Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(3):944-7

Evaluation of adropin and clusterin levels in patients with Sjögren's syndrome

Turan Akdag[1], Dilek Tezcan[2], Ali Unlu[3], Duygu Eryavuz Onmaz[3], Zeynep Ozel[4]

[1]Necmettin Erbakan University, Meram Vocational School, Department of Herbal and Animal Production, Konya, Turkey
[2]Selcuk University, Faculty of Medicine, Department of Rheumatology, Konya, Turkey
[3]Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya, Turkey
[4]Selcuk University, Faculty of Veterinary, Department of Biostatistics, Konya, Turkey

Received 02 November 2021; Accepted 02 April 2022
Available online 13.06.2022 with doi: 10.5455/medscience. 2021.10.358

Abstract

Sjögren's syndrome (SS) is a progressive autoimmune disease characterized by lacrimal and salivary gland dysfunction. However, the underlying causes of the pathophysiology of SS disease are relatively unclear. New investigations suggest an association exists between the pathogenesis of SS disease and cytokines. This study purposes to investigate and examine the serum adropin and clusterin serum levels in patients with SS. A total of 88 participants, 44 patients with SS disease (2 men and 42 women) and 44 healthy subjects (2 men and 42 women) were enrolled in the study. Patients' ages, age of disease onset, gender, SS disease duration, localization, and social history were recorded. Adropin and clusterin levels were determined using the enzyme-linked immunosorbent assay (ELISA) method. The adropin levels were measured as 110.43 ng/L in the SS patient group and 80.06 ng/L in the healthy control group. A remarkable increase was determined in terms of the SS group's adropin levels compared to the healthy subjects (p=0.047). The clusterin levels were minimally higher in the SS patients compared to the non-SS patients, however, these differences were not statistically significant (p=0.110; 98.41 and 84.59 ng/ml, respectively). The average disease duration in the SS patient group was determined as 46.68 months. Also, the average Eular Sjögren's syndrome disease activity index (ESSDAI) in the patient with SS was found to be 5.98. Elevated adropin levels were determined in the SS patients. Thus, serum adropin may be a potential biomarker of SS disease. To clarify these findings, further investigations are needed.

Keywords: Sjögren's syndrome, adropin, clusterin

Introduction

Sjögren's syndrome (SS) is a progressive autoimmune disease characterized by lacrimal and salivary gland dysfunction [1]. As is common, SS patients experience dry eyes (xerophthalmia) and dry mouth (xerostomia) as a result of inflamed salivary and lacrimal glands [2]. Moreover, dry mouth and dry eyes may occur as a result of lymphocyte infiltration and exocrine gland dysfunction [3]. SS disease occurs in the glands and can involve the nervous, musculoskeletal, genitourinary, hematological, and vascular systems [4]. The prevalence of SS disease in the community has been reported at approximately 0.5%. Also, SS disease has been observed more frequently in women than men (women-to-men ratio=9:1) [5].

*Corresponding Author: Turan Akdag, Necmettin Erbakan University, Meram Vocational School, Department of Herbal and Animal Production, Konya, Turkey
E-mail: turanakdag570@gmail.com

The pathogenesis of SS disease has been not fully elucidated. While some reasons are effective in the disease pathogenesis, the primary cause of SS is difficult to determine. The American/European Consensus Conference developed classification criteria for SS disease [6]. SS disease is described as a complex disease triggered by genetic predisposition and environmental factors. Cytokines and some proteins are regulators of innate and adaptive immunity. Moreover, they play critical roles in the direction, control, amplitude, and course of inflammatory responses [7]. As such, newly identified cytokines and proteins may contribute to the diagnosis and prognosis of SS disease.

As a result of the energy homeostasis-associated (ENHO) gene, adropin consists of two exons on chromosome 9p13.3 in humans [8]. Adropin has a molecular weight of 4499.9 Da and is made up of 76 amino acids [9]. Recent studies have revealed that adropin plays a regulatory role in lipid metabolism regulation, insulin resistance development, and vascular endothelial cell protection [10]. Zhang et al. declared adropin to be able to decrease macrophage

infiltration by reducing fat accumulation [11]. As a chaperone protein, clusterin is expressed in many tissues and is encoded in chromosomes 8p12-8p21. This protein has a glycoprotein structure, consisting of 449 amino acids with a molecular weight of 75-80 kDa [12]. In vitro studies have demonstrated clusterin expression to be able to increase in conditions of cellular stress like heat shock and oxidative stress [13]. Moreover, clusterin may be involved in many physiological events such as inflammation, apoptosis, complement regulation, lipid transport, tissue remodeling, and differentiation [14]. Based on the literature, some inflammatory markers play crucial roles in the progression of SS disease. Kim et al. declared NOD-like receptor protein 3 (NLRP3) to be upregulated in primary SS patients and to also be associated with disease activity [16].

