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The evaluation of diagnostic efficiency for stromal herpes simplex keratitis by the combination of tear HSV-sIgA and HSV-DNA

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Abstract

Purpose To assess the differential diagnostic values for stromal herpes simplex keratitis (HSK) by using tear HSV-sIgA, tear HSV-DNA, and the combination.

Methods Tear samples for both eyes and the paired serum were collected from 187 stromal HSK and 56 controls. Enzyme-linked immune sorbent assay (ELISA) was used to analyze the tear HSV-sIgA and serum IgG/IgM/IgA. The levels of tear HSV-DNA were measured by polymerase chain reaction (PCR).

Results The positive rates for tear HSV-sIgA and HSV-DNA were 36.90% and 10.96% respectively in stromal HSK patients. Twelve showed positivity for both sIgA and DNA, while 46 cases were positive for sIgA or DNA. The sensitivity, specificity, PPV, and NPV for simultaneous measurement were 39.73%, 98.21%, 98.31%, and 38.46%. The total negative conversion rate of sIgA was 95.71%.

Conclusions The diagnostic efficiency of HSV-sIgA only is nearly equal to the combination of HSV-sIgA and HSV-DNA, and the positive result is optimum to achieve a reliable diagnosis of stromal HSK even in atypical or unsuspected cases.

Jini Qiu and Feifei Huang contributed equally to the work presented here, and should therefore be regarded as equivalent authors.

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Introduction

Herpes simplex keratitis (HSK) is one of the known types of keratitis around the world. Clinical phenotypes of HSK include epithelial keratitis, neurotrophic keratopathy, immune stromal keratitis, necrotizing stromal keratitis, and endotheliitis [1]. Among these, stromal keratitis is the leading cause of corneal blindness because of its high recurrence tendency [2]. The typical stromal HSK manifests as presence of circular stromal edema, infiltration, Descemet membrane folds, and deterioration of corneal sensitivity. Normally, the diagnosis of stromal HSK usually relies on a history of recurrence, together with typical clinical manifestations of the infected eyes [3]. However, for those stromal HSK patients without any characteristics and relapse history, a rapid and reliable differentiated diagnostic method can be extremely useful in clinical terms.

Successful HSV isolation from the corneal scrapings was once considered the "golden standard" for HSK diagnosis [4]. However, this technique is time-consuming, with low sensitivity, and requires a special laboratory for viral processing. The polymerase chain reaction (PCR), which is sensitive and has a relatively rapid processing time, is an alternative choice for DNA detection [5]. Many previous studies showed that PCR is a useful auxiliary method for HSK diagnosis. However, the different clinical manifestations and the previous treatment regimen can have a direct influence on diagnostic efficiency [6].

As the vital component of the ocular surface, tears are closely related with many ocular diseases. Tear secretory IgA (sIgA) is a dimeric IgA synthesized by subepithelial plasma cells, and then secreted into tears with the help of poly-

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immunoglobulin receptors. It is an essential immune defense factor in ocular immunity [7]. Antigen-specific sIgA in tears functions as ocular surface biomarkers for certain infections because it is rarely influenced by the concentrations in blood. In addition, tear sample collection is safer, more convenient, and more efficient compared with traditional corneal scraping. Therefore, many previous studies suggested that HSV-specific sIgA could be an adjunct to the diagnosis of herpes keratitis [8, 9].

Shoji et al. [10] reported the simultaneous measurement of tear HSV-DNA and HSV-sIgA in 59 suspected HSK eyes and 23 eyes from healthy volunteers. They found that the combination of these two laboratory methods enabled higher reliability in diagnosing HSK. However, because of the small numbers of patients enrolled, it is a limitation that the detailed investigations of stromal HSK, the major subtype of HSK, remain unknown. The aim of our study is to evaluate the diagnostic efficiency of tear HSV-DNA and HSV-sIgA simultaneously in stromal HSK patients, and to determine the relationship with detailed clinical forms.

