Original Article

Taiwan J Ophthalmol 2017;7:213-220

Access this article online

Quick Response Code:

Website:
www.e-tjo.org

DOI:
10.4103/tjo.tjo_102_17

Serum components and clinical efficacies of autologous serum eye drops in dry eye patients with active and inactive Sjogren syndrome

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Abstract:

PURPOSE: Autologous serum eye drops are considered safe and efficient for the treatment of various ocular surface disorders, including dry eye diseases (DED) caused by the primary and secondary Sjogren syndrome (SS). However, the serum components in patients of SS may be different from those of normal patients and can thus lead to unpredictable therapeutic effects. This study divided the SS patients into active and inactive types based on the erythrocyte sedimentation rate and the presence or absence of active rheumatoid arthritis.

METHODS: We compared the serum components of these two groups with standard and multiplex enzyme linked immunosorbent assay arrays and predicted the therapeutic effects of topical autologous serum for the treatment of DED with ocular surface disease index (OSDI) and Oxford Schema scale (OSS).

RESULTS: Hyaluronic acid and transforming growth factor b1 levels were significantly higher in the active SS group compared to the inactive SS group ($P < 0.01$), whereas epidermal growth factors, insulin growth factor 1, and fibroblast growth factor b had no significant differences between these two groups. Active SS group had significantly higher expressions of interleukin (IL) 1 beta, IL 6, and tumor necrosis factor alpha compared to inactive SS patients ($P < 0.05$). There were no statistical differences in therapeutic effects between these two groups, as measured with the OSDI or OSS.

CONCLUSION: Dividing the Sjogren dry eye patients into active and inactive groups may appear as a reasonable method to predict the quality of autologous serum eye drops, but there seems to be no significant predictability to the therapeutic effects.

Keywords:

Autologous serum, cytokine, dry eye, growth factor, Sjogren syndrome

Introduction

Dry eye disease (DED) is common and has prevalence rates of 7.4%–33.7% among the general population based on different populations and time periods.[1,2] The Beaver Dam population-based study found the DED prevalence rate to be 14% in adults 48–91 years of age,[3] and the Shipai study in the elderly population in Taiwan reported a prevalence of 33.7% with significantly more women than men.[1]

The etiologies and pathogenesis of DED have been long studied and are believed to be multifactorial.[4-8] Various treatment options were proposed, targeting different classifications and severities of the disease. These include lubrication for aqueous deficiency; lid cleansing, expression, and hot compression for meibomitis; tetracycline, steroids, cyclosporine, and other anti-inflammatory agents for halting the inflammation propagation on the ocular surface.[9-13] It has also been verified in tear component studies that inflammatory

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Submission: 15-10-2017
Accepted: 24-10-2017

How to cite this article: Ma IH, Chen LW, Tu WH, Lu CJ, Huang CJ, Chen WL. Serum components and clinical efficacies of autologous serum eye drops in dry eye patients with active and inactive Sjogren syndrome. Taiwan J Ophthalmol 2017;7:213-20.

cytokines and chemokines were elevated in the DED group.[14,15] In particular, T-cell recruitment plays an important role on the ocular surface condition in DED.[16-19] This finding provides evidence for treating DED patients with anti-inflammatory eye drops.[10,13]

In addition to the abovementioned treatment strategies, autologous serum has been used to treat patients with severe DED.[20-24] The effect of autologous serum lies in its content: Abundant epitheliotrophic growth factors including epithelial growth factor (EGF) and transforming growth factor (TGF); vitamin A and extracellular matrix molecules such as hyaluronic acid (HA). All these components were reported to facilitate the growth of epithelium.[23,25-27] Serum also contains bactericidal components that have been shown to prevent infectious processes,[23,24,27] as well as inhibitors of inflammatory cytokines.[24,25,28,29] Another advantage of autologous serum lies in its biomechanical properties. With pH level and osmolarity similar to those of natural tears,[25] autologous serum eye drops serve as a good tear substitute. Finally, the autologous serum is devoid of additives such as stabilizers or preservatives. The positive effects of autologous serum on ocular surface disorders could be demonstrated at a cellular level, and included goblet cell regeneration, decreased dysmorphic ocular surface epithelium, and the inhibition of apoptosis on the ocular surface cells during stress.[20,30,31]

