Received: 14 June 2023 | **Accepted:** 13 July 2023 **Genel Tıp Derg. Volume** 33/**Issue** 4 (August), 360-366
DOI: 10.54005/geneltip.1314282

ORIGINAL ARTICLE

Serum Inflammatory Cytokines and Growth Factors in Patients with Sjögren's Syndrome and Diabetes Mellitus

Sjögren Sendromlu ve Diabetik Hastalarda Serum Büyüme Faktörleri ve İnflamatuar Sitokin Düzeyleri

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How to cite ?

Acar Duyan Ş. N. , Bozkurt B. , Unlu A. , Yilmaz S. , Karaküçük Y. Serum Inflammatory Cytokines and Growth Factors in Patients with Sjögren's Syndrome and Diabetes Mellitus. Genel Tıp Dergisi. 2023; 33(4): 360-366.

ABSTRACT

Purpose: Sjögren's syndrome (SS) and diabetes mellitus (DM) are common causes of dry eye disease (DED) and autologous serum is used when artificial tears are not sufficient. Our aim was to compare serum growth factor and inflammatory cytokine levels of SS and DM patients with the healthy individuals.
Methods: Twenty-three SS patients (mean age 52.70±7.55 years), 25 diabetic retinopathy (DR) patients (mean age 56.68±6.53 years), and 23 healthy subjects (mean age 51.70±9.14 years) were included in the study. After detailed ophthalmological examination, Schirmer test, tear break-up time (TBUT) and Ocular Surface Disease Index (OSDI) scores were measured. Serum levels of six different proinflammatory interleukins (IL), five growth factors, matrix metalloproteinase-9, and fibronectin were measured by immunoassay. One-way ANOVA or Kruskal-Wallis tests and Dunn-Bonferroni post hoc analysis were used for comparison and p<0.05 was considered significant.
Results: Schirmer test and TBUT were significantly lower in the SS group (2.08±1.72 mm/5 min and 3.08±2.08 s) than in the DR (10.24±4.63 mm/5 min and 4.20±3.09 s) and control groups (13.30±5.95 mm/5 min and 9.00±1.75 s) (p<0.001). Among the parameters studied, mean serum IL-23 level was significantly higher in the SS group (156.66±207.94 pg/mL) than in the DM and control groups (73.48±95.91 and 69.59±105.39 pg/mL, respectively) (p<0.05). Serum insulin-like growth factor 1 (IGF-1) level was lowest in DM patients (DM: 12.89±21.09, SS: 30.77±19.85, and control: 27.08±21.93 ng/mL) (p<0.05). Sjögren's syndrome disease activity index (ESSDAI) showed a negative correlation with TBUT and a positive correlation with IL-1, IL-2 and fibronectin (p<0.005).
Conclusion: Except IL-23 and IGF-1, the contents of serum obtained from patients with SS and DM are similar with the healthy individuals. Therefore, autologous serum seems to be a good option to replace deficient tear fluid in these subjects.

Keywords: Inflammatory cytokines, Growth factors, Sjögren's syndrome, Diabetic mellitus, Autologous serum

