Lacritin Level in Tear Film of Rheumatoid Arthritis Patients

Rana Sorkhabi, MD[1] • Amir Ghorbanihaghjo, MD[2] • Mehrnoosh Ghasemi, MD[3]
Alireza Khabazi, MD[4] • Mohammadhosein Ahoor, MD[5]

Abstract

***Purpose*:** The main ocular manifestation of rheumatoid arthritis is dry eye. Lacritin is a secreted glycoprotein of tear and when applied topically in rabbits, it increased the volume of basal tear secretion. So the aim of this study was to compare Lacritin level in rheumatoid arthritis (RA) and control group.

***Methods*:** This cross-sectional study was done on 40 patients with RA and 48 healthy subjects as a control group. In all participants, tear break-up time (TBUT) and Schirmer test with anesthesia were accomplished. Tear samples were collected directly from the inferior lateral tear meniscus and were immediately stored at -80° C until use. Lacritin level of tears were assessed by enzyme-linked immunosorbent assay.

***Results*:** The mean age of participants was 44.7±16.3 and 43.3±18 years, respectively (p=0.70). Lacritin level in RA group (19.6±23.1 $^{ng}/_{ml}$) was significantly lower (31.9±23.3 $^{ng}/_{ml}$) than control (p=0.008). Pearson correlation between Lacritin level and TBUT, Schirmer value, ESR, and high-sensitivity C-creative protein (hs-CRP) in RA patients were not significant with p=0.27, 0.67, 0.09 and 0.07 accordingly.

***Conclusion*:** In early stages of RA in spite of normal tear production, Lacritin level decreased, however there is not any correlation between Lacritin level and TBUT, Schirmer value, ESR, and hs-CRP in these patients.

***Keywords*:** Lacritin, Rheumatoid Arthritis, Tear Film

Iranian Journal of Ophthalmology 2013;25(4):284-287 © 2013 by the Iranian Society of Ophthalmology

1. Associate Professor of Ophthalmology, Tabriz University of Medical Sciences, Tabriz, Iran
2. Associate Professor Biochemistary, Tabriz University of Medical Sciences, Tabriz, Iran
3. Ophthalmologist, Tabriz University of Medical Sciences, Tabriz, Iran
4. Assistant Professor of Rheumatology, Tabriz University of Medical Sciences, Tabriz, Iran
5. Assistant Professor of Ophthalmology, Tabriz University of Medical Sciences, Tabriz, Iran

Received: March 22, 2013
Accepted: August 8, 2013

Correspondence to: *Rana Sorkhabi, MD*
Associate Professor of Ophthalmology, Tabriz University of Medical Sciences, Tabriz, Iran, Email: sorkhabi_r@yahoo.com

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that primarily affects the peripheral joints in a symmetric pattern with predominant female involvement. Approximately 75% of patients with RA are seropositive. It is also associated with HLA-DR_4 and DR_1 alleles.[1]

Incidence and frequency figures for extraarticular RA vary according to study design.

It occurs in about 40% of all patients.[2] There are a variety of ocular manifestations of RA. The most common is keratoconjunctivitis sicca (KCS) or secondary Sjogren syndrome (SS), which is clinically evident in 15-25% of RA patients. Ocular sicca usually develops in the setting of long-standing rheumatoid disease, although it may be an early or presenting manifestation of RA.[3]

Lacritin is a 12.3 KDa glycoprotein that is typically released from human lacrimal cells during reflex tearing[4-6] and when applied topically in rabbits, it appears to increase the volume of basal tear secretion.[5] It has been shown that Lacritin was down regulated in tears from patients with blepharitis.[7]

According to above mentioned results, this hypothesis appears that down regulation of Lacritin may have a role in the pathogenesis of dry eye syndrome in patients with RA.

So, this study aimed to measure the Lacritin level of tear film in patients with RA without signs and symptoms of dry eye syndrome and compared the results with healthy subjects.

Methods

This cross-sectional study was conducted according to the principles in the Declaration of Helsinki. Ethical approval was obtained From the Ethics Committee of the Tabriz University of Medical Sciences. Written consent was obtained from all patients after explanation of the procedures and the study requirements.

A total of 40 consecutive patients with RA (32 female, 8 male, age range 25-65 years) and 48 controls (32 female, 16 male, age range 23-63 years) enrolled in this study. All patients underwent a thorough ocular examination and if they had diminished tear meniscus, punctuate epithelial keratopathy, corneal and conjunctival staining and any type of scleritis or keratitis were excluded.

Diagnosis of RA was made according to 2010 Rheumatoid Arthritis Classification Criteria[8] and all patients were in functional class I or II of the disease.[9] Healthy subjects without any ocular and systemic disease from relatives of patients in optometry clinic of Nikookari Eye Hospital voluntarily participated as controls. All patients with a history of other rheumatologic disease, ocular trauma, ocular surgery, use of ocular drug(s) and history of ocular involvement due to underlying disease were also excluded.

