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Detection of Herpes Simplex Virus Type 1 in Patients Affected by Conjunctivitis

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ABSTRACT

Herpes simplex virus (HSV) is a common human pathogen that causes severe infections in newborns and immunocompromised patients. Conjunctivitis or corneal epithelial keratitis is caused by HSV type 1 all over the world and at all times of the year. The present study was aimed at detecting HSV in patients suffering from conjunctivitis. One hundred and ten (110) clinical samples (90 patients and 20 controls, both males and females) of eye conjunctiva swabs were collected from patients of different ages. The samples were analyzed using qPCR and ELISA techniques. The qPCR results revealed that HSV was present in 47 (52.2%) of the 90 patients who were infected. Of these patients, 25 (48.0%) were males and 22 (57.8%) were females, indicating that females are more susceptible to infection. According to the results by age group, patients over 50 years old had a higher rate (81.8%), making young adults more susceptible to infection. The prevalence of HSV-positive results by ELISA was observed in 29 (32.3%) individuals, including 16 (30.7%) males and 13 (34.2%) females, indicating that females are more susceptible to infection. Adults over the age of 50 (54.5%) are more susceptible to infection, according to the age group's HSV-positive outcomes. HSV type 1 infection is highly prevalent among Iraqi conjunctivitis patients, with a statistically significant difference when compared to controls, based on the two techniques. The findings of this study indicate that qPCR is more accurate and reliable than the ELISA technique for detecting HSV type 1.

Keywords: Detection, ELISA, HSV, PCR, Virus.

Introduction

Bacteria and viruses are the most prevalent infectious agents. Noninfectious conjunctivitis can include allergic, toxic, and scar conjunctivitis. Conjunctivitis brought on by a virus is typically unilateral. The discharge is small and watery and may be associated with vesicular eyelid lesions. It is recommended to use oral and topical antivirals to shorten the course of the disease.[1] Human herpes virus (HHV) infections have been documented since ancient Greece when human infections with the herpes simplex virus (HSV) were first reported. Hippocrates used the term "herpes," which means "to creep or crawl," to describe how skin lesions spread. Vidal was the first to recognize HSV transmission from person to person in 1893, although the vesicular presence of lesions associated with HSV infections was widely established in the late eighteenth century. HSV was discovered for the first time in 1919.[2] However, it took decades before it was discovered that two HSV serotypes, HSV-1 and HSV-2 existed. Several other HHV family members have been identified over time. The new member, HHV-8, was only identified a decade ago. The last 20 years have witnessed a flood of new information about HHV's biological properties due to significant laboratory advancements.[3] Herpes simplex types 1 and 2 separated around 6 million years ago.[4] Herpes viruses cause infections that last a lifetime and therefore cannot be eliminated from the body.[5] One of the most prevalent human viral pathogens that can cause major clinical disease at any age is the herpes simplex virus type 1 (HSV-1).[6]

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Epithelial cells of the conjunctiva, eyelid tissue, and cornea, when there is a latent infection, are the sites where HSV-1 acutely proliferates.[7]

Periodic reactivation of latent neural infection can result in chronic ocular diseases, including conjunctivitis, keratitis, and blepharitis. Conjunctivitis, also known as pink eye, is an inflammation of the inner surface of the eyelid and the white area of the eye's outermost layer. It can have infectious and noninfectious origins.[8] This inflammatory reaction can lead to decreased sensation of the corn ea, scarring, and blindness.[9] Public awareness of HSV and its implications is crucial.[10] It has been demonstrated that acyclovir is an effective treatment option.[11] Healthy adults who were seronegative for HSV-1 by ELISA or viral serum neutralization assay showed asymptomatic shedding.[12] By 2020, there will be 15 million blind people in India, with ocular infections accounting for 15% of the country's overall burden.[13] Viruses can cause conjunctivitis, keratitis, keratoconjunctivitis, uveitis, chorioretinitis, iridocyclitis, and acute retinal necrosis syndrome, among other ocular infections.[14] The classic HSV-1 epidemiology of oral transmission in young children is changing.[15] In Israel's hospitals, HSV-1 infections were discovered in 38% of children between the ages of 2 and 4 and 54% of patients between the ages of 15 and 17. HSV-1 frequency in England and Wales ranged from 17 to 27% in children aged 1 to 14 years, likely reflecting maternal antibody status in part. It was 46 to 49% in neonates.[17] The present study was conducted to detect herpes simplex virus type 1 in patients affected by conjunctivitis.

