function test, carbon monoxide diffusion test or high-resolution tomography for interstitial lung disease; neurological examination and electromyography for peripheral neurologic involvement; urine microscopy and renal biopsy histopathology for glomerulonephritis; parotid gland USG for parotid gland swelling; joint examination and joint USG for arthritis; skin examination for purpura was used to detect lymphoma predictors in the PSS group. The ESSDAI score was used to evaluate systemic disease activity in PSS.[35] To calculate this index score, a total of 12 areas, 11 areas related to organ involvement and one biological place reflecting B-cell activity, were examined, and scores were given to the patients. According to the ESSDAI score, patients were divided into groups defining systemic disease

activity as low (5>), moderate (>5 and <14), or severe (>14). Individuals with hypertension, diabetes mellitus, chronic heart disease, or chronic lung disease were included in the study groups in minimum numbers, and individuals with any of these diseases were considered positive for the presence of comorbid disease. Informed consent was obtained from all individuals included in the study before the study. Dates are given in DD/MM/YYYY format.

Obtaining Serum Samples and Determination of Serum Netrin-1 Level

Venous blood samples in 10 mL vacuum tubes were centrifuged at 1300 x g for an average of 10 minutes. Serum samples were stored in eppendorf tubes at -80 °C until analysis. Serum netrin-1 levels were measured using a quantitative immunoassay method with the catalog number E-EL-H2328 from elabscience, lot number GZWTKZ5SWK, in Texas, USA. Before adding the specific detection antibody rich in biotin for netrin-1, serum samples and standards were incubated with their specific antibodies at 37 °C for approximately 1.5 hours after addition to the micro-ELISA plate wells. Biotin-rich human netrin-1-specific detection antibodies and Avidin-Horseradish Peroxidase (HRP) were then added, and the samples were then incubated at 37 °C for 30 minutes. After the free components were separated by washing, the substrate solution was added to all wells. After this process was completed, a blue color was observed only in the Avidin-HRP conjugate, human netrin-1 wells and biotin-rich detection antibodies. The enzyme-substrate reaction ended after the addition of the stopping solution, resulting in a yellow color in the reaction. A spectrophotometric microplate reader with a wavelength of 450 nm was used to detect optical density levels, which is considered an indirect indicator of Human netrin-1 concentrations. Serum levels of human netrin-1 were calculated using optical density standard curves. The sensitivity used for netrin-1 at levels in the range of 31.25 to 2.000 pg/mL was determined. Inter- and intra-assay precision of <10% was obtained for all levels of netrin-1 concentrations.

Statistical Analysis

The Statistical Packages for the Social Sciences (SPSS) version 22.0 package program was used for the evaluation of statistical analyzes, and the $p<0.05$ level was considered statistically significant. Normality distribution fit in continuous variables was determined using analytical methods such as Kolmogorov-Smirnov/Shapiro-Wilk tests and histogram/probability graphs. Descriptive statistical results were shown as mean and standard deviation for normally distributed variables, and median [interquartile range, (25%-75%)] for non-normally distributed variables. The Independent Samples t-test and Mann-Whitney U test were used to evaluate the statistical significance difference in pairwise comparisons. Spearman correlation test was used to evaluate the correlation analysis between continuous variables. One-Way ANOVA Post-hoc Tukey test was used to evaluate variables with normal distribution, and Independent Samples Kruskal-Wallis test was used to evaluate variables that did not show normal distribution. Bonferroni correction was performed before the One-Way ANOVA Post-hoc Tukey test and the Independent Samples Kruskal-Wallis test. The Fisher's exact tests and chi-square test were used to compare categorical data.

Results

This study was designed as an analytical case control study. The PSS group consisting of 52 patients with a mean age of 49.08±7.23 and a healthy control group consisting of 62 patients with a mean age of 51.06±9.41 were included in the study. Age, gender, body mass index, smoking and presence of comorbid disease in the control group were similar to the PSS group ($p<0.05$). No significant difference was found between PSS and control groups in comparison of median values for netrin-1 [90.3 (43.87-166.41), 111.6 (63.39-171.36), p=0.190, respectively]. Demographic characteristics and laboratory parameters of PSS and control groups are shown in Table 1.

There was no significant difference in median values of netrin-1 between patients with (n=4) and those without (n=48) interstitial lung disease (n=48) in the PPS group [respectively, 115.17 (35.3-395.2), 90.3 (47.2-162.5), p=0.882]. In PSS patients, the median values of netrin-1 were found to be similar between those with negative antibody (anti-SSA/RO52 and -SSB) (n=11) and those with any antibody positivity (anti-SSA/RO52 or -SSB) (n=41) [respectively, 90.54 (38.04-184.4), 90.06 (47.3-159.3), p=0.920].