In spite of wide clinical usage, topical autologous serum was recently found to produce variable treatment responses in patients with different etiologies of DED.[17,32] Serum of patients with secondary Sjogren syndrome (SS) was reported to have elevated pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-a), interleukin-1beta (IL-1b), IL-6, and IL-8. Accordingly, the secondary SS patients were suspected to respond poorly to autologous serum for the treatment of DED when compared with patients with primary SS.[32-34] Bradley *et al*., however, found no significant difference between serum growth factor levels in dry eye syndrome patients versus controls.[35]

In the clinic, not all DED patients have the same clinical response after using topical autologous serum for treatment. We thus believed that the quality and efficacy of autologous serum might not be the same for patients with different etiologies and severities of DEDs. To the best of our knowledge, no prior study has analyzed the serum components and the treatment effects of autologous serum for active and inactive SS. We thus classified our SS patients into active and inactive groups, analyzed their serum components and correlated their serum condition with the treatment outcome. Through this study, we aimed to provide a simple screening method to individualize therapy and determine the DED patients with SS who would likely benefit from topical autologous serum.

Materials and Methods

Patient enrollment and group sorting

From June 2015 to February 2016, the study enrolled 21 patients with primary or secondary SS diagnosed according to European Classification Criteria for Primary and Secondary SS.[21] All enrolled patients had severe DED that was intractable with topical lubricants. All patients continuously used autologous serum eye drops for at least 6 months. Twenty percent autologous serum drop was prescribed every 2 h during waking hours. This study was conducted under the guidance of Declaration of Helsinki and acquired written consent from each patient.

Enrolled patients were divided into active or inactive SS by the level of erythrocyte sediment rate (ESR). All patients, regardless of the underlying etiology of SS, were allocated to the active group for ESR higher than age plus ten, divided by two in women; and higher than age divided by two in men.[36] Patients who had rheumatoid arthritis with active inflammatory arthritis confirmed in a rheumatologist's clinic were also sorted into the active SS group.[37] Those patients who did not qualify for the active group were sorted into the inactive group

Measurement of dry eye severity

All patients took the questionnaire for ocular surface disease index (OSDI)[38] to derive a subjective symptomatic score and answered the preferred dry eye treatment modality questions according to their previous treatment experience in our hospital. The objective ocular surface condition was measured by Oxford Scheme Scale (OSS).[39] The measurement result used for analysis was the average of both eyes.

Serum preparation

Blood draw was performed on patients by venipuncture after at least 8 h of fasting. Twenty milliliters of full blood were acquired from each patient. The samples were set to settle in room temperature (20°C –25°C) for 2 h, then centrifuged at 3000 g for 15 min. The serum was then processed with sterile technique and stored at −80°C before further analysis.

Quantification of serum elements

IL-1b, IL-2, IL-4, IL-6, CXCL8 (IL-8), IL-17, tumor necrosis factor-a (TNF-a), EGF, and TGF-b1 were measured by a customized membrane array (Quantibody® custom array, RayBiotech) and signal scanned with GenePix® 4000B Microarray Scanner. HA, insulin growth factor-1 (IGF-1), and fibroblast growth factor-basic (FGF-b) were

quantified with standard enzyme-linked immunosorbent assay (ELISA), according to individual manufacturer's instructions (Biotech Trading Partner®, R and D Systems, Inc., and BioLegend, Inc., respectively).

Statistical analysis

Scores of OSDI, grades of OSS, and the concentrations of IL-1b, IL-2, IL-4, IL-6, CXCL8 (IL-8), IL-17, TNF-a, EGF, HA, IGF-1, and FGF-b were compared between active and inactive SS using Wilcoxon rank test. Statistical significance was considered to be a $P < 0.05$.

Results

Among 21 Sjogren dry eye patients under autologous serum treatment in this study, fifteen were sorted into the inactive SS group and six were sorted into the active SS group. All patients in the active disease group were secondary SS patients, whereas only 6 among 15 patients in the inactive SS group had secondary SS. The demographic data and clinical manifestations of these 21 patients are shown in Table 1, which reveals a predominance of female and middle-aged patients in the study cohort.

Table 2 provides a comparison of the epitheliotrophic factor levels that were found in the serum of these two groups. HA and TGF-b1 levels were significantly higher in the active SS group compared to the inactive SS group (P = of 0.02 and 0.01, respectively). In comparison, there were no significant differences for EGF, IGF-1, and FGF-b between these two groups ($P > 0.05$).