Materials and Methods

Sample collection

Eye conjunctiva swabs from 110 clinical samples (90 patients and 20 controls, both men and women) were used in the study. These samples came from patients of various ages who were hospitalized in Ibn Al Hathim and Alyarmuk in Baghdad between March and May 2020 and had symptoms of conjunctivitis. The samples were collected and

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deposited in a deep freezer with 1 ml of VTM medium. In order to process the samples, an HSV-1 IgG ELISA kit was used.

DNA extraction from swab samples

DNA was extracted from the virus obtained from the samples by using the viral nucleic acid extraction kit II (Geneaid, Japan), and the extraction was done according to the manufacturer's instructions.

Detection of HSV by quantitative polymerase chain reaction (qPCR)

HSV DNA was detected in the samples using the real-time polymerase chain reaction (qPCR) method with the PCR Max qPCR Kit (USA Company). The reaction mixture was prepared by combining 10 µl of lyophilized 2x qPCR master mix, 1 µl of HSV-1 primer/probe mix (brown), 1 µl of internal extraction control primer/probe mix (brown), and 3 µl of RNAse/DNAse free water (white) to obtain a total reaction volume of 15 µl. Appropriate reactions were provided for the positive and negative controls. An endogenous control reaction for each DNA sample was prepared by combining lyophilized 2x qPCR master mix (10 µl), endogenous control primer/probe mix (brown) (1 µl), and RNAse/DNAse free water (white) (4 µl) to obtain a total reaction volume of 15 µl. The control reaction provided critical information on the biological sample's consistency. A volume of 15 µl of each mix was pipetted into individual wells. A volume of 5 µl of DNA template was added to each well. For negative control wells, 5 µl of RNAse/DNAse free water was added. The final volume in each well was 20 µl. The amplification program included 50 cycles of UNG treatment (if required) at 37^{o}C for 15 minutes, enzyme activation at 95 ^{o}C for 2 minutes, denaturation at 95^{o}C for 10 seconds, and data collection at 60^{o}C for 60 sec.

Detection of HSV by enzyme-linked immunosorbent assay (ELISA)

HSV-1 human antibody class IgG was detected using the enzyme-linked immunosorbent assay (ELISA) on conjunctiva and tear swab samples. The HSV-1 IgG ELISA Kit (Germany Company) was used for the assay, and the protocol was conducted according to the manufacturer's instructions.

Statistical analysis

The statistical package for the social sciences (SPSS) was used for the statistical analysis.

Results and Discussion

Real-time PCR was used to detect HSV in samples from both groups and screen for the presence of anti-HSV antibodies by ELISA. It was observed in 47 out of 90 patients (52.2%). There was a statistically significant difference between the patient and the control group, as shown in Figure 1 and Table 1. According to the results (Table 2) from the analysis of the qPCR product on 2 % agarose gel electrophoresis, HSV-1 infections were detected in people of all ages, including both males and females. In the present study, 47 (52.2%) of the 90 patients had HSV infection; 25 (48.0%) of the patients were male, while 22 (57.8) were female. This indicates that females are more susceptible to infection. There was no statistically significant difference between males and females in terms of HSV-1 infection, as shown in Table 2.

Population dynamics significantly affect the prevalence of HSV infections. Therefore, the percentage of patients shedding HSV-1 DNA reported in the present study may only reflect the prevalence of HSV-1 and partly depend on the population sample. A large number of African-American female cohorts (54%) reflect the demographics of this southeastern region, which has a population of 67.3% of African-Americans and 53.1 % women, two important predictors of HSV-1 infection.[18] Age, stress, socioeconomic status, level of education, age of first intercourse, and total years of sexual activity are all recognized as significant predictors.[18] The number of people who were diagnosed with HSV based on their age groups is presented in Table 3. Adults are more prone to infection, as shown by the greater rate of HSV-positive results (81.8%) in patients who are over 50 years old. This result shows a higher number compared to other studies. More than 62% of Americans between the ages of 12 and 19 have HSV-1.[19]

Table 1: The percentage of patient and control sample results by PCR

Group	No.	Positive No. (%)	Negative No. (%)	Chi-Square (χ^2)
Patients	90	47 (52.2)	43 (47.7)	1.07 NS
Control	20	0 (0.00)	20 (100.0)	15.00 **
Total	110	47 (42.7)	63 (57.2)	5.31 *
Chi-Square (χ^2)	--	10.53 **	10.53 **	---

*: $p \leq 0.05$; **: $p \leq 0.01$; NS: Non-significant

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Table 2: Positive HSV-1 results by PCR in relation to gender

