İzmir Eğitim ve Araştırma Hastanesi Tıp Dergisi,2013;17:150-157

ARAŞTIRMA MAKALESİ

TEAR FUNCTION IN THE HEALTHY EYES OF UNILATERAL LATENT HERPETIC STROMAL KERATITIS PATIENTS

TEK TARAFLI HERPETİK STROMAL KERATİTLİ HASTALARIN SAĞLIKLI GÖZLERİNDE GÖZYAŞI İŞLEVİ

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ABSTRACT: This study was conducted to evaluate the tear function in the healthy eye of the patients with unilateral latent stromal herpetic keratitis.
Fourty two patients with unilateral latent stromal herpetic keratitis were acquired from the cornea department. Control group consisted of 44 people including 25 women and 19 men. A 5-minute conventional Schirmer I test without anesthesia was performed on closed eyes. Tear BUT was measured and corneal fluorescein staining pattern evaluated in both groups. t test was used for comparison of the mean age of the two groups. Chi square test was used for the comparison of sex, smoking habits and tear function values of control and test groups. t test for was used for independent groups and chi square test was used for the comparisons.

There were no statistically significant difference between two groups in terms of mean age, sex and cigarette use. Corneal staining was positive in 31.7 % in herpes group and 6.8 % in control group. This difference was statistically significant (χ2: 8,604, p:0,005). Mean Schirmer value was 9.0±8.2mm in herpes group, 19.0±9.8mm in control group

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(p:0.000). Mean BUT was 13.8±14.5 in herpes group and 21.8±12.7mm (p:0.009). No statistically significant difference was found between two groups in terms of HSV-1IgG,M or HSV-2IgG,M positivity.
The healthy eyes of the patients with herpetic stromal keratitis showed a decrease in tear secretion and tear film stability.

Key words: Herpetic keratitis, contrlateral eye, tear function.

ÖZET: Bu çalışmanın amac tek taraflı latent stromal herpetik keratitli hastaların sağlıklı gözlerindeki gözyaşı işlevinin değerlendirilmesidir.
Kornea bölümünden unilateral latent stromal herpetic keratitli 42 hasta ele alındı. Kontrol gurubu sağlıklı 25 kadın, 19 erkekten oluşmaktaydı. 5 dk geleneksel Schirmer I testi anestezisiz olarak kapaklar kapalıyken uygulandı. Her iki gurupta BUT süresi ölçüldü ve korneanın floressein boyanma özelliği değerlendirildi. Kontrol ve test grubunun yaş ortalamalarının karşılaştırmasında t testi kullanıldı. Cinsiyet,sigara alışkanlığı ve gözyaşı işlevi ile ilgili değerlerin karşılaştırılmasında ise ki kare testi kullanıldı.
İki gurup arasında ortalama yaş, cinsiyet ve sigara içme oranları arasında istatistiksel anlamlı bir fark yoktu. Korneada floressein boyanma herpes gurubunda %31.7, kontrol gurubunda %6.8 oranında görüldü. Aradaki fark istatistiksel olarak anlamlı idi (χ2: 8,604, p:0,005). Ortalama Schirmer değeri herpes gurubunda 9.0±8.2mm, kontrol gurubunda 19.0±9.8mm (p:0.000). Ortalama BUT herpes gurubunda 13.8±14.5, kontrol gurubunda 21.8±12.7mm idi (p:0.009). İki gurup arasında HSV-1IgG,M or HSV-2IgG,M seropozitifliği açısından istatistiksel anlamlı fark bulunmadı.
Unilateral latent stromal herpetic keratitli hastaların sağlıklı gözlerinde istirahat hali gözyaşı salgısında azalma, gözyaşı kırılma zamanında kısalma (BUT), korneanın floressein boyanmasında artış saptanmıştır. Özetle bu gözler de kuru göz bulguları taşımaktadır.

Anahtar kelimeler: Herpetik keratit, karşı göz, gözyaşı işlevi.

INTRODUCTION

Herpes simplex virus (HSV)-1 is the main infectious cause of blindness in developed countries(1). Although only approximately 20% of people develop disease from HSV-1, 60% to 80% are infected, have antibodies, and have sensory ganglia from which virus can be isolated. Ocular viral disease is caused by multiplying virus or hypersensitivity reaction to viral antigens(2). When the virus first infects the cornea, it has probably arrived from within via the terminal branches of the ophthalmic division of the trigeminal nerve.