ÖZ

Amaç: Sjögren sendromu (SS) ve diabetes mellitus (DM) kuru göz hastalığının yaygın nedenleridir ve yapay gözyaşı preperatları yeterli olmadığında otolog serum kullanılmaktadır. Amacımız SS ve DM hastalarının serum büyüme faktörü ve inflamatuar sitokin düzeylerini sağlıklı bireylerle karşılaştırmaktı.
Gereç ve Yöntem: Çalışmaya 23 SS hastası (ortalama yaş 52,70±7,55), 25 DM hastası (ortalama yaş 56,68±6,53) ve 23 sağlıklı birey (ortalama yaş 51,70±9,14) alındı. Ayrıntılı oftalmolojik muayene sonrasında Schirmer testi, gözyaşı kırılma zamanı (GKZ) ve Ocular Surface Disease Index (OSDI) skorları ölçüldü. Altı farklı proinflamatuar interlökin (IL), beş büyüme faktörü, matriks metaloproteinaz-9 ve fibronektinin serum seviyeleri, immünoassay ile ölçüldü. Karşılaştırma için tek yönlü ANOVA veya Kruskal-Wallis testleri ve Dunn-Bonferroni post hoc analizi kullanıldı ve p<0,05 anlamlı kabul edildi.
Bulgular: Schirmer testi ve GKZ, SS grubunda (2,08±1,72 mm/5 dk ve 3,08±2,08 sn), DR(10,24±4,63 mm/5 dk ve 4,20±3,09 sn) ve kontrol grubuna (13.30±5.95 mm/5 dk ve 9.00±1.75 sn) göre anlamlı olarak düşüktü (p<0,001). İncelenen parametrelerden ortalama serum IL-23 düzeyi SS grubunda (156,66±207,94 pg/mL), DM ve kontrol gruplarına göre (sırasıyla 73,48±95,91 ve 69,59±105,39 pg/mL) anlamlı olarak yüksekti (p< 0.05). Serum insülin benzeri büyüme faktörü 1 (IGF-1) düzeyi DM'li hastalarda en düşüktü (DM: 12,89±21,09, SS: 30,77±19,85 ve kontrol: 27,08±21,93 ng/mL) (p<0,05). Sjögren sendromu hastalık aktivite indeksi (ESSDAI) GKZ ile negatif, IL-1, IL-2 ve fibronektin ile pozitif korelasyon gösterdi (p<0.005).
Sonuç: IL-23 ve IGF-1 dışında SS ve DM'li hastalardan elde edilen serum içerikleri sağlıklı bireylerle benzerdir. Bu nedenle, otolog serum, bu kişilerde eksik olan gözyaşını yerine koymak için iyi bir seçenek gibi görünmektedir.

Anahtar Kelimeler: İnflamatuar sitokinler, Büyüme Faktörleri, Sjögren sendromu, Diabetes mellitus, Otolog serum

Introduction

Dry eye disease (DED) is common health problem with a prevalence ranging from 5% to 50% depending on the definition and the demographic features of the population studied (1). DED is defined as a multifactorial disease of the ocular surface characterized by impaired tear film homeostasis and associated ocular symptoms (2). Tear film hyperosmolarity, inflammation, and neurosensory abnormalities play a role in the pathogenesis. Risk factors for DED include older age, female gender, Asian population, rheumatological disorders, Sjögren's syndrome (SS), diabetes mellitus (DM), androgen deficiency, hormone replacement therapy, digital screen use, contact lens wear, graft-versus-host disease, and systemic medications such as antihistamines, antidepressants, anxiolytics, and isotretinoin.

Peer-Review: Double anonymized - Two External
Plagiarism Checks: Yes - iThenticate
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SS is an autoimmune disease that primarily affects the salivary and lacrimal glands and is characterized by periductal lymphocytic infiltration, local tissue damage, and exocrine gland dysfunction leading to dry eye and mouth (3,4). Patients are diagnosed as having primary SS after excluding hepatitis C, sarcoidosis, and other systemic autoimmune diseases, whereas secondary SS is associated with other connective tissue disorders such as rheumatoid arthritis, systemic lupus erythematosus, and scleroderma. In a study conducted in the United States, SS was found responsible for severe aqueous deficient dry eye in 11.6% of the cases (5). SS has also been shown to have an evaporative component with associated meibomian gland dysfunction (6).

The relationship between DED and DM is not well understood. Approximately half of diabetic patients were shown to have dry eye symptoms associated with the disease duration and DM complications (7-11). In diabetic patients, impaired corneal innervation by the trigeminal nerve leads to neurotrophic keratopathy (NK), which is characterized by reduced corneal sensation and reflex lacrimation, cornea epithelial healing problems, and ulceration (12,13).