In all participants, tear break-up time (TBUT) and Schirmer test with anesthesia were accomplished. Tear samples were collected directly from the inferior lateral tear meniscus of the right eye of each patient, using a blunt glass capillary tube. Care was taken to minimize stimulation to the eye, to avoid collecting reflex tear.

The tear samples were immediately stored until measurement at -80° C. Lacritin concentrations of tear were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Uscn, Life Science Inc). According to the manufacturer, no significant cross-reactivity or interference between human Lacritin and analogs were observed by this assay.

For statistical analysis, Statistical package for Social Science version 17 (SPSS In., Chicago, IL) was used. Non-parametric tests were used to compare the results between two groups (Man-Whitney U test). The Pearson correlation test was used to analyze the relationship between clinical, TBUT, Schirmer value, ESR, high-sensitivity C-creative protein (hs-CRP) and Lacritin level in tear from patients with RA. For all tests $p<0.05$ was considered to be significant.

Results

The demographic and tear film measures of the patients were shown in table 1. There was no significant difference in age, sex ratio and TBUT between the case and the control.

Although Schirmer test values were in normal range but in both, they were higher in patients with RA.

Mean of Lacritin level in RA patients (19.6±23.1 $^{ng}/_{ml}$) was significantly lower (31.9±23.3 $^{ng}/_{ml}$) than control group (p=0.008). The characteristics of RA patients were shown in table 2. Table 3 demonstrates that there was not correlation between Lacritin and TBUT, Schirmer test values, ESR, hs-CRP.

Table 1. Demographic and tear film measure of the patients in both groups

	Case n=40	Control n=48	p-value
Age (gr)	44.7±16.3	43.3±18	0.70
Sex ratio (female/male)	32/8 (80%)	32/16 (66.7%)	0.23
Tear break-up time (min)	11±4.2	12±1.8	0.32
Schirmer test (mm)	16.9±8.1	22.3±6.8	0.001

Table 2. Characteristics of rheumatoid arthritis patients

Variable	Mean±SD
hs-CRP (mg/L)	2.9±0.7
ESR at 1 st hr	25.5±20.4
Time of disease (month)	61.6±60.6

hs-CRP: high-sensitivity C-creative protein

Table 3. Pearson correlation between Lacritin level and other measures of patients with rheumatoid arthritis

	Lacritin	
	r	p
Tear break-up time	0.14	0.27
Schirmer value	0.05	0.67
ESR	0.2	0.09
hs-CRP	0.2	0.07

hs-CRP: high-sensitivity C-creative protein

Discussion

RA is a chronic systemic inflammatory disease of unknown cause that primarily affects the peripheral joints in a symmetric pattern. Ocular involvement is a frequent finding in these patients and may be the leading clinical manifestation of disease.[10] The most common ocular manifestation is KCS or SS with incidence between 15 to 25% in literature. The keratoconjunctivitis in RA is classically described as an aqueous tear deficiency.[2,3] It remains a matter of debate whether KCS or SS represents a separate coexisting entity from a rheumatic disease or is a clinical manifestation as part of a rheumatic disorder. A study suggests that the etiology of dry eye may be different in RA patients with secondary SS and patients without SS.[11]

Tear film is a rich source of growth factors, protease, protease inhibitors, antioxidants, mucins, and lipids that has been only partially characterized.[12] Comparison of tears from Sjogren's syndrome dry eye versus normal subjects by mass spectrometry revealed seven proteins peaks down regulated and three upregulated.[13]

Lacritin is a glycoprotein that is typically released from human lacrimal acinar cells during reflex tearing and can be detected in mixed reflex and basal human tear by ELISA and western blotting. Lacritin is also produced by corneal, conjunctival, meibomian and salivary epithelia as one of the most eye-restricted genes.[14,15] Thus, Lacritin appears to be a multifunctional eye-specific factor with a potential role in tear secretion and corneal epithelium.[16] In this study our aim was to compare the Lacritin level of tear film in patients with early stages of RA without ocular complain versus normal subjects in order to identify the role of this glycoprotein in early stages of RA disease before frank ocular involvement. We found that Lacritin level was significantly lower in RA patients compared to the controls.

In a few previous studies it has been shown that Lacritin down regulated in inflammatory ocular surface disease[7,17] such as blepharitis and contact lens-related dry eye. Recently, Nicholas and Green reported that Lacritin is selectively down regulated more than any other tear protein in contact lens-related dry eye.[18] In a recent study Csosz and coworkers found that Lacritin level increased in tear film of patients with diabetic retinopathy.[19] It was demonstrated that the Lacritin is required for tear production and proliferation of several cell types providing a proliferative milieu possibly required for progression of the disease.[20] Samudre and coworkers in an experimental study in rabbit showed that recombinant Lacritin acutely simulates basal tear flow that its effects sustained for at least 240 minutes. One week after treatment discontinuation basal tearing was still 50% over baseline.[16]