Once an initial lesion has developed, it is possible that virus may persist in the cornea particularly if virus has entered the stroma(3).
HSV-1 keratitis was reported as unilateral in 81-90 % of the patients. Only atopy, HIV infection, and other forms of immunosuppression may cause bilateral disease(4). The healthy eyes of unilateral herpes keratitis patients are usually overlooked and considered normal. However, recent studies revealed that these eyes have certain

ocular surface abnormalities either. Keijser et al showed that the herpes virus may affect the tear secretion mechanism of the other eye(1).
The unstable tear film and the altered corneal permeability in dry eye, leave the cornea vulnerable to sterile ulceration and microbial infection which may lead to reduced vision, blindness, and even loss of the eye(5). Dry eye has not been listed as a causative factor for HSV corneal disease in a large review study about herpes virus epidemiology(4). Whereas, there are some studies stating ocular surface have more influence on HSV recurrences than previously credited(3,6).
The purpose of this study was to assess the steady state tear secretion, tear break up time (BUT) and fluorescein staining in uninvolved eyes of the patients with latent stromal herpetic keratitis and to compare these data with those of healthy volunteers to obtain information about the ocular surface of these eyes.

MATERIAL AND METHOD

Informed consents of all participants were taken before enrollement into the study. This study was performed under the rules of Helsinki declaration. Fourty two patients with unilateral latent stromal herpetic keratitis were acquired from the cornea department of the Izmir Bozyaka Education and Research Hospital between December 2009 and February 2010. This group of patients was classified as Herpes group and measurements were taken from the healthy eyes of this group. A group of healthy volunteers from the same clinic were recruited for comparison.

Furthermore, nine healthy volunteers from the staff of the clinic were added into the study. Control group consisted of 44 people including 25 women and 19 men. All participants had to have a clear cornea on slitlamp examination and could not wear contact lenses or have any systemic disease which can cause dry eye. Patients with active herpetic epithelial infection were excluded, because affected epithelium could stimulate reflex tears and mask a possible decrease in tear flow on the contrlateral eye. Eleven sick eyes of herpes group patients had previously undergone corneal transplantation and three eyes had amniotic membrane transplantation. Subjects who had ocular surgery on the eyes which were planned to be measured were excluded from both groups**.** Four subjects of the control group were diagnosed as cataract and two of them dry eye. These two dry eye patients were not excluded from the control group to ensure the homogeneity between the two groups. Because the control group subjects within the same age group with herpes patients might have dry eye by chance. There were 2 diabetic patients in control group and 3 patients in herpes group. None of the patients from each group were not using antidepressants which can cause dry eye.
Detailed history was taken from the patients. They were asked if they had any ocular or systemic disease or not. Any medication or cigarette use, labial or ocular herpes story were asked. All tests took place in the morning to avoid a bias in the tear production caused by circadian rhythm. Before measurements, an ophthalmologist examined the surface of the cornea with a slitlamp without using fluorescein.

A 5-minute conventional Schirmer I test without anesthesia was performed on closed eyes by placing a commercially available 5x35-mm paper strip (Clement Clarke Int Ltd, London, England) over the lower lid margin at the junction of the middle and lateral third of the lid margin. At the end of 5 minutes, wet part of the Schirmer strip were recorded into the inquiry form in milimeters. When the strip became totally wet in few minutes due to the reflex tears caused by conjunctival irritation, the test was repeated after topical anesthesia, and this value was recorded as the Schirmer score. Fluorescein (Fluorescein 2%, Doka Medical Inc., Eskişehir, Turkey) was instilled into lower cul de sac and presence of corneal fluorescein staining was checked under blue-light of a biomicroscope. Patients showing punctate or diffuse staining were recorded as fluorescein staining positive. Subjects showing no fluorescein staining were accepted as staining negative. Later on, tear BUT was measured. Patients were instructed to blink, and the precorneal tear film was examined under blue-light illumination using a biomicroscope with an x10 objective. While subjects were keeping their eyes open, tear BUT were recorded in seconds. The mean value of a total of 3 measurements was recorded. Both eyes of the control group were measured to check the similarity of the measurements. Means of the Schirmer and BUT scores of the two eyes were used for the statistical analysis. Better fluorescein pattern was chosen since these eyes represent the normal state better. Blood samples were taken from the subjects for HSV-1 immunoglobulin G (HSV-1 IgG) , HSV-1 IgM, HSV-2 IgG and HSV-2 IgM analysis with ELISA method.