Artificial tears are the first-line therapy for dry eye. However, they neither treat the underlying pathology of DED nor contain the essential growth factors for ocular surface epithelial health (14-16). Serum, the liquid component of blood that separates out after clotting, is biochemically similar to natural tears (16,17). Blood-derived ophthalmic drops can be made from the patient's own blood (autologous serum or platelet-rich plasma) or that of homologous donors (allogeneic peripheral blood serum). Autologous serum has been used by ophthalmologists since 1975 and was proven to be effective and safe for the treatment of severe DED, refractory cornea epithelial defects, and NK (16-23). Several studies showed that blood-derived ophthalmic drops promote corneal wound healing, inhibit inflammatory cytokine secretion from conjunctival epithelial cells and increase the number of goblet cells and conjunctival expression of mucin (24,25). Autologous serum was shown to alleviate dry eye symptoms and improve dry eye tests and conjunctival impression cytology (23,26,27).

Autologous serum is commonly used in SS patients with DED and non-healing corneal epithelial defects, such as neurotrophic keratopathy (NK). Serum obtained from patients with SS might contain high amounts of proinflammatory cytokines, especially during active disease if untreated or not treated properly, which can lead to unpredictable therapeutic effects and possibly even worsen the inflammation when applied to the ocular surface (28,29). Similarly, the possible increase of serum inflammatory cytokines and a decline in growth factors call into question whether autologous serum from diabetic patients can replace deficient tear fluid (30,31).

This study aimed to compare serum growth factor and inflammatory cytokine levels of SS and DM patients with healthy individuals. Correlations between serum contents, SS disease activity index score, HbA1c, diabetic organ involvement, and dry eye parameters were also analyzed.

Materials and Methods

Patient Recruitment

The protocol was approved by a clinical research ethics committee (Selçuk University Medical Faculty, 2020/504) and followed the tenets of the 1964 Helsinki Declaration. The study was designed prospectively. Written informed consent was obtained from the participants before their inclusion in the study.

The SS group included women diagnosed with primary SS using the American-European Consensus classification criteria (32). The patients' demographic features, organ involvement, joint involvement, blood tests, ESSDAI scores, and medical treatments were recorded. The ESSDAI consists of 12 parts: cutaneous, respiratory, kidney, joint, muscle, peripheral and central nervous systems, hematological, glandular, structural, lymphadenopathic, and biological. Each area is divided into 3-4 activity levels. ESSDAI scores are classified into three degrees of disease activity: a score less than 5 is regarded as low activity, 5-13 as moderate activity, and >14 as high activity (33). The DM group included women with type 2 DM and non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR), who were using topical lubricants with the diagnosis of DED (34). Exclusion criteria were comorbid autoimmune disorders and active infection. Patients with diabetic retinopathy (DR) who received intravitreal anti-vascular endothelial growth factor (anti-VEGF) injection or dexamethasone implant in the last one year were not included in the study since anti-VEGF treatment might affect the results of the study. Demographic features, duration of DM, blood tests including HbA1c levels, and organ involvement were recorded. The control group included age-matched healthy women who had no systemic or ocular problems other than refractive error.

After a detailed eye examination, optic coherence tomographic measurements of the peripapillary retina and macula were obtained (Spectralis® OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) and dry eye tests were performed. Tear break-up time (TBUT) is an indicator of tear film stability and commonly used in the assessment of evaporative dry eye. Sodium fluorescein is installed into the lower conjunctiva and the patient is asked to look straight ahead and blink a few times. Under blue light, the time between the last blink and the first dry spot is recorded. TBUT more than 10 seconds is considered normal, 5 to 10 seconds marginal, and <5 seconds was considered as abnormal. The Schirmer test is performed by folding the Whatman paper strip at the notch and hooking the folded end over the temporal one-third of the lower lid margin. The score is the measured length of wetting from the notch, after a period of 5 min. A value <5 mm was defined as definitely decreased tear production. The Oxford scheme were used for assessment of ocular surface damage. The cornea and temporal and nasal

parts of the conjunctiva were evaluated and ocular surface staining (OSS) was graded as mild (stage 0-1), moderate (stage 2 or 3), or severe (stage 4-5) using the Oxford grading scheme. The OSDI includes 6 questions related to visual disturbance (blurred vision, or poor vision) or visual function (problems reading, working on a computer, or watching TV, driving at night) (35). In the SS group, severe dry eye patients with TBUT <5 seconds, Schirmer I test result <5 mm/5 min, and OSS >1 (moderate to severe staining) were included. All participants completed the OSDI questionnaire. Corneal sensitivity was measured using the Luneau Cochet-Bonnet esthesiometer (Western Ophthalmics, USA) in the DM group.