Materials and methods

Patients

A total of 187 patients older than 18 (129 men and 58 women) attending Eye Ear Nose and Throat Hospital, Fudan University diagnosed with unilateral active HSV-related stromal keratitis during the period of Sep 2011 to Dec 2015 were enrolled in this study. The mean age was 51 ± 13 (range 0–85 years). All the stromal HSK patients were treated with topical eye-drops, and no oral antiviral therapies were employed previously. Fifty-six controls were selected from patients with other ocular diseases, involving pterygium, leucoma, corneal dystrophy, cataract, and microbial keratitis confirmed by confocal microscopy, smear, or culturing. Ethics approval for the use of human subjects was obtained from the research ethics committee of EYE and ENT hospital, and informed consent was obtained from each patient.

Sample collection

Tear samples from the lower fornices of both eyes were collected into capillary tubes then expelled into Eppendorf tubes. The volume of each sample was usually 5 to 20 ul. Anesthesia was not used during the process. Paired serum samples were also collected. In cases in which the quantity of tears was insufficient, only the HSV-sIgA assay was performed. About 5 ml venous blood was collected for HSV specific antibodies testing. All the specimens were transported immediately and stored at −80 °C until processed.

Enzyme-linked immunosorbent assays

All serum and tear specimens were assayed by enzyme-linked immunosorbent assay (Virion/Serion classic HSV 1+2 Ig G/IgM/IgA; order number ESR105G, ESR105M, ESR105A, Würzburg, Germany). According to the manufacturer's instructions, only Serion reagents were used for the test procedure. Microtest plates were coated with antigens, which constituted the solid phase. Patient samples were added to the plates, and any antibodies specific for antigen present would bind to the solid phase. After dilution with phosphate-buffered saline, samples and standard sera were pipetted into the microtest wells, which were incubated for 60 minutes at 37 °C in moist chambers, then thoroughly washed four times with washing solution by automated washer. IgG/IgM/IgA conjugates were added to the appropriate wells and incubated for another 30 minutes, then washed as before. Every well was then covered with substrate solution followed by 30 minutes incubation. Finally, stopping solution was used to stop the reaction. The microplate reader (Sunrise-Basic, Tecan, Austria) was used to read the extinctions at 620 nm. Values were considered positive if sample/cutoff (s/co) values were greater than 1. Antibody activities in units per milliliter were determined from the standard curve with the corrected values.

Real-time polymerase chain reaction

The detection of HSV DNA was carried out using the Liferiver HSV 1+2RT-PCR kit (Shanghai, China). Reactions were set up and performed according to the manufacturer's instructions, and were executed by the vitro medical diagnostic device intended for use on the Loche 480 instrument. All reactions were performed in a total volume of 40 ul. The reaction conditions were as follows: pre-denaturation at 37 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 95 °C for 5 s, and extension at 60 °C for 30s.

Statistical analysis

Statistical analysis was carried out by using Statistical Package for Social Sciences software (SPSS 19.0 for windows, IBM), and a $p < 0.05$ was considered statistically significant. Data were presented as mean $\pm$ SD. Qualitative variables were analyzed using the x^2 test and Fisher's exact test. The nonparametric Mann–Whitney U test was used for comparison between groups with heterogeneity. Receiver operator characteristic curve (ROC) analysis was used for evaluating the diagnostic power.

Results

Positive and negative results were determined according to the following criteria: specimens with ≥30 copies/sample of HSV DNA and for HSV-sIgAs/co ≥1 were deemed positive.

In total, 187 patients who had been diagnosed with monocular stromal HSK and 56 controls with other ocular diseases were enrolled in this study. Among the 187 cases, 139 presented with epithelial defects. The positive rates of serum HSV-specific IgG, IgM, and IgA were 100%, 1.60% (3/187), and 51.87% (97/187) in the stromal HSK group compared with 92.86% (52/56), 5.36% (3/56), and 23.21% (13/56) in controls. None of the three serum IgM-positive cases in controls showed any symptoms of HSV infection.