Statistics: SPSS 15.0 for Windows statistical package programme was used for gathering data. t test was used for comparison of the mean age of the two groups; since there were more than 30 subjects in both groups. Chi square test was used for the comparison of sex, smoking habits and tear function values of control and test groups. Data were interpreted in 95% confidence interval. When p value was below 0.05; the differences were considered as statistically significant.

RESULTS

There were no statistically significant difference between two groups in terms of mean age, sex and cigarette use. In other words, subjects of two groups showed homogeneity (Table 1). The comparison of two groups in terms of Schirmer test and BUT values may be seen on Table 2. Schirmer scores, showing the tear production under physiological conditions, were significantly lower in herpes group than the control group (Fisher exact p:0.000).

	Groups		p	Statistical test
	Herpes (n=42)	Control (n=44)		
MeanAge ± Standard deviation	47.9 ± 18.2 (7.0-77.0)	46.4 ± 20.3 (19.0-80.0)	0.720	t test
Sex	21 female (50.0 %) 21 male (50.0 %)	25 female (56.8 %) 19 male (43.2 %)	0.666	chi square
Cigarette use	28 (66.7 %)	36 (81.8 %)	0.140	chi square

Table 1. Mean age, sex distrubition and cigarette use values of herpes and control groups.

Test	Group	n	Mean	SD	p*
Schirmer test (mm/5min)	Herpes	42	9,00	8,24	0,000
	Control	44	19,05	9,78	
BUT (sec)	Herpes	41	13,85	14,51	0,009
	Control	44	21,80	12,65	

Table 2. Mean Schirmer test and BUT values of herpes and control groups.

*Fisher chi square test

Test	Group	n	Number of eyes / (%)	p*
Schirmer test (<5mm/5min)	Herpes	42	15 (%35.7)	0,004
	Control	44	4 (%9.1)	
BUT (<10sec)	Herpes	41	20 (%48.8)	0,002
	Control	44	7 (%15.9)	

Table 3. Comparison of the ratio of the eyes with low Schirmer test scores under 5mm/5min and short BUT values under 10 seconds in both groups.

* Fisher chi square test

Test	Group	n	Positive	Negative	Fisher p
HSV1IgG	Herpes	32	31 (96.9 %)	1 (3.1 %)	1.000
	Control	32	31 (96.9 %)	1 (3.1 %)	
HSV1IgM	Herpes	31	1 (3.2 %)	30 (96.8 %)	0.492
	Control	32	0 (0.0 %)	32 (100.0 %)	
HSV2IgG	Herpes	32	1 (3.1 %)	31 (96,9 %)	0.355
	Control	32	4 (12.5 %)	28 (87,5%)	

Table 4. Serological analysis of herpes and control groups.

*Fisher chi square test

Similarly, BUT scores reflecting the stability of the tear film were significantly decreased in herpes group compared to normal subjects (Fisher exact p:0.009). The healthy eyes of the herpes group patients also showed significantly more corneal staining than the control group. Fluorescein staining was positive in 13/41 (31.7%) of herpes group eyes and 3/44 (6.8%) of control eyes. This difference was statistically significant ($\chi 2$: 8,604, Fisher exact p:0,005). Since average values may be misleading, the ratio of subjects with Schirmer test scores under 5mm/5min and subjects with BUT values under 10 seconds were compared between two groups. As may be seen in Table 3, the ratio of the eyes with pathologically low Schirmer and BUT scores was significantly higher in herpes group than the control group.

Serological analysis for HSV-1 and HSV-2 immunoglobulins were also performed on the blood samples of the patients with herpetic stromal keratitis and control group subjects to compare the humoral immunity against the two virus strains in

both groups. No statistically significant difference was found between two groups in terms of positivity of these spesific immunoglobulins. HSV-2IgM was negative in all subjects.

Labial herpes history was positive in 62.9% of herpes group patients and 40.9% of control group subjects. This difference was not found to be statistically significant. (χ2:3.757, Fisher p: 0.071).

DISCUSSION

HSV keratitis frequently cause a reduction in tearing that follows hypoesthesia in infected eye(3). In their small series, Keiser et al showed that unilateral herpes infection might cause dryness in the other eye as well(1). Our study was conducted to investigate further on this phenomenon in a larger case series. In addition to measurement of the tear secretion, BUT and fluorescein staining were also evaluated. Our results revealed that the mean Schirmer scores of the uninvolved eyes of the herpes group patients were significantly lower than the control group. Besides, these eyes showed more corneal staining and shorter tear BUT than the control eyes. These data indicate that, most of the uninvolved eyes of patients with unilateral HSV keratitis are not healthy and suffering from dryness. Because of the dryness and tear film instability, these eyes develope epitheliopathy which leads corneal staining(5).

