ORIGINAL ARTICLE

Impacts of Polymerase Chain Reaction Confirmed Herpes Simplex Virus type-1 Keratitis on Patients Attending Ophthalmology Center Mansoura University

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ABSTRACT

Background: *Herpes simplex keratitis (HSK) is a significant corneal disease leading to visual impairment, with an estimated 1.8 million cases worldwide annually and over 230,000 new instances of monocular vision impairment reported in 2016, caused by the herpes simplex virus (HSV), particularly type 1 (HSV-1). HSK has a prolonged course and high recurrence rate, negatively impacting patients' lives and potentially causing blindness if untreated.* ***Objectives:*** *this study aimed to determine the proportion of confirmed HSV type 1 from total clinically suspected viral corneal infection at Ophthalmology Center, Mansoura University, and also to recognize associated risk factors that may increase the severity of the infection.* ***Methodology****: this research identified 23 positive cases out of 100 patients with suspected viral corneal infection by traditional PCR analysis targeting the DNA polymerase gene of HSV type-1.* ***Results:*** *this study did not reveal any statistically significant association between the evaluated risk factors and the occurrence or severity of HSK, but we observed a tendency for higher infection rates among specific demographics such as: patients in their fourth and fifth decades of life, males, and individuals residing in rural areas. In addition, univariate regression analysis showed that keratoplasty was the only predictor that had a statistically significant association with any poor outcome and recurrent infection.* ***Conclusion:*** *this study revealed that corneal ulceration and scarring were the most common complications of HSK, necessitating urgent management to protect against potential vision loss.*

INTRODUCTION

Herpetic ocular infection is a major cause of blindness worldwide. It is well known that herpes viruses including *Herpes Simplex Viruses* (HSV), *Varicella Zoster Virus* (VZV) and *Cytomegalovirus* (CMV) are involved in the pathogenesis of many ocular diseases including keratitis, keratoconjunctivitis, uveitis, iridocyclitis and acute retinal necrosis (ARN) syndrome [1].

Infectious keratitis (IK) is a disease of the cornea caused by bacteria, fungi, protozoa and viruses. Viral keratitis, most commonly in the form of *Herpes Simplex* keratitis (HSK), represents a common cause of infectious keratitis. Several studies documented HSK as a primary cause of infectious keratitis (24%). The annual incidence of HSK has been estimated at 8.4 per 100,000 [2,3].

Primary ocular infection is usually asymptomatic, depending on immunological status of the host. Once the primary infection resolves, the virus becomes latent and stores its genome in the nucleus of the host cell [4].

The recurrent ocular manifestations include epithelial and stromal keratitis. Epithelial keratitis is clinically presented, as dendritic lesions with terminal buds, swollen borders and intraepithelial cell infiltration which may coalesce to give a geographical or amoeboid ulcer, especially in patients with local or systemic immunosuppression and stromal keratitis appears opaque or whitened on physical examination due to stromal infiltration [5].

The most common risk factors of viral ocular infections include contact lens wear, ocular trauma, ocular surface diseases (OSDs) (e.g., dry eye diseases, lid diseases), post-corneal surgery (e.g., keratoplasty, cataract surgery) and systemic diseases (e.g., diabetes, hypertension, immunosuppression) which may increase the severity of the infection [6].

Herpetic ocular infections in particular, represent a significant global burden of disease so rapid definitive diagnosis and recognition of the associated risk factors of these infections has become urgent given the availability of specific antiviral drugs and to prevent potential sight-threatening sequelae of these viral ocular infections [7].

This study aimed to determine the proportion of confirmed HSV type 1 from total clinically suspected viral corneal infection at Ophthalmology Center, Mansoura University, and also to recognize associated risk factors that may increase the severity of the infection.

METHODOLOGY

The study's protocol was approved by Institutional Review Board (IRB), Faculty of Medicine, Mansoura University; code number: MD. 22.09.2120.

This was a cross-sectional study with follow up; it was conducted over a period of 18 months from August 2022 to January 2024. Samples were collected from patients attending Ophthalmology Center, Faculty of Medicine, Mansoura University, Egypt.

Hundred conjunctival swabs were collected from patients with clinically suspected viral corneal infection. Sociodemographic and clinical data were collected from patients, including: age, sex, occupation, place of residence, history of diabetes, hypertension, immunocompromised state, dry eye diseases, lid diseases, contact lens wear, post-corneal surgery, and recent eye trauma.