Levels of HSV-sIgA in tears

For all 187 stromal HSK patients, the tear HSV-sIgA positive rate was 36.90% (69/187) in infected eyes compared with 5.88% (11/187) in uninfected eyes. One hundred and thirty-nine cases (74.33%) of the infected eyes presented with epithelial lesions; and among the 139 eyes, 60 (43.17%) were also HSV-sIgA positive. Only one of the 56 controls was HSV-sIgA positive. The concentration of tear HSV-sIgA was significantly higher in stromal HSK eyes than that in paired unaffected eyes and control cases ($p < 0.05$) (Fig. 1a b); for stromal HSK eyes with epithelial defects, the HSV-sIgA concentration was higher

than in those manifesting no epithelial disorders (Fig. 1c). In addition, the levels of sIgA for the 11 patients with both eyes showing HSV-sIgA positivity still remained higher in sick eyes than unaffected eyes (Fig. 1d). The clinical characteristics of the 187 patients and the correlation with tear sIgA are summarized in Table 1.

Levels of HSV-DNA in tears

In total, 146 stromal HSK patients and 56 controls were detected for tear HSV-DNA levels. The positive rates for infected eyes and uninfected eyes were 10.96% (16/146) and zero respectively. Fifteen of the 110 fluorescence staining positive patients (13.64%) were detected as HSV-DNA. Figure 2 showed HSV-DNA copies of the 16 positive samples. None of the controls showed tear HSV-DNA positivity. Details of the 146 stromal HSK patients and the correlation with tear HSV-DNA were summarized in Table 2.

Simultaneous measurements of tear HSV-sIgA and HSV-DNA

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for tear HSV-sIgA were 36.90%, 98.21%, 98.57%, and 31.79%; and for HSV-DNA, were 10.96%, 100%, 10.96%, and 30.11% respectively. For the 146 patients measured by the two methods simultaneously,

Fig. 1 Comparison of HSV-sIgA in tears. **a** For stromal HSK patients, the concentrations of tear HSV-sIgA were significantly higher in sick eyes than healthy eyes. **b** Compared with control group, the HSV-sIgA levels were significantly higher in sick eyes. **c** Fluorescence staining positive stromal keratitis patients had a higher tear HSV-sIgA level. **d** The levels of sIgA for patients with both eyes HSV-sIgA positivity still remained higher in sick eyes than unaffected eyes
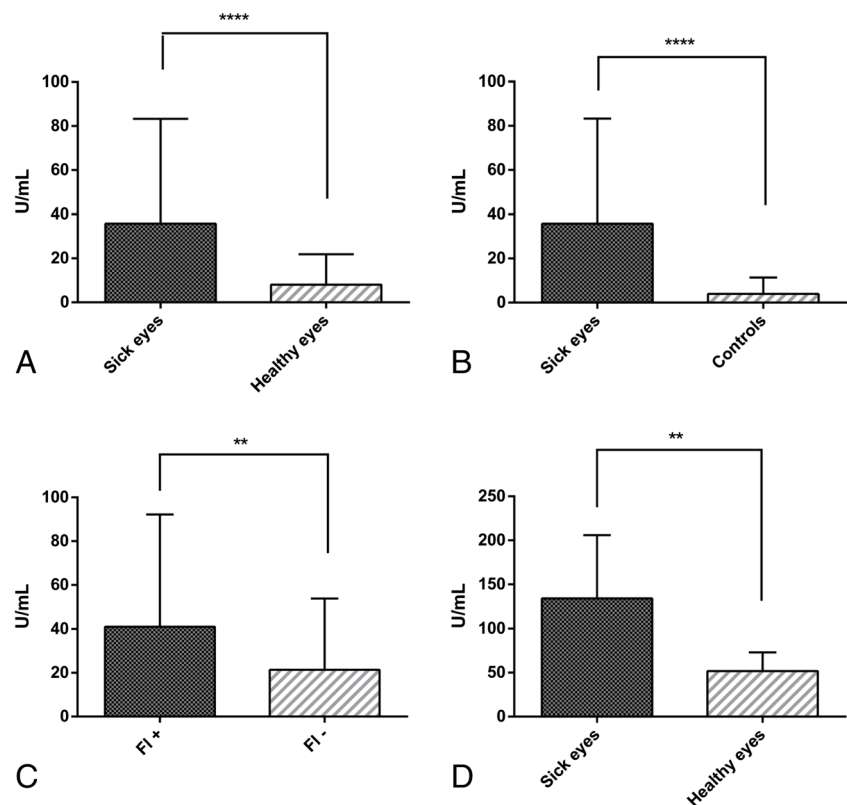
Table 1 Clinical characteristics of HSK patients and correlation with sIgA in tears