Table 3 provides a comparison of the pro-inflammatory cytokines in the serum of these two groups. Active SS group had significantly higher expressions of IL-1b, IL-6, and TNF-alpha compared to the inactive SS group ($P < 0.05$). For other proinflammatory cytokines, there was also a trend toward higher expressions in the active SS group, but the results did not meet statistical significance ($P = 0.058$, $P = 0.099$ and $P = 0.092$, respectively).

In Tables 2 and 3, we show the median expression levels of each component. The results are also expressed by means ± standard deviations

Figures 1 and 2 are boxplots for epitheliotrophic factors and pro-inflammatory cytokines. The active SS group tended to have wider spread of data for most growth factors and cytokines examined in this study. The expression levels of HA, TGF-b1, IL-1b, IL-6, and TNF-a were significantly higher in the active SS group. Figure 3 is the boxplot for the treatment outcomes that were assessed using OSDI and OSS. There seems to be no statistical differences between the two groups of SS patients.

Table 1: Demographics and disease activity in patients with Sjogren dry eye

Disease activity	Age	Sex	ESR	Active arthritis documented[a]	Diagnosis
Inactive	63	Female	NA	None	RA
Inactive	78	Female	13	None	Primary SS
Inactive	66	Female	13	None	Primary SS
Inactive	62	Female	20	None	Primary SS
Inactive	81	Female	NA	None	Primary SS
Inactive	72	Female	7	None	Primary SS
Inactive	81	Female	31	None	Primary SS
Inactive	73	Female	10	None	RA
Inactive	74	Female	22	None	RA
Inactive	48	Male	6	None	Primary SS
Inactive	63	Female	25	None	Primary SS
Inactive	51	Female	12	None	RA
Inactive	60	Female	NA	None	SLE
Inactive	39	Female	2	None	RA
Inactive	67	Female	18	None	Primary SS
Active	64	Female	35	Both arthralgia and tenderness	RA
Active	63	Female	6	Both arthralgia and tenderness	RA
Active	34	Female	544	None	SLE
Active	59	Female	29	Both arthralgia and tenderness	RA
Active	41	Female	21	Both arthralgia and tenderness	RA
Active	57	Female	76	None	RA

[a]Active arthritis documented by rheumatologists. RA=Rheumatoid arthritis, SLE=Systemic lupus erythematosus, SS=Sjogren syndrome, ESR=Erythrocyte sedimentation rate, NA=Not available

Table 2: Concentrations of epitheliotrophic factors and extracellular matrix components in patients with active and inactive Sjogren dry eyes

	Inactive SS		Active SS		*P**
	Mean±SD	Median	Mean±SD	Median	
IGF1 (pg/mL)	3645.8±1821.4	3409.6	2823.6±1545.5	3473.9	0.24
HA (ng/mL)	121.5±87.7	85.8	250.6±131.1	241.0	0.02
FGF-b (pg/mL)	1065.4±983.5	607.0	4383.5±7253.6	1153.2	0.59
EGF (pg/mL)	1499.7±514.6	1431.0	1869.2±764.1	1789.0	0.32
TGFb1 (pg/mL)	1406.0±980.9	1377.0	3701.0±1988.8	2994.0	0.01

*Wilcoxon rank test. SS=Sjogren syndrome, IGF-1=Insulin growth factor-1, HA=Hyaluronic acid, FGF-b=Fibroblast growth factor-b, EGF=Epidermal growth factor, TGFb1=Transforming growth factor, SD=Standard deviation

Discussion

Topical autologous serum was first used to treat ocular chemical burns back in the 1970s.[40] Soon after, the therapeutic indications broadened to cover persistent or recurrent epithelial defects,[26,41-43] neurotrophic keratopathy,[44] superior limbic keratoconjunctivitis,[45] and various etiologies of DED.[20-22,25] The advantages of topical autologous serum in treating ocular surface diseases can be explained by the abundant corneal epitheliotrophic factors that facilitate epithelium growth,[23-27] its similar biomechanical and biochemical properties to those of tears,[23,25] and its absence of