Samples transported in vials containing 1.2 mL viral transport medium **(DNA Technology, Russia)** to Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, then stored in the freezers at -70°C until later usage in genomic DNA extraction for detection of DNA polymerase gene of HSV type 1 by traditional PCR[7].

Extraction of DNA was done using Exgene™ Viral DNA/RNA kits **(GeneAll®, Korea).** All extraction procedures were done according to manufacturer's protocols. The concentrations and purity of all extracted DNA were measured using the NanoDrop™ 2000/2000c spectrophotometer (**Thermo Scientific, USA**).

Initially, PCR reaction mixture was carried out in 25µl. Amplification done by initial denaturation stage at 94°C for 5-minute followed by 45 cycles of denaturation step at 94°C for 45 sec, annealing step at 57°C for 45 sec, primer extension at 72°C for 45 sec, and final extension step at 72°C for 7 min [8]. Then every sample of conjunctival swab was examined to check for the existence of DNA polymerase gene by traditional type PCR using primers illustrated in **table (1)**.

Table 1: Primer Set and Primer-Sequence used in the PCR[8]

Primer Set	Primer – Sequence	Amplification region	Product size (base pairs)
HSV 1	Forward- 5 ATCAACTTCGACTGGCCCTT 3	DNA polymerase gene	179
	Reverse- 5 CCGTACATGTCGATGTTCAC 3		

Statistical analysis:

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 24). The normality of data was first tested with one-sample Kolmogorov-Smirnov test.

Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test while Fisher exact test was used when expected cell count less than 5.

Continuous variables were presented as mean ± SD (standard deviation) for normally distributed data. Significant variables on univariate analysis entered into Logistic regression model using the forward statistical technique to predict the most significant determinants and to control for possible interactions and confounding effects. The results were considered significant when the $p \leq 0.05$.

RESULTS

Out of the 100 virally suspected patients with keratitis, 23 (23%) were positive for *Herpes Simplex* virus genome by PCR as shown in **figure (1).**

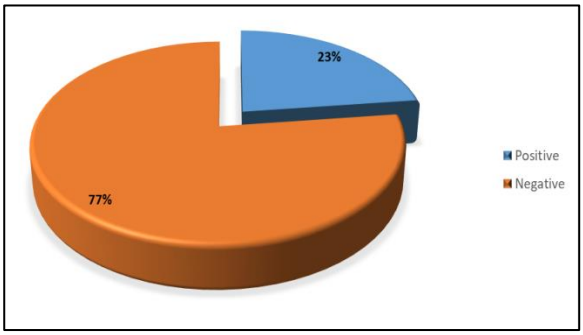

Fig. 1: Prevalence of HSK genome positivity by PCR

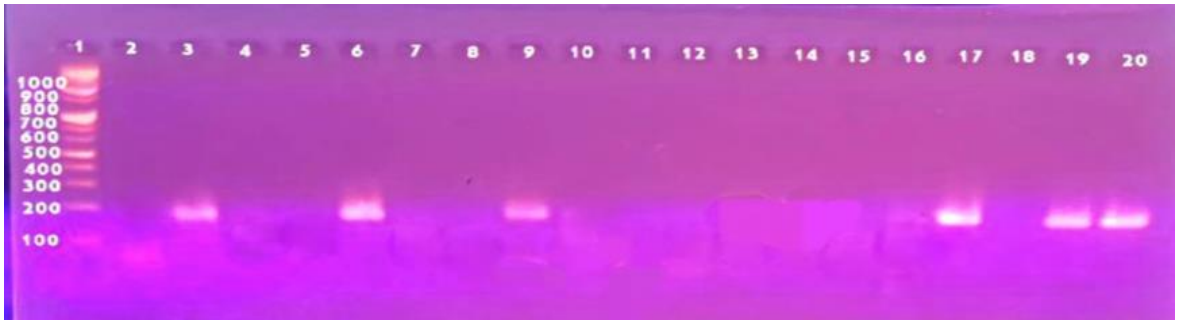

Photo 1: Agarose gel electrophoresis for HSV DNA polymerase gene amplicons in conjunctival swabs
Lane 1 represents 100-bp DNA molecular size marker, lane 3,6,9,17,19,20 are positive samples for the gene (179 bp)

Photo 2: Agarose gel electrophoresis for HSV DNA polymerase gene amplicons in conjunctival swabs
Lane 1 represents 100-bp DNA molecular size marker, lane 2,3 are positive samples for the gene (179 bp)

The mean age of the positive patients was 55.74 years ± SD 11.13. 56.5% of patients were above 50 years old, with predominance of male sex (78.3 %) and rural area (60.9 %), as summarized in **table (2).**