Clinical characteristics		Patients (n)	Sick eye		P value
			sIgA −	sIgA+	
Age (years)	≤40	43	28	15	0.755
	>40	144	90	54	
Gender	Male	129	78	51	0.256
	Female	58	40	18	
Episode	First	68	47	21	0.197
	Recurrent	119	71	48	
Fluorescence staining	Negative	47	38	9	0.003[a]
	Positive	139	79	60	
Edema and infiltration	Negative	0	0	0	NA
	Positive	187	187	187	
PK and/or hypopyon	Negative	112	66	46	0.148
	Positive	75	52	23	
Serum IgG	Negative	0	0	0	NA
	Positive	187	187	187	
Serum IgM	Negative	184	116	68	0.897
	Positive	3	2	1	
Serum IgA	Negative	90	66	24	0.005[a]
	Positive	97	52	45	
Healthy eye sIgA	Negative	176	118	58	0.000[a]
	Positive	11	0	11	

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; sIgA, secretory immunoglobulin

a = statistically significant

12 showed positive results for both sIgA and DNA, and 46 (four positive for HSV-DNA and 42 positive for HSV-sIgA) cases were positive for sIgA or DNA. The simultaneous diagnostic rate was 39.73% (58/146), which was defined as a positive rate for HSV-DNA and/or HSV-sIgA. The sensitivity, specificity, PPV and NPV for simultaneous measurement were 39.73%, 98.21%, 98.31%, and 38.46%. The ROC was used to evaluate the diagnostic performance of tear sIgA, DNA, and the combination of them. The areas under the curve for sIgA, DNA, and simultaneous measurement were 0.676 ($p = 0.000$, 95% CI = 0.602-0–750), 0.555 ($p = 0.228$, 95% CI = 0.470–0.639) and 0.690 ($p = 0.000$, 95% CI = 0.617–0.762) (Fig. 3).

Discussion

Stromal HSK is the leading cause of corneal blindness worldwide. Although the diagnosis of HSK with typical manifestations is straightforward, some clinical features of stromal HSK might be obscure, leading to the possibility of misdiagnosis and the relative inappropriate treatment [11]. One previous study indicated that only 34.2% of all clinically suspected cases of HSK could be diagnosed correctly [12]. So it is vital to screen out a rapid noninvasive and reliable method for the diagnosis of stromal HSK, especially those suspected cases without typical characteristics. The appropriate sample should be closely related to the disease and the collecting process safe and rapid. So we chose tears, as the vital component of the ocular surface, as the testing samples in the present study.