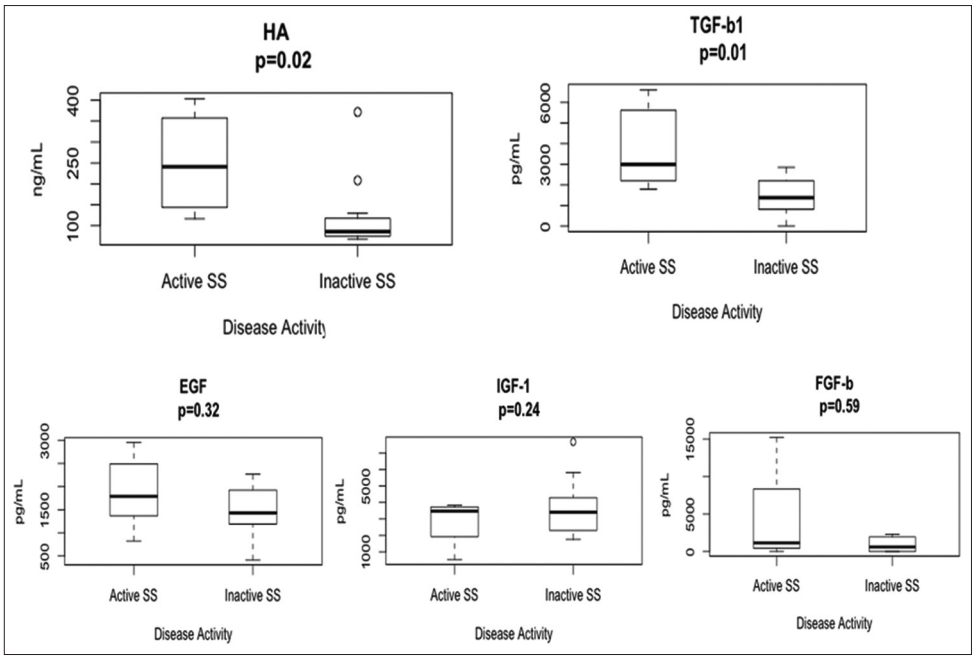

Figure 1: Boxplots for epitheliotrophic factors in serum of active and inactive SS. SS: Sjogren syndrome, IGF-1: Insulin Growth factor-1, HA: Hyaluronic acid, FGF-b: Fibroblast growth factor-beta, EGF: Epidermal growth factor, TGFb1: Transforming growth factor

Table 3: Concentrations of pro-inflammatory cytokines in patients with active and inactive Sjogren dry eyes

	Inactive SS		Active SS		*P**
	Mean±SD	Median	Mean±SD	Median	
IL17 (pg/mL)	14.4±15.2	5.0	9.8±10.2	9.0	0.34
IL1b (pg/mL)	2.1±1.6	2.0	14.7±14.1	9.5	0.01
IL2 (pg/mL)	12.7±7.1	13.0	21.3±9.9	19.5	0.06
IL4 (pg/mL)	1.9±2.3	1.0	5.2±3.9	4.5	0.10
IL6 (pg/mL)	4.8±2.9	6.0	20.3±17.8	11.0	0.004
IL8 (pg/mL)	46.0±26.7	46.0	80.2±48.6	76.5	0.09
TNFa (pg/mL)	116.5±66.0	109.0	244.7±87.3	220.0	0.002

*Wilcoxon rank test. IL=Interleukin, TNFa=Tumor necrosis factor-alpha, SD=Standard deviation, SS=Sjogren syndrome

stabilizers and preservatives that are associated with corneal toxicity.

Recently, different expression levels of tear cytokines were found and compared among the normal population, non-Sjogren dry eye patients and Sjogren dry eye patients.[15,46-48] Accordingly, the serum levels of cytokines in different patients could be different and the measurements could be used to guide the usage of autologous serum eye drops to prevent possible side effects. It has been reported that the levels of IL-1b, IL-2, IL-4, IL-6, IL-17A, TNF-a, and TNF-b were elevated in patients with SS.[49-52] Both IL-1b secreting and TNF-a secreting circulating lymphocytes significantly increased in Sjogren dry eye patients.[51] Moreover, the amount of IL-1b secreting lymphocytes in the peripheral blood correlated with the SS disease status.[51] Recently, Hwang *et al*. looked into the differences between primary and secondary SS. They noticed the elevation of tumor necrosis factor a, IL-1b, IL-6, and IL-8 in the serum of patients with secondary SS.[32] However, many Sjogren dry eye patients may have received treatments for their autoimmune disease before visiting the eye clinic. The disease activity, in addition to the classification of SS, should be noted before the use of autologous serum eye drops for DED.