Table 2: Association between HSV infection and demographic data

Demographic data	Total	HSV type 1 keratitis		Test of significance	p value
		Positive (n=23)	Negative (n=77)		
Age categories					
≤50 y	33	10 (43.5%)	23 (29.9%)	$\chi 2=1.84$	0.223
>50 y	67	13 (56.5%)	54 (70.1%)		
Sex					
Male	64	18 (78.3%)	46 (59.7%)	$\chi 2=2.63$	0.104
Female	36	5 (21.7%)	31 (40.3%)		
Resident					
Urban	52	9 (39.1%)	43 (55.8%)	$\chi 2=1.98$	0.159
Rural	48	14 (60.9%)	34 (44.2%)		

According to the distribution of age; 35% of positive cases were at fourth and fifth decade, as shown in **figure (2).**

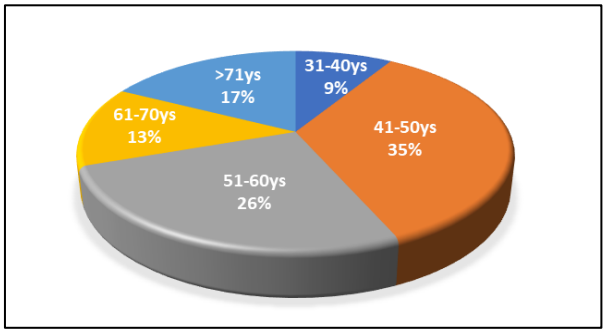

Fig. 2: Distribution of HSK genome-positive patients by age

The association between HSV infection and medical history is summarized in **table (3).** 39.1% of HSK positive patients were diabetic, 30.4% of HSK positive patients were hypertensive, 8.7% of HSK positive patients were immunocompromised.

Table 3: Association between HSV infection and medical history

Medical history	Total	HSV type 1 keratitis		Test of significance	p value
		Positive (n=23)	Negative (n=77)		
Diabetes					
Yes	37	9 (39.1%)	28 (36.4%)	$\chi2$=0.058	0.809
No	63	14 (60.9%)	49 (63.6%)		
Hypertension					
Yes	26	7 (30.4%)	19 (24.7%)	$\chi2$=0.305	0.581
No	74	16 (69.6%)	58 (75.3%)		
Immunocompromised					
Yes	6	2 (8.7%)	4 (5.2%)	FET	1.0
No	94	21 (91.3%)	73 (94.8%)		

$\chi2$: Chi square test, FET: Fisher exact test

The association between HSV infection and ocular history is summarized in **table (4).** 26.1 % of HSK positive patients had history of dry eye diseases, 21.7 % of HSK positive patients had history of keratoplasty, 17.4 % of HSK positive patients had history of cataract surgery, no detected association between contact lens wear, ocular trauma and herpes simplex positive keratitis.

Table 4: Association between HSV infection and ocular history

Ocular history	Total	HSV type 1 keratitis		Test of significance	p value
		Positive (n=23)	Negative (n=77)		
Dry eye disease					
Yes	18	6 (26.1%)	12 (15.6%)	$\chi2$=1.32	0.250
No	82	17 (73.9%)	65 (84.4%)		
Keratoplasty					
Yes	20	5 (21.7%)	15 (19.5%)	$\chi2$=0.056	0.812
No	80	18 (78.3%)	62 (80.5%)		
Cataract surgery					
Yes	30	4 (17.4%)	26 (33.8%)	$\chi2$=2.26	0.133
No	70	19 (82.6%)	51 (66.2%)		
Contact lens					
Yes	4	0 (0%)	4 (5.2%)	FET	0.571
No	96	23 (100%)	73 (94.8%)		
Ocular trauma					
Yes	2	0 (0%)	2 (2.6%)	FET	1.0
No	98	23 (100%)	75 (97.4%)		

$\chi2$: Chi square test, FET: Fisher exact test

The association between HSV infection and complications is summarized in **figure (3).** 91.3% of positive HSK patients had cornel ulcer, from which 69.6 % developed corneal scar, 8.7 % of positive HSK patients developed glaucoma and 4.3 % of positive HSK patients exposed to recurrent infection.