In this study also we found that in RA patients even before appearance of symptomatic dry eye, the level of this glycoprotein decreased and there was not correlation between the level of Lacritin and TBUT, Schirmer values, ESR and hs-CRP. To the best of our knowledge, this is the first study evaluating Lacritin in tear film of RA patients, and the small sample size, lack of patient's follow-up and enrolment of patients only in early stages of disease are important limitations of this study, so we must interpret our findings with caution. However reevaluating of these patients in a longitudinal study would be more useful for documentation of the downregulation of this glycoprotein which occurs independent to inflammatory status of disease and it even may be present in early stages and even before frank ocular involvement. If such a result documented; applying the recombinant form of this glycoprotein could be a subject of a clinical trial for studying the preventive role of Lacritin in occurring of dry eye syndrome in RA patients. Therefore, further studies and clinical trials are needed to advance our understanding of the role of Lacritin in occurrence and prevention of dry eye in RA patients.

Conclusion

In conclusion we have shown that in early stages of RA, in spite of normal tear production, Lacritin level has been decreased.

Acknowledgments

This work is supported by a grant from the vice-chancellor for research of the Tabriz University of Medical Sciences, Tabriz, Iran. We express our appreciation to all the patients as well.

References

1. Shinzato M, Yamamoto J, Hirata CE, Goldberg AC, Yoshinari NH, Bonfá E. Eye disease in a patient with rheumatoid arthritis. Postgrad Med J 1999;75(889):676-7.
2. Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Occurrence of extra-articular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. J Rheumatol 2002;29(1):62-7.
3. Matsuo T, Kono R, Matsuo N, Ezawa K, Natsumeda M, Soda K, et al. Incidence of ocular complications in rheumatoid arthritis and the relation of keratoconjunctivitis sicca with its systemic activity. Scand J Rheumatol 1997;26(2):113-6.
4. Ma P, Wang N, McKown RL, Raab RW, Laurie GW. Focus on molecules: lacritin. Exp Eye Res 2008;86(3):457-8.
5. McKown RL, Wang N, Raab RW, Karnati R, Zhang Y, Williams PB, et al. Lacritin and other new proteins of the lacrimal functional unit. Exp Eye Res 2009;88(5):848-58.
6. Morimoto-Tochigi A, Walkup RD, Nakajima E, Shearer TR, Azuma M. Mechanism for carbachol-induced secretion of lacritin in cultured monkey lacrimal acinar cells. Invest Ophthalmol Vis Sci 2010;51(9):4395-406.
7. Koo BS, Lee DY, Ha HS, Kim JC, Kim CW. Comparative analysis of the tear protein expression in blepharitis patients using two-dimensional electrophoresis. J Proteome Res 2005;4(3):719-24.
8. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69(9):1580-8.
9. Hochberg MC, Chang RW, Dwosh I, Lindsey S, Pincus T, Wolfe F. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 1992;35(5):498-502.
10. Feist E, Pleyer U. [Diseases of the outer eye in rheumatoid arthritis]. J Rheumatol 2010;69(5):403-10. [Article in German]
11. Fujita M, Igarashi T, Kurai T, Sakane M, Yoshino S, Takahashi H. Correlation between dry eye and rheumatoid arthritis activity. Am J Ophthalmol 2005;140(5):808-13.
12. de Souza GA, Godog LM, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. Genome Biol 2006;7(8):R72.
13. Tomosugi N, Kitagawa K, Takahashi N, Sugai S, Ishikawa I. Diagnostic potential of tear proteomic patters in Sjögren's syndrome. J proteome Res 2005;4(3):820-5.
14. Nakajima T, Walkup RD, Tochigi A, Shearer TR, Azuma M. Establishment of an appropriate animal model for lacritin studies: cloning and characterization of lacritin in monkey eyes. Exp Eye Res 2007;85(5):651-8.
15. Tsai PS, Evans JE, Green KM, Sullivan RM, Schaumberg DA, Richards SM, et al. Proteomic analysis of human meibomian gland secretions. Br J Ophthalmol 2006;90(3): 372-7.
16. Samudre S, Lattanzio FA Jr, Lossen V, Hosseini A, Sheppard JD Jr, McKown RI, et al. Lacritin, a novel human tear glycoprotein, promotes sustained basal tearing and is well tolerated. Invest Ophthalmol Vis Sci 2011;52(9):6265-70.
17. Green-Church KB, Nichols JJ. Mass spectrometry-based proteomic analyses of contact lens deposition. Mol Vis 2008;14:291-7.
18. Nicholas JJ, Green-Church KB. Mass spectrometry-based proteomic analyses in contact lens- related dry eye. Cornea 2009;28(10):1109-17.
19. Csősz É, Boross P, Csutak A, Berta A, Tóth F, Póliska S, et al. Quantitative analysis of proteins in tear fluid of patients with diabetic retinopathy. J Proteomics 2012;75(7);2196-204.
20. Wang J, Wang N, Xie J, Walton SC, Mckown RL, Raab RW, et al. Restricted epithelial proliferation by lacritin via PKC alpha-dependent NFAT and mTOR pathways. J Cell Biol 2006;174(5):689-700.