Research has reported adropin levels to decrease in various inflammatory diseases, having a negative correlation with the expression levels of inflammatory cytokines and the anti-inflammatory process. Adropin has also been declared to display a very important regulative role in inflammatory factors and immunity [19]. Significant changes have been reported in plasma protein oxidation, TNF-α, nitrotyrosine, myeloperoxidase activity, and GSH levels in SS patients, and an association has also been proposed to exist between oxidative stress and SS disease [20].

The present study aims to determine serum adropin and clusterin levels in SS patients and to evaluate their possible relation to SS disease.

Materials and Methods

Forty-four patients (age>18 years) diagnosed with SS disease in the rheumatology clinic of Selcuk University Medical Faculty Hospital were enrolled in the study. The control subjects were formed from 44 participants (ages and gender matching the SS group) without any known systemic or rheumatological disease. The control group sample consists of participants who'd been admitted to the rheumatology outpatient clinic for routine check-ups. Age, age of disease onset, gender, disease duration (in months), localization, social history, and Eular Sjögren's syndrome disease activity index (ESSDAI) of the SS patients were questioned and recorded. ESSDAI scores describe the following: ESSDAI<5=low activity, 5≤ESSDAI≤13=moderate activity, and ESSDAI≥14=high activity [15]. Participants with congestive heart failure, hypertension, hyperlipidemia, chronic renal failure, diabetes mellitus, thyroid disorders, cerebrovascular diseases, or peripheral vascular diseases were excluded from the study. Obtained blood samples were placed in biochemistry tubes and centrifuged at 3.000 rpm for 12 minutes at 5°C. Then the sera were placed in serum tubes and kept at -80°C until the assay. The study approval was obtained from the local ethics committee of Selcuk University Faculty of Medicine (Approval date and number: 04.03.2021/2021-114). Written and informed consent was obtained from all participants.

Measurements of serum adropin and clusterin

The measurement for adropin was measured using the human adropin enzyme-linked immunosorbent assay commercial kit (Human Adropin ELISA Kit, Bioassay Technology Laboratory, Shanghai, China Cat No: E3231Hu). The minimum detectable concentration was 2.49 ng/L, the detection sequence was 5-10 ng/L, and intra- and inter-assay precision was <8% and <10%, respectively. The clusterin levels were determined using the human clusterin enzyme-linked immunosorbent assay commercial kit (Human Clusterin ELISA Kit, Bioassay Technology Laboratory, Shanghai, China Cat No: E1189Hu) following the manufacturer's instructions. The minimum detectable sensitivity was 0.31 ng/ml, detection sequence was 0.5-300 ng/ml, and intra- and inter-assay precision was <8% and <10%, respectively. The absorbance of samples was determined using a microtiter plate reader at 450 nm (ELx800TM, Bio-Tech Instruments, USA).

Statistical analysis

Statistical analysis was performed with SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. NY, IBM Corp). Variables were determined as mean±standard deviation and mean (maximum-minimum) percentage and frequency values. Homogeneity of the variance was performed using Levene's test. Normality was checked using the Shapiro-Wilk test. To compare the differences between the two groups, the Mann-Whitney-U test was used for the nonparametric test prerequisites. Cut-off scores for the patients and the healthy individuals were evaluated by measurement parameters using ROC analysis.

Results

The characteristic features and laboratory measurements for the SS patients and healthy subjects are given in Tables 1 and 2, respectively. The mean age of the 88 participants was found to be 47.92 years. The average disease duration (mean) was determined as 46.68 months in the SS patient group. In addition, the mean ESSDAI was found to be 5.98 in the SS patient group. As shown in Table 3, the serum adropin levels were measured as 110.43 ng/L in the SS patients group and 80.06 ng/L in the healthy subject group. A significant increase was determined in terms of adropin levels in the SS group, compared to the subject group (p=0.047). The clusterin level showed a minimal increase in the SS patients compared to the non-SS group, however, this difference is not statistically significant (p=0.110, 98.41, and 84.59 ng/ml, respectively). As shown in Table 5, a negative correlation exists between clusterin level and age (p=0.036) and a negative correlation between clusterin level and disease duration (p=0.050). Also, the AUC (area under the curve), selectivity, and sensitivity values are shown in Figure 1.