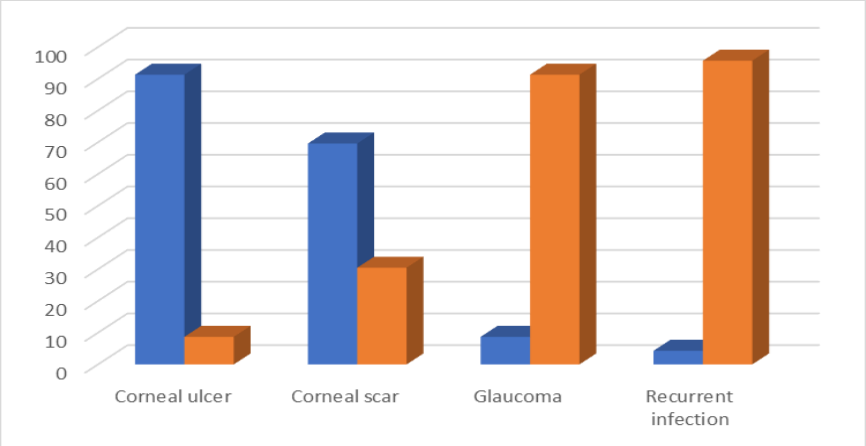

Fig. 3: Association between HSK genome positivity and complications

Univariate regression analysis showed that keratoplasty was the only predictor that had a statistically significant association with any poor outcome and recurrent infection. Age over 50 years, male sex, dry eye and cataract surgery might be associated with poorer outcomes in terms of corneal ulcer and scar but these associations were not statistically significant, as shown in **table (5).**

Table 5: Univariate regression analysis for independent predictors of poor outcome

Independent/ Predictors	Any poor outcome		Corneal ulcer		Corneal scar		Glaucoma		Recurrent infection	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Age >50 y	1.69 (0.7-3.9)	0.23	2.1 (0.8-5.9)	0.14	1.8 (0.7-4.5)	0.21	1.5 (0.15-15)	0.73	1.52 (0.3-8.0)	0.62
Male Sex	2.1 (0.9-4.9)	0.08	1.8 (0.6-4.9)	0.26	2.19 (0.9-5.4)	0.09	0.58 (0.06-5.8)	0.64	0.93 (0.2-4.2)	0.93
DM	1.06 (0.5-2.4)	0.88	1.1 (0.4-2.8)	0.98	1.63 (0.7-3.8)	0.25	1.74 (0.2-12.9)	0.58	0.22 (0.03-1.9)	0.17
Hypertension	0.86 (0.4-2.1)	0.75	0.5 (0.2-1.5)	0.24	1.2 (0.5-2.9)	0.76	-	-	0.9 (0.2-5)	0.95
Immuno-compromised	0.59 (0.1-3.4)	0.56	-	-	0.34 (.04-3.01)	0.33	6.1 (0.5-69.3)	0.15	-	-
Dry eye	0.4 (0.13-1.2)	0.11	0.4 (0.1-1.2)	0.09	0.63 (0.2-1.9)	0.42	-	-	0.6 (0.1-5.5)	0.67
Keratoplasty	2.8 (1.1-7.7)	0.04*	2.4 (0.5-11)	0.26	2.1 (0.8-5.6)	0.15	-	-	4.7 (1.1-21)	0.04*
Cataract surgery	1.3 (0.6-3.1)	0.51	1.3 (0.4-3.8)	0.69	2.3 (0.9-5.6)	0.06	0.8 (0.1-7.7)	0.82	0.3 (0.04-2.6)	0.28

DISCUSSION

Herpes Simplex keratitis is a significant corneal disease-causing visual impairment, with around 1.8 million annual cases globally and 230,000 new cases of monocular vision impairment reported in 2016 [1]. In the present study, 23% of cases were PCR-confirmed HSK, a study conducted in Egypt by Ting et al. [9], observed that 15% of infectious keratitis was caused by HSV type 1.

The mean age of the positive patients in this study was 55.74 years which agreed with Erdem et al. [10], whose patients' mean age was 56 years, the most common age group distribution was between 41-50 years (35%) which agreed with Das et al. [11], who found the most common age group of the patients at their research was between 41 and 50 years (18.58%), but contrasting with older age averages reported by Rangel et al. [12], and Grubešić et al.[13].

Ocular involvement was unilateral in 100% of the cases in our study, in the Das et al. [11] **,** study unilateral involvement was found in 92.91% of the patients while in the Grubešić et al. [13], study ocular involvement was unilateral in 88% of the cases and bilateral in 12% so it's assumed that the most common presentation is unilateral HSK.

Although it wasn't statistically significant but the incidence in male infection (78.3%) was higher than female (21.7%), which agreed with Grubešić et al. [13], and Sinha & Dulani [14], who reported the incidence of HSK was slightly towards the male population, also a greater number of patients (60.9%) were from rural areas, echoing findings by Das et al. [15].