In this study, we collected all DED patients with primary and secondary SS and simply divided them into active and inactive groups according to the levels of ESR. We used the ESR elevation criteria[36] and the clinical observation of active arthritis by rheumatologists as the definition of active SS. This classifying method is convenient for ophthalmologists since they do not review the complex systemic and medication histories of these patients before prescribing topical autologous serum for the treatment of their DED. In this study, we evaluated several important epitheliotrophic growth factors and extracellular matrix components by membrane array and ELISA. The results showed no significant differences in the expression of EGF, FGF, and IGF between active and inactive SS, whereas the level of HA and TGF-b were significantly higher in the active SS group.

The expression of EGF, FGF, and IGF has been associated with epithelial proliferation and migration.[53-55] HA is an extracellular matrix component as well as an indicator of connective tissue turnover. Although the serum level of HA was not elevated in the primary SS patients presented with dry mouth,[56] it was well-documented to be elevated in rheumatoid arthritis, chronic liver and lung diseases.[56-58] TGF-b1 regulates the epithelial and

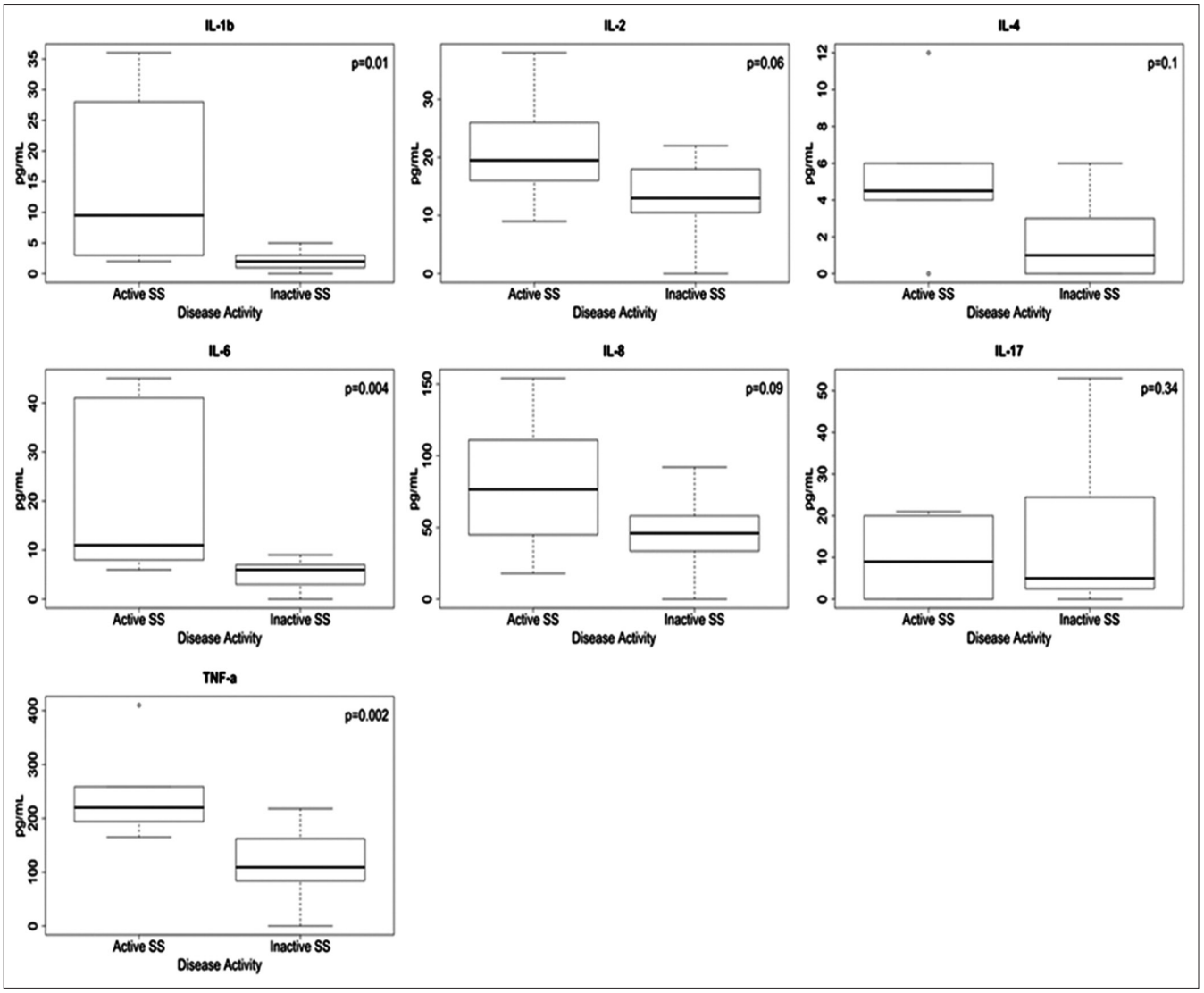

Figure 2: Boxplots for pro-inflammatory factors in serum of active and inactive SS. IL: Interleukin, TNFa: Tumor necrosis factor-alpha

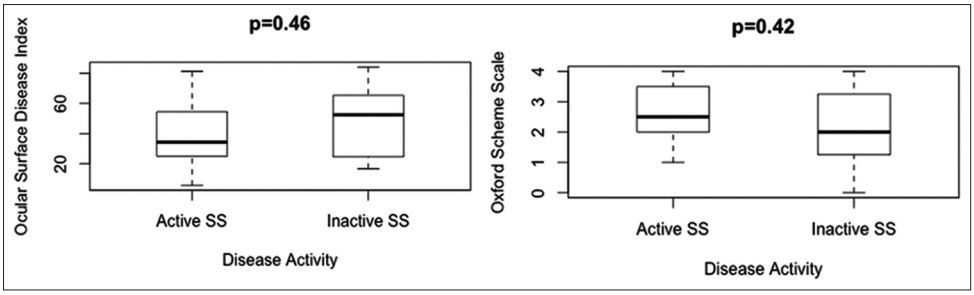

Figure 3: Boxplots for treatment outcomes using serum of active and inactive Sjogren syndrome

stromal repair[59] and limits self-immunity by decreasing the self-reactive T-cells.[49,60,61] It may also regulate IL-17, which leads to excessive metalloproteinase production and can cause ocular surface disruption.[62-65] The upregulation of HA and TGF-b1 may thus theoretically influence the therapeutic effects of topical autologous serum.

Clinical observation of the poorer response to autologous serum in patients with secondary SS compared to those with primary SS has been reported,[32] and the elevated levels of proinflammatory cytokines were thought to play a key role.[17,32,33,66,67] It has been demonstrated that patients receiving anti-inflammatory treatments may have decreased inflammatory cytokines in tears.[68] In