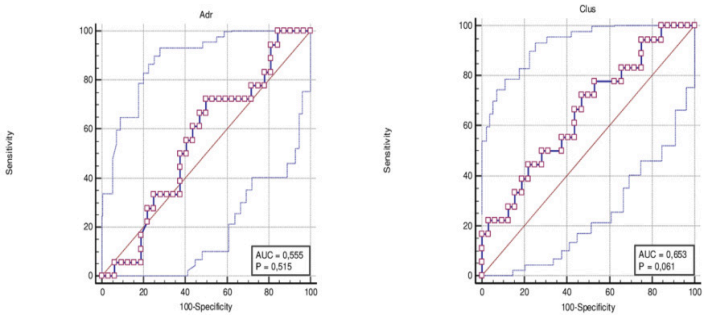

Figure 1. ROC (Receiver operating characteristic) analyses for adropin and clusterin

Table 1. Characteristics of the SS patients and healthy group

	n	Minimum	Maximum	Mean	Std. Deviation
Age, years	88	24.00	71.00	47.92	12.40
Duration time of disease, month	44	12.00	96.00	46.68	22.84
ESSDAI, score	44	3.00	16.00	5.98	3.22
Adropin, ng/L	88	4.70	598.04	182.21	176.46
Clusterin, ng/ml	88	43.22	198.27	95.36	33.55
Hgb, g/dl	44	9.9	17.4	12.93	1.34
WBC, $10^3/mm^3$	44	3.2	11.4	6.06	1.75
PLT, $10^3/mm^3$	44	77	427	243.76	69.20
NEU#, $10^3/mm^3$	44	1.6	8.9	3.41	1.31
LYM#,$10^3/mm^3$	44	0.7	4.4	1.95	0.68
MO#, 103/mm^3	44	0.1	1.1	0.53	0.18
CRP, mg/L	44	1.0	31.0	5.55	6.07
ESR, mm/hour	44	2	94	21.22	16.97

ESSDAI: EULAR Sjögren's syndrome disease activity index, Hgb: hemoglobin, WBC: white blood cell, PLT: platelet, NEU#: neutrophil, LYM#: lymphocyte, MO#: monocyte, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

Table 2. Descriptive statistics regarding adropin and clusterin for both groups

Group		n	Minimum	Maximum	Mean	Std. Deviation
Control	adropin	44	4.70	535.82	147.38	161.71
	clusterin	44	44.79	165.25	90.62	34.11
Patient	adropin	44	18.50	598.04	217.04	185.16
	clusterin	44	43.22	198.27	100.09	32.63

Table 3. Comparison of adropin and clusterin values between the SS patients and healthy group

	Control	Patient	Test Statistics	p
Adropin, ng/L	80.06 (4.70-535.82)	110.43 (18.50-598.04)	-1.989	0.047€*
Clusterin, ng/ml	84.59 (44.79-165.25)	98.41 (43.22-198.27)	-1.596	0.110€

*p<0.05, €Mann Whitney-U

Table 4. Predictive power of adropin and clusterin regarding SS disease

	cut-off	AUC(95%CI)	p	sensitivity	specificity	PPV	NPV
Adropin	202.948	0.555(0.407-0.695)	0.515	72.22%	50.00 %	44.8 %	76.2%
Clusterin	86.246	0.653(0.505-0.782)	0.061	72.22%	53.13 %	46.4%	77.3%

AUC: area under the curve, CI: confidence interval, NPV: negative predictive value, PPV: positive predictive value

Table 5. Correlations of clinical and demographic variables

		Clus	Adr	Hgb	WBC	PLT	NEUT	LYMP	MONO	CRP	ESR	Age
Adr	r	0.077										
	p	0.595										
Hgb	r	0.113	0.003									
	p	0.435	0.982									
WBC	r	0.010	-0.123	.366**								
	p	0.946	0.396	0.009								
PLT	r	-0.179	-0.056	-0.083	0.263							
	p	0.214	0.701	0.566	0.065							
NEU	r	0.076	-0.114	.440**	.879**	0.229						
	p	0.599	0.431	0.001	0.001	0.109						
LYM	r	-0.025	-0.079	0.238	.787**	0.259	.455**					
	p	0.863	0.586	0.097	0.001	0.069	0.001					
MO	r	-0.031	-0.122	0.173	.749**	0.228	.643**	.550**				
	p	0.830	0.397	0.229	0.001	0.111	0.001	0.001				
CRP	r	0.096	-0.009	0.237	.340*	0.181	.296*	.293*	0.225			
	p	0.509	0.953	0.098	0.016	0.209	0.037	0.039	0.116			
ESR	r	-0.167	-0.104	-0.268	0.018	0.223	0.154	-0.209	-0.082	0.139		
	p	0.246	0.472	0.060	0.902	0.120	0.286	0.145	0.573	0.337		
Age	r	-.297*	0.094	0.220	-0.001	-0.141	-0.103	0.115	0.037	0.149	-0.076	
	p	0.036	0.515	0.125	0.996	0.329	0.477	0.427	0.801	0.302	0.602	
DT of D	r	-0.279	0.018	-0.091	-0.203	.304*	-0.181	-0.178	-0.198	0.099	0.147	0.123
	p	0.050	0.904	0.529	0.158	0.032	0.207	0.215	0.168	0.493	0.309	0.393