In the current study, there wasn't statistically significant relationship between diabetes and HSK, only 9 out of 23 (39.1%) of patients were diabetic, but Kaiserman et al. [16], indicated that among people 60 to 79 years old, the prevalence of herpetic eye disease was much higher in diabetics than in non-diabetics; however, there was no significant difference found in those who were younger than this age group, this could possibly come from a shorter course of diabetes in younger age that hasn't yet affected the vascular and immune systems [17].

Although OSDs as dry eye was not a statistically significant factor, this research found a potential association with poorer outcomes based on univariate regression analysis. This observation agreed with Sluch et al.[18], who reported a high incidence of dry eye among his patients with poorer outcomes and proposed that ocular surface disease was a significant risk factor for herpetic eye disease, also the pro-inflammatory environment caused by dry eye might facilitate disease reactivation.

The occurrence of newly acquired HSK was 14 times greater in corneal transplant patients compared to the normal population as mentioned by Tsatsos et al. [19], but in this study, there was no significant relationship.

In the present study, 17.4% of positive HSK patients had previous cataract surgery, Barequet & Wasserzug [20], who described only 3 cases of HSK after cataract surgery in comparison to 14 patients after keratoplasty in the same period, suggested that HSK following cataract surgery was a far less common complication than that after keratoplasty.

In this research, (91.3%) had ulcer at the time of presentation, 16 cases of them developed corneal scar and opacity affecting visual acuity with different degrees and that was similar with previous studies done by Sluch et al**.** [18] and Kim et al. [21], who reported similar rates of corneal scarring at 76.5% and 76.4%, respectively.

In a recent study by Rosenberg et al**.** [6], 8.16% of their patients developed glaucoma, a rate that aligns closely with the findings in this study (8.7%). In contrast, Miserocchi et al. [22], reported a significantly higher incidence of glaucoma at 38%. This discrepancy may be attributed to the longer follow-up period in Miserocchi et al.'s research. Additionally, the association of keratitis with uveitis could be a factor, as Gorla & Brown [23], found that glaucoma was a more common complication in cases of keratouveitis.

In the present study, 4.3 % of the patients had recurrent infection in 12 months follow up period, in a study conducted by Hsiao et al. [24], (45%) of their patients developed recurrent HSK after 35.3 months mean follow-up time. This significant difference in recurrence rates could be attributed to the extended follow-up period in the Hsiao et al. study, suggesting that HSK may have a higher likelihood of recurrence over a longer duration.

It was also observed that all previous complications occurred in patients who initially diagnosed with ulcers and who confirmed positive early in the duration of this study, this was probably because these patients had a longer follow-up time. Conversely, the two patients who sought treatment before ulceration experienced resolution of their infections without complications during the follow-up period. This underscores the critical importance of early diagnosis and management in preventing poor prognosis and complications associated with HSK.

Also, the high prevalence of positive patients in rural areas might indeed overshadow the statistical significance of clinical and medical risk factors in infection reactivation or complication development. This is likely due to additional factors prevalent in these regions, such as low medication compliance and poor adherence to infection control measures.

The limitations of this study include a small sample size, retrospective data collection with its inherent biases, and a short follow-up period, particularly for patients confirmed positive later in the study. Therefore, future research with larger sample sizes, extended follow-up periods, and more detailed assessments of risk factors is essential to better understand the determinants of HSK severity and complications. This could help in the effort to avoid the poor sequelae associated with ocular *Herpes Simplex* infection.

CONCLUSIONS

This study did not reveal any statistically significant association between the evaluated risk factors and the occurrence or severity of HSK, there was a tendency for higher infection rates among specific demographics such as: patients in their fourth and fifth decades of life, males, and individuals residing in rural areas. In addition, univariate regression analysis showed that keratoplasty was the only predictor that had a statistically significant association with any poor outcome and recurrent infection. Age over 50 years, male sex and dry eye might be associated with poorer

outcomes, but these associations were not statistically significant. The present study also revealed that corneal ulceration and scarring were the most common complications of HSK, necessitating urgent management to protect against potential vision loss.

Recommendations

Implementing regular screening protocols for patients presenting with corneal infections, especially those suspected of viral etiology, can aid in early detection of HSK. This can potentially reduce the risk of complications such as corneal scarring and glaucoma.

Further long-term follow-up researches are needed to better understand the determinants of HSK severity and complications.

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Submission declaration: The manuscript has not been published elsewhere and has not been submitted simultaneously for publication elsewhere.

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