HSV-specific tear sIgA reacting with HSK has been described previously as an adjunct to the diagnosis of herpetic keratitis [9, 13, 14]. For patients with a history of herpetic disease, its concentration of infectious eyes was not higher than the other healthy eyes as the inflammation was relieved [15]. The sensitivity, specificity, PPV, and NPV for tear HSV-sIgA were 36.90%, 98.21%, 98.57%, and 31.79% in our study. This

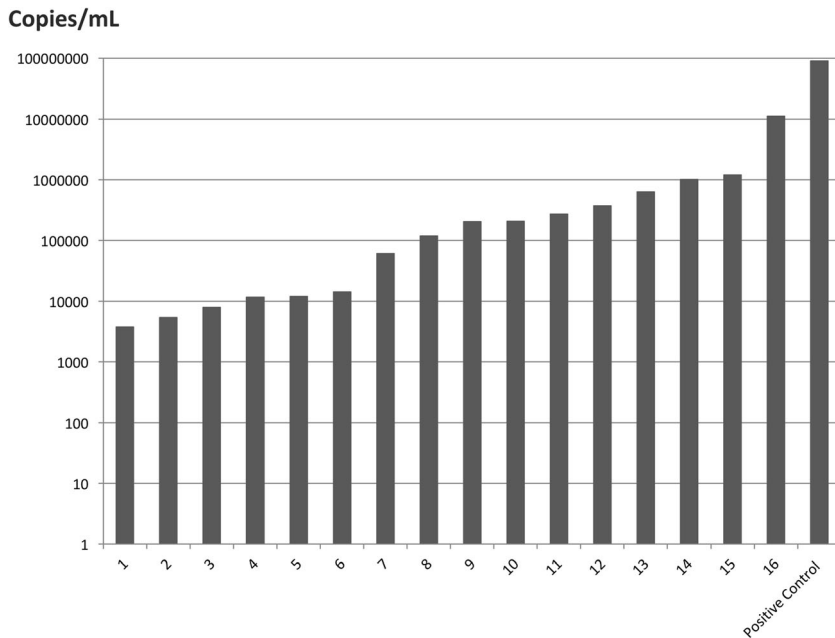**Fig. 2** Tear HSV-DNA copies of the 16 positive samples

**Table 2** Clinical characteristics of HSK patients and correlation with HSV-DNA in tears

Clinical characteristics		Patients (n)	Sick eye		P value
			DNA−	DNA+	
Age (years)	≤40	32	30	2	0.307
	>40	114	100	14	
Gender	Male	104	93	11	0.817
	Female	42	37	5	
Episode	First	44	40	4	0.630
	Recurrent	102	90	12	
Fluorescence staining	Negative	36	35	1	0.041[a]
	Positive	110	95	15	
Edema and infiltration	Negative	0	0	0	NA
	Positive	146	130	16	
PK and/or hypopyon	Negative	85	75	10	0.713
	Positive	61	55	6	
Serum IgG	Negative	0	0	0	NA
	Positive	146	130	16	
Serum IgM	Negative	145	129	16	1.000
	Positive	1	1	0	
Serum IgA	Negative	78	67	11	0.193
	Positive	68	63	5	
Infected eye sIgA	Negative	92	88	4	0.001[a]
	Positive	54	42	12	

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; sIgA, secretory immunoglobulin

a = statistically significant

result suggested the potential significance of HSV-sIgA as an auxiliary differential diagnostic method for atypical stromal keratitis. Although the positive rate was not high enough to suggest that HSV-sIgA could be a gold standard, the positivity indicated that those patients with no typical manifestations might well be HSK. Our results showed that the tear HSV-sIgA levels was significantly higher in the patients with epithelial lesion. It indicated that the increase in HSV-sIgA concentration might be due to the rise in the HSV-DNA load on the ocular surface. However, even for the stromal herpetic keratitis without any epithelial defects, the tear HSV-sIgA of infected eyes still remained much higher than the uninfected eyes or the