Adr: adropin, Clus: clusterin, Hgb: hemoglobin, WBC: white blood cell, PLT: platelet, NEU: neutrophil, LYM: lymphocyte, MO: monocyte, CRP: c-reactive protein, ESR: erythrocyte sedimentation rate, DT of D: duration time of disease

Discussion

The study determined a significant increase to be present in terms of the adropin levels in the SS patients. Moreover, clusterin levels were minimally higher in the SS patients compared to the non-SS group. Also, negative correlations were observed between clusterin level and age; and between clusterin level and disease duration.

A recent study declared serum lipoprotein-associated phospholipase A2 (Lp-PLA2) to possibly serve as a new biomarker for B cell lymphoproliferation in SS disease [19]. Recently, a study proposed that patients with primary SS had a higher prevalence of carotid atherosclerosis and higher serum levels of calprotectin [20].

Chen et al.'s study demonstrated low adropin levels to be related to nonalcoholic fatty liver disease and oxidative stress severity [21]. Another study emphasized adropin levels to be elevated in systemic sclerosis and Behçet's disease patients compared to the healthy controls ($p=0.023$ and $p<0.001$, respectively) [22]. A recent study observed higher levels of adropin in Kawasaki disease (KD) compared to the controls. The study also proposed that adropin may be related to the pathogenesis of KD and be usable as a biomarker in KD [23]. We similarly found a remarkable increase in terms of serum adropin levels with 110.43 ng/L in the SS group compared to 80.06 ng/L in the control group ($p=0.047$; see Table 3). Therefore, adropin may be a potential biomarker able to distinguish between SS patients and those without SS. The average disease duration was determined as 46.68 and the average ESSDAI score as 5.98 in the SS group (Table 1).

One study reported clusterin levels to be significantly lower in psoriasis Vulgaris patients compared to their control group.

The study also suggested reduced levels of clusterin in psoriatic Vulgaris patients to likely be associated with the disease as well as a marker of systemic inflammatory activity [24].

Interestingly, another study revealed clusterin levels to increase in neuromyelitis optica spectrum disorder (NMOSD) in primary SS patients compared to those without SS (298.33±184.52 ng/ml versus 173.49±63.03 ng/ml, p<0.01) [25]. However, a recent study declared clusterin levels to be higher in patients with psoriasis Vulgaris compared to the control groups [26]. Kropáčková et al. determined clusterin levels to be higher in early rheumatoid arthritis patients and proposed a relationship to exist between clusterin levels and disease activity through treatment response [27]. The study determined SS patients' average clusterin levels be 98.41 ng/ml and the control group's to be 84.59 ng/ml (p=0.110). As a result and when compared to our results, clusterin may not be directly associated with SS.

Conclusion

In conclusion, no data are available on the circulating levels of adropin in those with SS, and our study is the first to investigate adropin levels in SS patients. Increased adropin levels were observed in SS patients compared to the healthy group. As such, adropin may be useful as a prognostic tool in SS disease. The study has some limitations such as it having been conducted in one center, the sample size, the nature of the disease, and the differences in patients' characteristics. Further investigations with larger samples are required to verify the results and clinical significance of adropin levels in SS disease.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
The study approval was taken from the local ethics committee of Selcuk University Faculty of Medicine (Approval date and number: 04.03.2021/2021-114).

Author Contributions:
The contribution of both authors was equal in reviewing the literatüre, designing the study, being prepared for the ethics committee, and analyzing and reporting the data.

References

1. Thanou-Stavraki A, James JA. Primary Sjögren's Syndrome: Current and prospective therapies. Semin Arthritis Rheum. 2008;37:273-92.

2. Harris ED. Approaches to the treatment of sjögren syndrome. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, editors. 4th ed. Philadelphia: WB Saunders Company;1993

3. Ramos-Casals M, Brito-Zerón P. Emerging biological therapies in primary Sjögren's syndrome. Rheumatology (Oxford). 2007;46:1389-96.