In the multiple comparisons made in terms of netrin-1 levels between groups receiving different types of treatment in PSS, netrin-1 levels were found to be similar between groups receiving other types of treatment (p=0.342). There was no significant difference in netrin-1 levels between those who received any treatment for PSS and those who did not receive the same treatment ($p>0.05$). The comparison of netrin-1 levels according to medical treatment subtypes in the PSS group is shown in Table 2.

While netrin-1 serum levels are associated only with presence of lymphopenia [yes=159.34 (91.2-213.8),

Table 1. Comparison of demographic characteristics and laboratory results between groups

Parameters	PSS group	Control group	p-value
Gender female/male, n	47/5, 52	55/7, 62	0.509
Age, mean ± SD (years)	49.08±7.23	51.06±9.41	0.164
Body mass index, mean ± SD	23.1±5.14	24.9±6.4	0.18
Smoking, n (%)	3 (5.7)	1 (1.6)	0.33
Presence of comorbid disease			
Hypertension, n	3	5	0.726
Chronic obstructive pulmonary disease, n	3	1	0.330
Coronary artery disease, n	1	2	0.665
Diabetes mellitus, n	1	5	0.217
Disease duration, median (IQR) [years]	12 (7-16)		
Hemoglobin, mean ± SD [x10^9/L]	14.2±1.7	13.4±1.07	0.094
Platelets, mean ± SD [x10^9/L]	260.60±55.06	271.71±13.16	0.253
WBC, mean ± SD [x10^9/L]	5.97±1.95	6.16±2.12	0.062
Neutrophil, mean ± SD [x10^9/L]	4.61±1.51	4.98±1.74	0.072
Lymphocyte, mean ± SD [x10^9/L]	1.35±0.43	1.39±0.71	0.056
Creatinine, mean ± SD [mg/dL]	0.74±0.21	0.63±0.19	0.414
ALT, mean ± SD [U/L]	17.07±6.8	18.26±7.32	0.881
LDH, mean ± SD [U/L]	236.71±36.3	194.52±25.4	0.102
CRP, median (IQR) [mg/L]	2.9 (0.9-6.8)	3.5 (1.1-7.9)	0.095
ESR, median (IQR) [mm/h]	18 (9-24)	13 (8-17)	0.061
Spot urine protein/creatinine ratio	160±31.3	143±22.5	0.231
Netrin-1 levels, median (IQR) [pg/mL]	90.3 (43.87-166.41)	111.6 (63.39-171.36)	0.190
ESSDAI, median (IQR)	6.5 (3.25-14)		
RF, median (IQR) [IU/mL]	10 (9-33)		
C3, median (IQR) [g/L]	1.1 (1-1.28)		
C4, median (IQR) [g/L]	0.2 (0.18-0.3)		
IgG, median (IQR) [g/L]	13.2 (10.6-18.1)		
Presence of interstitial lung disease, n (%)	4 (9.6)		
Antibody negativity (anti-SSA/RO52 and -SSB), n (%)	11 (21.1)		

ALT: Alanine aminotransferase, C3: Complement-3, C4: Complement-4, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ESSDAI: EULAR Sjogren's syndrome disease activity index, IgG: Immunoglobulin G, LDH: Lactate dehydrogenase, RF: Rheumatoid factor, SD: Standard deviation, WBC: White blood cells

Table 2. Netrin-1 levels according to the types of medical treatment used in the PSS group

Medical therapy		n	Netrin-1 levels median (IQR) [pg/mL]	p-value
Hydroxychloroquine	yes	48	85.6 (42.08-161.5)	0.097
	no	4	95.62 (52.1-183.7)	
Corticosteroids	yes	11	90.54 (35.2-169.21)	0.439
	no	41	90.06 (49.24-159.85)	
Methotrexate	yes	7	85.7 (32.4-149.3)	0.167
	no	45	91.3 (47.3-174.5)	
Mycophenolate mofetil	yes	2	88.5 (40.3-156.9)	0.234
	no	50	92.12 (45.1-167.3)	
Rituximab	yes	2	91.4 (55.5-169.5)	0.42
	no	50	94.14 (60.1-172.3)	
Pilocarpine	yes	8	84.8 (39.6-149.3)	0.132
	no	44	91.3 (47.3-155.9)	

IQR: Interquartile range, PSS: Primary Sjogren's syndrome

no=81.29 (42.3-147.5) p=0.014], which is a predictive marker of lymphoma in PSS, it is not correlated with other features (p>0.05). The relationship between the factors predicting lymphoma and netrin-1 levels in PSS is shown in Table 3.

While there was a significant correlation between netrin-1 and spot urine protein amount (r=0.278, p=0.046) and lymphocyte level (r=-0.343, p=0.013), no significant correlation was found between other study parameters (p>0.05) (Table 4).