Biochemical Measurements

Approximately 30 ml of blood was collected from each subject in sterile vacutainer tubes containing serum separator gel without additive. The blood was left to clot at room temperature for 2 hours and then centrifuged at 3500 rpm for 15 minutes. The serum was separated in a laminar flow cabinet and stored at -80°C until analysis. Serum levels of interleukin-1β (IL-1β), IL-2, IL-6, IL-17, IL-17 receptor A (IL-17RA), IL-23, epidermal growth factor (EGF), nerve growth factor (NGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1), matrix metalloproteinase-9 (MMP-9), and fibronectin were measured using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotech Co. Ltd, USA; ELK Biotech Co. Ltd, USA) in accordance with the manufacturer's instructions, and the absorbance values were determined in the CLARIOstar plus microplate reader. The analyte concentration in each sample was determined using a calibration chart based on the standards. All kits have intra- and inter-assay coefficient of variation percentages <10%.

Statistical Analysis

SPSS version 21 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Data are expressed as mean ± standard deviation, range (min-max), and percentage. Categorical data were compared using chi-square and Fisher's exact tests. The Kolmogorov-Smirnov test was used was used to verify whether numerical data were normally distributed. According to the results of normality analysis, the parameters were compared between the groups using parametric one-way ANOVA or non-parametric Kruskal-Wallis tests and Dunn-Bonferroni post hoc analysis. For pairwise comparisons, either Mann-Whitney U or Student's t test was used. Pearson or Spearman correlation analysis was used to examine the correlations between autologous serum contents, dry eye tests, ESSDAI scores, and HbA1c. A p value <0.05 was considered statistically significant.

Results

Twenty-three women with SS (mean age 52.70±7.55 years), 25 women with DR (mean age 56.68±6.53 years), and 23 healthy women (mean age 51.70±9.14 years) were included in the study with no statistical age difference among the groups (p=0.069) (Table 1). Mean SS duration was 6.04±3.62 years and the mean ESSDAI score was 5.95±2.30. Disease activity was low in 39.1% (n=9) and moderate in 47.8% (n=11) of the patients. Patients were using systemic immunosuppressive or immunoregulatory medications including corticosteroids, hydroxychloroquine, methotrexate, and/or azathioprine.

Patients with SS had statistically significantly higher mean OSDI (32.77±16.25) and Oxford staining score (1.47±1.16) and significantly lower mean Schirmer I test result (2.08±1.72 mm/5 min) than in the DR (6.11±4.35, 0.56±0.65, and 10.24±4.63 mm/5 min, respectively) and control groups (6.59±5.06, 0.00±0.00, and 13.30±5.95 mm/5 min, respectively) (p<0.001 for all) (Table 1). Mean TBUT was shorter in both DR (4.20±3.09 s) and SS (3.08±2.08 s) patients compared to the control group (9.00±1.75 s) (p<0.001).

Except IL-23 and IGF-1, there were no significant differences in the levels of inflammatory cytokines and growth factors among the three groups (Table 2). Mean serum IL-23 level was statistically significantly higher in the SS group (156.66±207.94 pg/mL) than the DR and control groups (73.48±95.91 pg/mL and 69.59±105.39 pg/mL, respectively) (p=0.049) Mean serum IGF-1 level was lower in the DR group (12.89±21.09 ng/mL) compared to the SS and control groups (30.77±19.85 ng/mL and 27.08±21.93 ng/mL, respectively; p<0.001).

ESSDAI scores were moderately correlated with TBUT (r=-0.48), serum IL-1 levels (r=0.5), IL-2 levels (r=0.7), and fibronectin levels (r=0.66) (p<0.005 for all). Other biochemical parameters did not show statistically significant correlation with ESSDAI scores.