Fig. 3 Receiver operator characteristic curve analysis for diagnostic power of sIgA, DNA, and the combination of sIgA and DNA
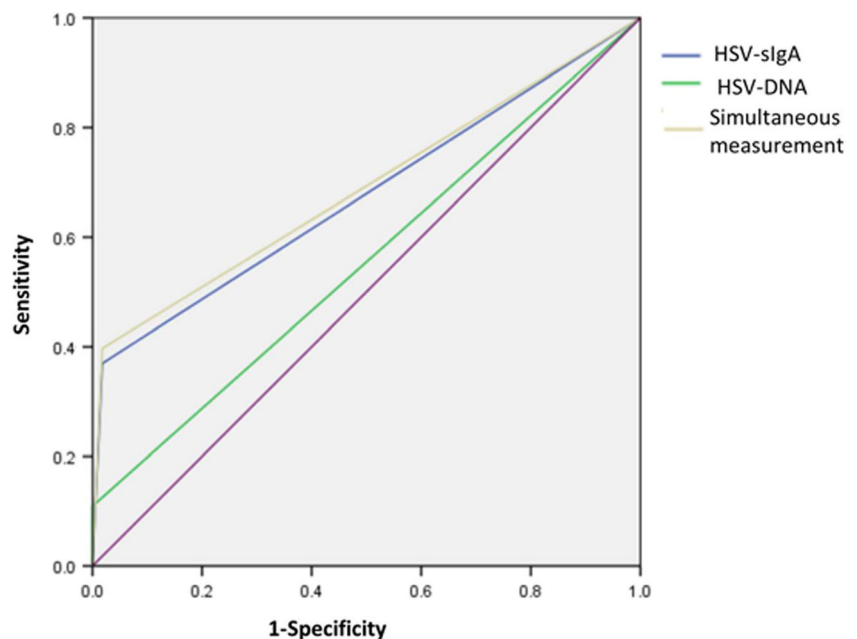
healthy controls. This phenomenon implied that the local immunological processes and inflammation might also increase the HSV-sIgA levels.

For decades, several studies have reported PCR-based laboratory investigations as a valuable tool for HSK diagnosis. The positive rate of corneal scrapings or combined samples (epithelial scrapings, tear fluid, and aqueous humor) in HSK-suspected patients is relatively high, while it is not so sensitive if only tear samples are tested [16, 17]. It is suggested that when the concentration of virus in corneal epithelial scrapings is lower than 10^5 copies/ml, HSV-DNA is seldom detected in tears. On the contrary, if the concentration is higher than 10^5 copies/ml, the tear positive rate increases to 80% [18]. Patients with corneal ulcers had much greater chances of tear HSV-DNA positivity [19]. Furthermore, the virus copy number detected by PCR in atypical epithelial keratitis and stromal keratitis is low [5]. The tear HSV-DNA positive rate for stromal HSK cases was low in our study (10.96%), and the patients with epithelial lesion had a slightly higher detection rate (13.64%). It has been demonstrated that clinical aspects of stromal HSK are not only caused by HSV duplication in cornea, but are also closely related to local mucosal immunological process. So it is not appropriate that only PCR, the HSV-DNA detecting method, is employed as a rapid diagnostic method for stromal HSK.

Shoji et al. evaluated HSK diagnostic efficiency by simultaneous measurement of tear HSV DNA and sIgA. The PPV and NPV obtained by the combined methods were 90.9% and 61.3% respectively. The authors suggested that the combination of laboratory detection of HSV DNA and HSV-sIgA using tear samples enabled higher reliability in diagnosis [10]. However, one limitation of this research is that no detailed investigation of stromal HSK has been identified. Our present study focuses on the evaluation of tear HSV-sIgA, HSV-DNA, and the combination for stromal HSK diagnosis. The sensitivity, specificity, PPV, and NPV for simultaneous measurement and HSV-sIgA only were not significantly different (39.73%, 98.21%, 98.31%, and 38.46% vs 36.90%, 98.21%, 98.57%, and 31.79%). It was already known that the HSV DNA levels in the disciform keratitis were significantly lower than those in dendritic/geographic keratitis. So we suggest that for stromal HSK, especially those without any epithelial defects, the diagnostic efficiency of combining the two laboratory examinations is not much better compared with just testing the tear HSV-sIgA.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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