Discussion

The absence of anti-SSA or anti-SSB antibodies in one-third of PSS patients and the lack of markers that can be used in disease activation and lymphoma prediction have revealed the necessity of investigating new autoantibodies associated with PSS. It has been previously reported that netrin-1 may be related to different mechanisms in the pathogenesis of some lymphomas,[32] multiple myeloma,[33] solid tumors,[36] and SSc,[31] a connective tissue disease. In this study, we investigated the utility of netrin-1 as an indicator

Table 3. The relationship between lymphoma predictive markers and netrin-1 levels in the PSS group

Lymphoma predictive markers		n	Netrin-1 levels median (IQR) [pg/mL]	p-value
Hypocomplementemia (C3 or C4)	yes	18	76.68 (40.6-156.1)	0.459
	no	34	91.52 (47.4-173.4)	
Hypocomplementemia (C4)	yes	8	75.5 (39.2-212.6)	0.794
	no	44	90.3 (48.4-163.2)	
Hypocomplementemia (C3)	yes	14	68.45 (39.4-135.9)	0.227
	no	38	93.72 (47.4-173.4)	
Hypergammaglobulinemia (IgG)	yes	9	165.6 (79.4-238.8)	0.082
	no	43	77.89 (41.8-149.5)	
RF positivity	yes	15	81.29 (42.8-149.5)	0.724
	no	37	94.94 (44.23-175.5)	
Anti-SSA/RO52 positivity	yes	37	90.06 (38.5-169.2)	0.824
	no	15	90.54 (38.5-169.2)	
Anti-SSB positivity	yes	23	77.89 (35.6-149.5)	0.173
	no	29	94.94 (57.1-176.8)	
Purpura	yes	1	92.5	0.962
	no	51	90.06 (42.8 -166.6)	
Lymphadenopathy	yes	5	153.03 (59.3-175.8)	0.449
	no	47	90.06 (41.8-166.6)	
Splenomegaly	yes	3	165.6 (145.4-249.3)	0.094
	no	49	81.29 (42.3-159.8)	
ESSDAI	low	22	79.59 (47.4-146.4)	0.289
	moderate	19	90.06 (35.6-166.6)	
	high	11	153.03 (50.9-228.3)	
Parotid gland enlargement	yes	1	309.02	0.154
	no	51	90.06 (42.8-165.6)	
Monoclonal gammopathy	yes	3	126.01 (72.1-186)	0.429
	no	49	90.06 (42.3-166.1)	
Glomerulonephritis	yes	3	187.55 (116.1-249.3)	0.086
	no	49	81.29 (42.3-159.3)	
Leukopenia	yes	9	165.6 (70.4-235.9)	0.058
	no	43	81.29 (42.3-147.5)	
Neutropenia	yes	6	145.8 (77.8-229.3)	0.198
	no	46	79.59 (42.5-156.4)	
Lymphopenia	yes	16	159.34 (91.2-213.8)	0.014
	no	38	70.63 (41.01-116.3)	
Gender	Female	47	97.14 (56.5-169.7)	0.546
	Male	5	109.43 (51.5-152.7)	

C3: Complement-3, C4: Complement-4, ESSDAI: EULAR Sjogren's syndrome disease activity index, IgG: Immunoglobulin G, LDH: Lactate dehydrogenase, RF: Rheumatoid factor

Table 4. Correlation analysis results between netrin-1 and some parameters

Parameter r (p)	Age	Lymphocyte	CRP	C3	C4	IgG	AntiSSA\ RO52	AntiSSB	RF	ESSDAI	UPCR
Netrin-1	0.002 (0.981)	-0.343 (0.013)	0.056 (0.694)	-0.049 (0.729)	0.071 (0.618)	0.371 (0.074)	-0.039 (0.784)	-0.220 (116)	-0.031 (0.833)	0.067 (0.637)	0.278 (0.046)

C3: Complement-3, C4: Complement-4, CRP: C-reactive protein, ESSDAI: EULAR Sjogren's syndrome disease activity index, R: Spearman correlation coefficient, rF: Rheumatoid factor, UPCR: Spot urine protein/creatinine ratio

of disease activity in PSS and its relationship with known predictors of lymphoma. Our results showed that netrin-1 levels were not high in the serum of PSS patients, and there was no significant correlation between disease activity and ESSDAI score. In addition, this study showed a substantial relationship between netrin-1 levels and lymphopenia, one of the known predictors of lymphoma in PSS. Still, no critical relationship exists between other predictors.