Within the DR group, 10 patients (40%) had NPDR and 15 patients (60%) had PDR. Patients in the NPDR and PDR subgroups were compared in terms of age, duration of DM, duration of insulin use, HbA1c levels, corneal sensitivity, and macular thickness (Table 3). Other than the duration of DM, there were no differences in duration of insulin use, HbA1c, corneal sensitivity, macular thickness, or dry eye parameters between NPDR and PDR patients (p>0.05). In addition, serum contents did not differ between the NPDR and PDR subgroups (p>0.05) (Table 4).

Table 1. Demographic features and dry eye tests in the control, diabetic retinopathy (DR) and Sjögren's syndrome (SS) groups (mean ± SD)

	Control (n=23)	DR (n=25)	SS (n=23)	p
Age (years)	51.70±9.14	56.68±6.53	52.7±7.55	0.069
OSDI	6.59±5.06	6.11±4.35	32.8±16.2*	**<0.001**
Schirmer (mm)	13.30±5.95*	10.24±4.63*	2.08±1.72*	**<0.001**
TBUT (s)	9.00±1.75*	4.20±3.09	3.08±2.08	**<0.001**
OSS	0.00±0.00*	0.56±0.65*	1.47±1.16*	**<0.001**

*It refers to the group(s) from which the difference originates, **Kruskal-Wallis test.

OSDI Ocular Surface Disease Index, *TBUT* tear break-up time, *OSS* ocular surface staining

Table 2. Serum inflammatory cytokine, growth factor, and fibronectin levels in the control, diabetic retinopathy, and Sjögren's syndrome groups (mean ± SD)

	Control	Diabetic Retinopathy	Sjögren's Syndrome	P value
IL-1 (pg/mL)	6.45±2.12	6.89±2.99	6.31±2.40	0.756
IL-2 (pg/mL)	27.78±21.11	32.02±31.57	20.27±8.75	0.543
IL-6 (pg/mL)	9.94±5.67	11.14±7.13	14.14±12.73	0.554
IL-17A (pg/mL)	22.00±2.02	23.57±7.62	31.48±30.85	0.848
IL-17RA (pg/mL)	592.45±670.75	578.9±530.37	518.17±623.05	0.177
IL-23 (pg/mL)	69.59±105.39	73.48±95.91	**156.66±207.9***	**0.049**
EGF (pg/mL)	210.57±79.12	214.80±56.61	231.82±50.00	0.446
MMP9 (ng/mL)	4.76±3.56	4.06±3.17	3.37±2.61	0.420
Fibronectin (ng/mL)	11.73±7.71	14.23±14.15	12.86±17.44	0.350
IGF-1 (ng/mL)	27.08±21.93	**12.89±21.09***	30.77±19.85	**0.001**
VEGF-A (pg/mL)	53.05±30.79	51.92±34.81	53.80±36.67	0.405
TGF (ng/mL)	6.42±4.93	7.93±9.62	5.36±1.56	0.860
NGF (pg/mL)	117.47±235.00	139.15±276.3	113.41±211.97	0.935

*Indicates the group from which the difference originates.

IL interleukin, *EGF* epidermal growth factor, *MMP-9* matrix metalloproteinase-9, *IGF-1* insulin-like growth factor, *VEGF-A* vascular endothelial growth factor, *TGF* transforming growth factor, *NGF* nerve growth factor

Table 3. Demographic features, corneal sensitivity, macular thickness, and dry eye parameters in non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) subgroups (mean ± SD)

	NPDR (n=10)	PDR (n=15)	p
Age (years)	58.10±3.69	55.73±7.86	0.386
Disease Duration (years)	15.10±8.02	23.60±8.60	**0.020**
Insulin Usage Duration (years)	14.85±9.02	14.36±7.69	0.907
HbA1c (%)	8.52±1.41	9.81±2.24	0.092
Corneal Sensitivity	3.77±2.10	4.03±1.56	0.757
Macular Thickness (µm)	353.30±137.49	363.93±124.83	0.846
OSDI	5.20±4.59	6.71±4.24	0.419
Schirmer(mm)	9.60±4.37	10.66±4.90	0.845
TBUT (s)	4.20±3.04	4.20±3.23	0.932
OSS	0.70±0.48	0.46±0.74	0.203