our study, we demonstrated the elevation of IL-1b, IL-6, TNF-alpha, IL-2, IL-4, and IL-8 in serum of active SS (with the first three factors achieving statistical significance). The elevation of these cytokines in active SS may damage the corneal surface and lead to harming effects from the use of autologous serum eye drops. To the best of our knowledge, no prior study had assessed the different levels of pro-inflammatory cytokines in the autologous serum of active and inactive SS patients. This study results suggest that classifying SS patients according to their disease activity may provide a good method to predict the quality of topical autologous serum eye drops.

In this study, we not only measured the serum components in patients with active and inactive SS but also correlated the serum quality to the treatment results. Our results indicated no significant differences in OSDI and OSS between the active and inactive SS groups. Several reasons may explain this discrepancy between the serum components and the therapeutic results. First, the small sample size of the study could have led to poor statistical power. Second, the wide distribution of the measured data for serum components, especially in the active SS group, could have masked the real effects. Third, the systemic autoimmune medications could have accumulated in the serum and affected the ocular surface condition after it was used as topical eye drops. Finally, SS is a complex disease entity and patients' lifestyles and habits of drug usage could all affect the study outcomes.

Conclusion

We devised a simple way of classifying patients with SS into active and inactive groups according to their ESR and the presence or absence of active rheumatoid arthritis. The two groups were found to have different levels of serum components, with the inactive group exhibiting better corneal epitheliotrophic abilities and lesser harmful effects. However, the differences in serum quality did not seem to lead to different therapeutic effects. Further large-scaled studies are needed to confirm our study results.

Financial support and sponsorship

Supported, in part, by the National Taiwan University Hospital(NTUH) Plan Asia One, the Department of Medical Research at NTUH and NTUH grant 106-S3519.

Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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