There are a limited number of studies investigating the relationship of netrin-1 with rheumatological diseases. In two of these studies, the relationship between netrin-1 and antibodies in rheumatoid arthritis (RA) synovial tissues,[30,37] while in other studies, netrin-1 levels were investigated in the serum of patients with familial Mediterranean fever (FMF) [38] and SSc[31] patients. It was determined that there was a significant decrease in inflammation and joint erosion in the group treated with anti-netrin-1/anti-Unc5b monoclonal antibody injection (n=6) compared to the untreated group (n=10) from 8-week-old arthritis mice (p<0.001).[30] Unlike this study, Schubert et al.[37] reported that the expression of UNC5B (4-fold) and UNC5C (769-fold) from netrin-1 receptors was higher in RA (n=5) and osteoarthritis (OA) (n=6) synovial tissues compared to healthy subjects (n=3) synovial tissues. They also reported that treatment of RA and OA synovial tissues with netrin-1 results in the inhibition of the migration of synovial fibroblasts, and thus netrin-1 could reduce cartilage degeneration. In a study where we evaluated serum netrin-1 levels between FMF patients (n=42) and healthy controls (n=44), we found netrin-1 levels to be similar between the two groups (p=0.19).[38] In another study, we evaluated netrin-1 levels in the sera of SSc patients (n=56) compared to healthy controls (n=58). In this study, we found that netrin-1 levels were significantly higher in the sera of SSc patients (p<0.0001).[31] Studies supporting the possible contribution of netrin-1 to the pathogenesis of SSc showed that profibrotic cytokine and extracellular matrix protein synthesis in SSc were induced by M2 macrophages[39,40] and netrin-1 increased the expression of M2 macrophage markers.[41-43] It has also been reported that netrin-1 promotes the development of fibrosis in human SSc lung cell culture and bleomycin-induced mouse lung.[44,45] In this study, we compared serum netrin-1 levels between PSS patients (n=52) and healthy controls (n=62), we found netrin-1 levels to be similar between the groups, and we did not find a significant correlation between netrin-1 and the disease activation indicator ESSDAI score (p>0.05). Although the data on the relationship of netrin-1 with rheumatological diseases come mostly from small-scale or cross-sectional studies, further randomized controlled studies are needed to better understand its role in rheumatological diseases.

A study by Broutier et al.[32] using a transgenic mouse model showed that netrin-1 contributes to the development of DLBCL and MCL by inhibiting apoptosis on DCC receptors. In addition, this study determined that there was a decrease in tumor cell density and an increase in DNA fragmentation in tumor cell lines with antibodies blocking netrin-1. Nagoshi et al.[33] showed that transcriptional dysregulation of the netrin-1 receptor DCC in multiple myeloma cell lines plays a role in the progression of plasma cell malignancy. Netrin-1 has been found to cause the inhibition of p53-related apoptosis by stabilizing inactive p53 expression through its receptors.[46,47] It has been reported to be associated with hepatocellular, breast, lung, colorectal, and pancreatic cancers.[34] These associations of netrin-1 with lymphoid and solid malignant diseases prompted us to investigate its relationship with lymphoma predictors in PSS. In our study, a significant relationship was found between netrin-1 and only lymphopenia, but no significant relationship was found between other lymphoma predictive factors.

Study Limitations

The limitations of this study are that it is a cross-sectional study, there is an insufficient number of subjects in some groups that predict lymphoma, and ectopic germinal-like structures and focus scores were not evaluated in salivary gland biopsy. In our study, although the median values of netrin-1 were higher in some lymphoma predictor subgroups (hypergammaglobulinemia, lymphadenopathy, splenomegaly, ESSDAI, parotid gland enlargement, monoclonal gammopathy, glomerulonephritis, leukopenia, neutropenia), no statistically significant results could be obtained. This may be because the statistical results are affected due to the small number of subjects in the subgroups. Although we did not find a significant relationship between netrin-1 and most of the variables that predict lymphoma in PSS in this study, the close relationship of netrin-1

with lymphoproliferative and solid malignancies makes it necessary to investigate its role in the development of lymphoma in PSS with further prospective studies.

Conclusion

Serum netrin-1 levels are not high in PSS and there is no significant correlation between netrin-1 and lymphoma predictive values, except lymphopenia, and the ESSDAI score, which is an indicator of disease activity.

Ethics

Ethics Committee Approval: The study was approved by the ethics committee of the University where the study was conducted (Ankara City Hospital Ethics Committee - approval number: 460; date: 30.09.2020).

Informed Consent: Informed consent was obtained from all individuals included in the study before the study.

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Authorship Contributions

Concept: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E., Design: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E., Data Collection or Processing: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E., Analysis or Interpretation: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E., Literature Search: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E., Writing: A.K., H.E.K., E.F.O., Y.M., E.A., K.O., Ö.E., Ş.E.

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