HbA1c hemoglobin A1c, *OSDI* Ocular Surface Disease Index, *TBUT* break-up time, *OSS* ocular surface stain

Table 4. Serum inflammatory cytokine, growth factor, and fibronectin levels in the non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) subgroups (mean ± SD)

	NPDR (n=10)	PDR (n=15)	p
IL-1 (pg/ml)	5.96±1.65	7.51±3.55	0.157
IL-2 (pg/ml)	25.65±21.97	36.26±36.75	0.739
IL-6 (pg/ml)	12.85±9.74	10.01±4.78	0.868
IL-17A (pg/ml)	21.88±3.33	24.69±9.44	0.134
IL-17RA (pg/ml)	589.66±512.46	571.72±559.69	0.824
IL-23 (pg/ml)	82.15±106.24	67.70±91.78	0.698
EGF (pg/ml)	191.72±74.36	230.18±36.12	0.134
MMP-9 (ng/ml)	3.97±2.94	4.11±3.42	0.912
Fibronectin (ng/ml)	11.49±11.33	16.05±15.86	0.292
IGF-1 (ng/ml)	11.69±15.32	13.70±24.70	0.698
VEGF-A (pg/ml)	44.30±27.75	57.00±38.90	0.292
TGF (ng/ml)	4.93±1.07	9.93±12.14	0.174
NGF (pg/ml)	90.59±141.00	171.52±339.52	0.405

IL interleukin, *EGF* epidermal growth factor, *MMP-9* matrix metalloproteinase-9, *IGF-1* insulin-like growth factor, *VEGF-A* vascular endothelial growth factor, *TGF* transforming growth factor, *NGF* nerve growth factor

Discussion

Autologous serum not only serves as a tear substitute that lubricates the ocular surface, but also contains growth factors, vitamins, and proteins that allow it to replace natural tears (23,27,36). However, the serum composition of a healthy person might differ from that of patients with autoimmune disorders such as SS or diabetic people with end organ involvement. Serum containing unfavorable factors may even exacerbate ocular surface inflammation and lead to adverse effects when applied therapeutically. Some studies in the literature have demonstrated higher concentrations of serum inflammatory cytokines in patients with SS, especially when associated with a rheumatological disorder or during active disease (28,29,37,38). In a study by Ma et al. (28), patients with SS were divided into active and inactive groups based on erythrocyte sedimentation rate and rheumatoid arthritis activity. Although the serum of patients with active disease contained higher levels of IL-β, IL 6, and TNF-a ($p<0.05$), the therapeutic efficacy of autologous serum eye drops was similar in the active and inactive groups.

Hwang et al. (29) compared serum concentrations of proinflammatory cytokines between subjects with primary and secondary SS and evaluated the clinical efficacy of 4 weeks of 50% autologous serum treatment in the management of DED. Using multiplex immunobead assay, TNF-a, IL-1β, IL-6, and IL-8 levels were found higher in secondary SS than primary SS ($p<0.01$). Additionally, ocular symptoms, OSS, and TBUT improved remarkably in patients with primary SS, while no improvement was observed in the secondary SS group ($p>0.05$).

The IL-23/IL-17 pathway plays an important role in the pathogenesis of many autoimmune diseases such as SS, psoriasis, spondyloarthritis, rheumatoid arthritis, Crohn's disease, and multiple sclerosis (37-40). Katsifis et al. (37) showed that IL-17-expressing cells were present in the minor salivary glands of primary SS patients and that IL-17 protein expression increased in parallel with disease activity, as demonstrated by higher biopsy focus scores ($p<0.001$). In addition, serum levels of IL-17, IL-6, IL-23, and IL-12 were significantly elevated with no change in immunomodulatory regulatory T cells. Mean serum IL-23 level was 100 pg/ml in SS patients and 20 pg/ml in healthy subjects.37 Using multiplex assays, López-Villalobos et al. (38) also detected higher IL-23 levels in patients with primary SS (29 pg/ml) compared to control subjects (9 pg/ml). According to principal component analysis, the group with severe disease had higher cytokine concentrations, antibody levels, and damage index scores. In our study, except for IL-23 and IGF-1, the levels of cytokines, growth factors, and fibronectin were similar among the three groups. Consistent with previous studies, we found elevated serum IL-23 levels in SS patients compared to DR patients and healthy subjects ($p<0.05$). IL-17A and IL-6 levels were also higher in the SS group, but the difference was insignificant. All of our SS patients were under systemic immunoregulatory/ immunosuppressive treatment and none had an ESSDAI value greater than