4. Schattner A, Friedman J, Klepfish A, et al. Immune cytopenias as the presenting finding in primary Sjögren's syndrome. OJM. 2000;93:825-9.

5. Fox RI. Sjögren's syndrome. Lancet. 2005;366:321-31.

6. Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol. 2017:76:9-16.

7. Roescher N, Tak PP, Illei GG. Cytokines in Sjögren's syndrome. Oral Dis. 2009;15:519-26.

8. Kumar KG, Trevaskis JL, Lam DD, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab. 2008;8:468-81.

9. Cakmak BD, Dundar B, Acikgoz AS, et al. The relationship between maternal and umbilical cord adropin levels with the presence and severity of preeclampsia. J Perinat Med. 2017;45:879-85.

10. Sato K, Yamashita T, Shirai R, et al. Adropin contributes to anti-atherosclerosis by suppressing monocyte-endothelial cell adhesion and smooth muscle cell proliferation. Int J Mol Sci. 2018;19:1293.

11. Zhang S, Chen Q, Lin X, et al. A review of adropin as the medium of dialogue between energy regulation and immune regulation. Oxid Med Cell Longev. 2020;2020:3947806.

12. Kılıçarslan S, Tekcan A, Demir HD, et al. Investigation of Association with Clusterin Rs11136000 Gene Polymorphism of Pseudoexfoliation Syndrome. Bozok Med J. 2017;7:1-6.

13. Viard I, Wehrli P, Jornot L, et al. Clusterin gene expression mediates resistance to apoptotic cell death induced by heat shock and oxidative stress. J Invest Dermatol. 1999;112:290-6.

14. Trougakos IP, Gonos ES. Oxidative stress in malignant progression: the role of clusterin, a sensitive cellular biosensor of free radicals. Adv Cancer Res. 2009;104:171-210.

15. Steinfeld S, Sutcliffe N, Sumida T, et al. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. RMD Open. 2015;1:e000022.

16. Kim SK, Choe JY, Lee GH. Enhanced expression of NLRP3 inflammasome-related inflammation in peripheral blood mononuclear cells in Sjögren's syndrome. Clin Chim Acta. 2017;474:147-54.

17. Zhang S, Chen Q, Lin X, et al. A review of adropin as the medium of dialogue between energy regulation and immune regulation. Oxid Med Cell Longev. 2020;3947806:7.

18. Pagano G, Castello G, Pallardó FV. Sjøgren's syndrome-associated oxidative stress and mitochondrial dysfunction: prospects for chemoprevention trials. Free Radic Res. 2013;47:71-3.

19. Nezos A, Kotsifaki E, Skarlis C, et al. Lipoprotein-associated Phospholipase A2: A new biomarker for lymphoma development in sjögren's syndrome [abstract]. Arthritis Rheumatol. 2020;72.

20. Balarini GM, Zandonade E, Tanure L, et al. Serum calprotectin is a biomarker of carotid atherosclerosis in patients with primary Sjögren's syndrome. Clin Exp Rheumatol. 2016;34:1006-12.

21. Chen X, Sun X, Shen T, et al. Lower adropin expression is associated with oxidative stress and severity of nonalcoholic fatty liver disease. Free Radic Biol Med. 2020;160:191-8.

22. Yolbas S, Kara M, Yilmaz M, et al. Serum adropin level and ENHO gene expression in systemic sclerosis. Clin Rheumatol. 2016;35:1535-40.

23. Yang M, Pei Q, Zhang J, et al. Association between adropin and coronary artery lesions in children with Kawasaki disease. Eur J Pediatr. 2021;180:2253-59.

24. García-Rodríguez S, Arias-Santiago S, Perandrés-López R, et al. Decreased plasma levels of clusterin in patients with psoriasis. Actas Dermosifiliogr. 2013;104:497-503.

25. Qiao L, Deng C, Wang Q, et al. Serum clusterin and complement factor h may be biomarkers differentiate primary sjögren's syndrome with and without neuromyelitis optica spectrum disorder. Front Immunol. 2019;10:2527.

26. Holmannova D, Borsky P, Borska L, et al. Metabolic syndrome, clusterin and elafin in patients with psoriasis vulgaris. Int J Mol Sci. 2020;21:5617.

27. Kropáčková T, Mann H, Růžičková O, et al. Clusterin serum levels are elevated in patients with early rheumatoid arthritis and predict disease activity and treatment response. Sci Rep. 2021;11:11525.