Nevertheless, there are limitations to the present study. Although the Schirmer test is easy to perform, it is usually critised as inaccurate or not suitable for research purposes. Instead a scanning fluorometer could be used for measuring the tear secretion quantitatively(1). Tear BUT measurement has certain disadvantages as well. The instillation of fluorescein can destabilize the tear film. The measurement of BUT in the absence of fluorescein was reported to give more accurate assessment of tear stability(7). However, there are some studies suggesting conventional tests are still valid, since there are no other golden standard tests available(8). Age and cigarette use being well known risk factors for dry eye were taken into consideration in our study(9). Dry-eye state is usually defined as a Schirmer test of under 10mm of wetting at 5min or a tear BUT of shorter than 10 seconds(10). Schirmer score was considered to be low if it was under 5mm to make our results more reliable.

Dry eye disease has a prevalence of 14-15% in general population of Caucasians(7,9). In our study, the percentage of the control subjects with a Schirmer score under 5mm was 9.1% and a BUT score under 10sec was 15.9% which were both close to this prevalence. Whereas, herpes group eyes had percentages of 35.7% and 48.8% respectively which are higher than normal population. Keijser et al suggested two possible explanations for the dryness in the contrlateral eyes of the patients with unilateral stromal herpetic keratitis: The tear secretion mechanism of the healthy eye is also affected by the HSV, or the dryness is not caused by the herpes infection but predisposes to chronic recurrences of stromal herpes(1). There are arguments that support either statement. It has been shown that HSV does not affect the tear production of the lacrimal glands, therefore the reduction of tear secretion are thought to be a result of reduced stimulus due to hypoesthesia(1,10). Herpes virus has been detected on the contrlateral side of the brain stem in unilaterally infected mice. Thus, contrlateral denervation has been suggested to be the cause of the corneal hypoesthesia resulting in less stimulus to the lacrimal glands(11).

HSV recurrences are typically with the same strain and may be triggered by upper respiratory tract infection, hormonal changes, seasonal conditions, ultraviolet exposure, psychological stress, ocular trauma, trigeminal nerve manipulation and excimer laser surgery. These recurrences are usually seen in the same eye, and the other eye is usually protected from infection with some defence mechanism(12). A healthy corneal epithelium and tear film is the most important parts of this mechanism(5). Corneal nerves are also

important by relaying sensory information leading to reflexive movements to protect the eye(13). Dry eye has not been considered as a risk factor for ocular HSV recurrences(2,4,7). However, Sheppard et al showed that the treatment of the dry eye reduces the recurrences(14). Although both herpes and control group subjects of our study have already been infected by HSV-1, only the subjects having dry eye intented to have corneal involvement. In view of these reports and our findings, it may be suggested that dryness can stimulate the HSV reactivations in corneal stroma.

In Europe, almost 80% of the adult population has antibodies against HSV-1(1). Serological results of our patients and control subjects revealed that HSV-1 IgG was positive in almost all participants (96.9 %). It seems that the HSV-1 seropositivity of Turkish people over 40 is almost 100%. In contrary, HSV-2 infection is rare in either groups (3.1% in herpes group, 12.5% in control group).

One of the as yet unresolved questions is the precise state of the virus in different parts of the cornea and at different times during keratitis and indeed in the trigeminal ganglion in patients with keratitis(3). Human herpes viruses can infect the eye and be excreted subsequently in tears. HSV-1 and HSV-2 were found in two samples from patients with metaherpetic corneal scarring with multiplex PCR method(15). This technique may be used in detecting HSV activity in uninvolved eyes of these patients in future research.

In conclusion**;** the results of this study showed that the uninvolved eyes of the patients with unilateral herpetic stromal keratitis may have a reduced tear secretion and an unstable tear film leading corneal fluorescein staining. Therefore, clinicians should carefully inspect these eyes in terms of dry eye signs, and perform simple tests where necessary. Thus, it would be possible to protect these eyes from complications of dry eye by lubrication.

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Yazının alınma tarihi:10.05.2013

Kabül tarihi:10.06.2013

Online basım:01.07.2013