14, which means that their disease was under control.

Additionally, we investigated the relationship between serum components and ESSDAI scores, organ involvement, and dry eye tests. ESSDAI scores showed significant correlations with IL-1, IL-2, and fibronectin levels (r=0.5, 0.7, and 0.66, respectively; p<0.005 for all). No correlation was found between ESSDAI and Schirmer test (r=-0.29), while a moderate correlation was found with TBUT (r=-0.48), and a borderline weak correlation with OSDI (r=0.23)(p<0.05).

The pathogenesis of DR is influenced by a variety of vascular, inflammatory, and neuronal mechanisms. The release of inflammatory mediators is a result of long-term hyperglycemia, which alters retinal vascular hemodynamics. Koleva-Georgieva et al. (39) compared serum cytokine levels in 39 type 2 DM patients (11 without DR, 17 with NPDR, 11 with PDR) and 38 healthy individuals and determined that serum IL-1β, IL-6, TNF-a, and VEGF levels were higher in the DM group (p<0.05). In subgroup analysis, cytokine levels were higher in the PDR subjects than the NPDR subjects (p<0.05 for all). Similarly, Hernández-Da Mota et al. (40) found higher levels of serum TNF-a and IL-6 in the DR group compared to DM subjects without retinopathy and healthy controls, although the difference was statistically significant only for serum TNF-a level (p<0.05). However, such differences were not observed in some studies (40,41). Chen et al. (41) compared serum inflammatory and regulatory cytokines in 29 type 2 DM patients and 30 healthy subjects using Flowcytomix Technology and found no differences in serum cytokine levels except for serum IL-22, which was lower in DM patients. Ozturk et al. (42) compared serum inflammatory cytokines and VEGF among 28 healthy subjects, 31 DM patients without DR, 49 patients with NPDR, and 46 patients with PDR using multiplex bead immunoassay and found no significant differences between the groups except in serum VEGF level, which was higher in DM subjects, with a more pronounced increase in those with PDR. In our study, except for IGF-1, there were no significant differences in serum inflammatory cytokine levels and growth factors between patients with DR and healthy controls. These parameters did not even differ statistically between PDR and NPDR, which might be related to the small number of patients within each group and the wide range of cytokine and growth factor levels.

The multifunctional cell factor IGF-1 plays role in the proliferation, maturation, and migration of cells and is structurally similar to insulin. It is a non-selective polypeptide neurotrophic factor that not only shortens corneal epithelial wound healing time, but also promotes peripheral nerve regeneration by preventing neuronal apoptosis and promoting axon elongation (43). Low IGF-1 was proposed to be associated with poor metabolic control, and some studies revealed an association between low serum IGF-1 levels and the development of type 2 diabetes and DR (44-46). In a survey including 5,511 subjects over 18 years of age, lower serum IGF-1 levels were positively associated with DM (44). In a cohort of 1,126 individuals with type 2 DM (573 without retinopathy, 301 with NPDR, and 252 with PDR) and 348 healthy subjects, IGF-1 was found to increase susceptibility to type 2 DM susceptibility as well as advanced DR, while VEGF-A was associated with early DR stage (45). In another study including 159 patients with and without DR and 110 healthy volunteers, DR patients had lower serum levels of free IGF-1 than diabetics without DR (46). Consistent with these studies, we found lower serum IGF-1 levels in people with diabetes (12.89±21.09 ng/mL) compared to healthy subjects (27.08±21.93 ng/mL) (p<0.001). In diabetes, reduced adhesion of the corneal epithelium to the basement membrane leads to fragile cornea epithelium and poor wound healing. This together with corneal neuropathy makes patients with DM susceptible to refractory corneal erosions and infections (47). In an animal model of NK, substance P and IGF-1 eyedrops were found to restore barrier function and accelerate healing of the corneal epithelium, and promote regeneration of the peripheral nerves by preventing neuronal apoptosis (48). Therefore, the serum of DM subjects with lower levels of IGF-1 might have inferior therapeutic efficacy in corneal healing compared to healthy serum, which necessitates further investigation.

Several ocular and systemic diseases including DM might damage the trigeminal cranial nerve axons and result in the development of neurotrophic keratopathy (NK) (12,13). NGF, which is essential for the development and maintenance of neurons, can be used in the treatment of NK. Lambiase et al. (49) reported complete corneal healing after 10 days to 6 weeks of treatment with topical NGF eye drops in 12 patients with stage 2 and 3 NK, with most showing a remarkable improvement in corneal sensitivity. In a similar study, topical NGF resulted in corneal healing within 6 weeks of treatment in refractory eyes with grade 2 and 3 NK (50). Recently, a recombinant human NGF eye drop (6 times daily for 6 weeks) was shown to restore corneal sensitivity and stimulate corneal healing in 21 patients with NK (most commonly caused by herpetic keratitis) (51). In our study, none of our DM patients had recurrent corneal epithelial defects or neurotrophic keratopathy detected with Cochet-Bonnet esthesiometer. Mean serum NGF levels were similar in people with DR and healthy subjects. Only one patient with diabetic foot involvement was found to have serum EGF, TGF-β1, and NGF levels that were markedly below average. This suggests that the autologous serum of diabetic subjects may be used in DED. However, further studies are needed to better understand the variations in cytokines and growth factors in diabetic patients, especially those with neuropathy.

Serum fibronectin of elevated molecular weight was proposed as one of the diagnostic criteria in SS patients. (52) Silvestre et al. (53) reported the presence of multiple protein bands, including fibronectin, in the saliva of patients with SS which were not present in healthy individuals, and fibronectin fragments were

more notable in active disease. The authors concluded that the presence of fibronectin peptides is a potential indicator of salivary gland destruction. In our study, there was no difference in serum fibronectin levels between the SS group and healthy subjects. However, we showed a statistically significant correlation between ESSDAI score and serum fibronectin levels (r=0.66), which supports the hypothesis that fibronectin may be a biomarker of disease activity ($p<0.05$). Fibronectin has also been shown to have a role in the development of DR (54-56). Seghieri et al. (54) evaluated the relationship between plasma fibronectin and vascular complications of DM and found higher fibronectin levels in the diabetic group; however, no association was found with DM stage. Lee et al. (55) investigated whether type IV collagen and fibronectin plasma levels were related to the presence of diabetic microangiopathy. They found higher serum fibronectin and collagen type 4 levels in diabetic patients, although fibronectin levels did not differ between diabetic patients with and without microangiopathy. In this study, we also found higher serum fibronectin levels in the PDR group than in the NPDR and control groups, but the difference was not significant ($p>0.05$).

In conclusion, except IL-23 and IGF-1, we detected no significant differences in serum inflammatory cytokine levels or growth factors among inactive SS patients under treatment, patients with DR, and healthy individuals. Therefore, autologous serum seems to be a good option to replace deficient tear fluid in these subjects.

Author Contributions

Conception: Şule Nur Acar Duyan, Banu Bozkurt, Data Collection and Processing: Şule Nur Acar Duyan, Ali Ünlü, Design:Şule Nur Acar Duyan, Banu Bozkurt, Supervision: Banu Bozkurt, Sema Yılmaz, Analysis and Interpretation: Şule Nur Acar Duyan, Banu Bozkurt, Literature Review: Şule Nur Acar Duyan, Writer: Şule Nur Acar Duyan, Critical Review: Banu Bozkurt, Yalçın Karaküçük

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