Gender	Positive No. (%)	Negative No. (%)	Total No. (%)	Chi-Square (χ^2)
Males	25 (48.0)	27 (51.9)	52	1.06 NS
Females	22 (57.8)	16 (42.1)	38	5.48 *
Total	47 (52.2)	43 (47.7)	90	1.66 NS
Chi-Square (χ^2)	1.38 NS	4.77 *	--	---

*: $p \leq 0.05$; NS: Non-significant

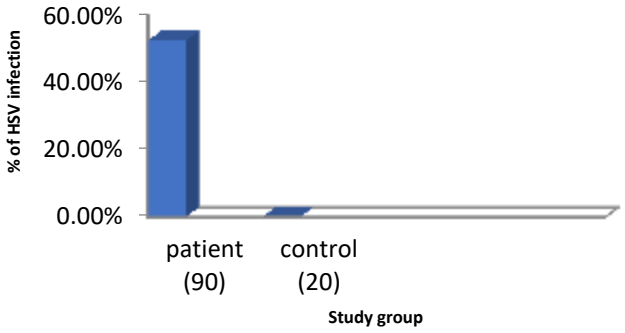

Figure 1: The prevalence of HSV infection by RT-PCR between patient and control groups.

Anti-HSV antibodies (IgG) were detected in tears from study groups using the enzyme-linked immunosorbent assay (Figure 2). Positivity was observed in 29 (32.3%) patients. A statistical analysis of all of these results, as depicted in Table 4, revealed a highly significant difference between the patient and control distributions for every test. As shown in Table 5, gender analysis revealed that 29 samples, including 16 (30.7%) males and 13 (34.2%) females, were positive. There was no statistically significant difference between males and females in terms of herpes simplex virus type 1 infection (Table 5). In one study, all participants over the age of 60 had HSV-1 seropositivity.[20]

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Table 3: Positive HSV-1 results by PCR in relation to age group of the patients

Age groups (year)	No. of patients	Positive No. (%)	Negative No. (%)	Chi-Square (χ^2)
1-20	20	5 (25.0)	15 (75.0)	12.83 **
20-50	60	33 (55.0)	27 (45.0)	4.41 *
More than 50	11	9 (81.8)	2 (18.1)	13.55 **
Total	90	47 (52.2)	44 (48.8)	
Chi-Square (χ^2)	---	11.53 **	11.53 **	---

*: p≤0.05; **: p≤0.01

Table 4: The percentage of patient and control sample results by ELISA

Group	No.	Positive No. (%)	Negative No. (%)	Chi-Square (χ^2)
Patients	90	29 (32.3)	61 (67.7)	9.26 **
Control	20	(0.00)	20 (100.0)	15.00 **
Total	110	29 (26.3)	81 (73.6)	12.52 **
Chi-Square (χ^2)	--	9.41 **	9.41 **	---

** (P≤0.01).

**: p ≤ 0.01

Table 5: Positive of HSV-1 results by ELISA in relation to gender

Gender	Positive No. (%)	Negative No. (%)	Total No. (%)	Chi-Square (χ^2)
Males	16 (30.7)	36 (69.2)	52	10.66 **
Females	13 (34.2)	25 (65.7)	38	9.05 **
Total	29 (32.2)	61 (67.7)	90	9.73 **
Chi-Square (χ^2)	1.29 NS	1.29 NS	---	---

**: p ≤ 0.01; NS: Non-Significant

Table 6: Positive HSV-1 results by ELISA in relation to age group of the patients

Age groups (year)	No. of patients	Positive No. (%)	Negative No.(%)	Chi-Square (χ^2)
1-20	19	2 (10.5)	17(89.4)	13.97 **
20-50	60	21 (35.0)	39 (65.0)	9.12 **
More than 50	11	6 (54.5)	5 (45.4)	4.51 *
Total	90	29 (32.2)	61 (67.7)	9.73 **
Chi-Square (χ^2)	---	11.08 **	11.08 **	---

*: p ≤ 0.05; **: p ≤ 0.01

According to this study's age results, people between the ages of 20 and 50 had the highest positive number, which was 21 (35.0%). The statistical analysis of all of these observations revealed a highly significant variation in the age distribution for each patient analyzed, as shown in Table 6. In a New Mexico study, HSV-1 was found in 59% of Navajo children aged 1 to 5 and in 79% of those aged 6 to 15 years.[21] A similar observation was made in Syria among children and young adults (55% among 1–5-year-olds and 80% among 11 to 20-year-olds).[16] In Germany, HSV-1 prevalence was lower in children aged 1–5 years (31%) and increased to 44–49% in those aged 6–16 years.[22]

Comparison between qPCR and ELISA outcomes

To detect HSV-1 infection, the results of the qPCR and ELISA tests were compared.

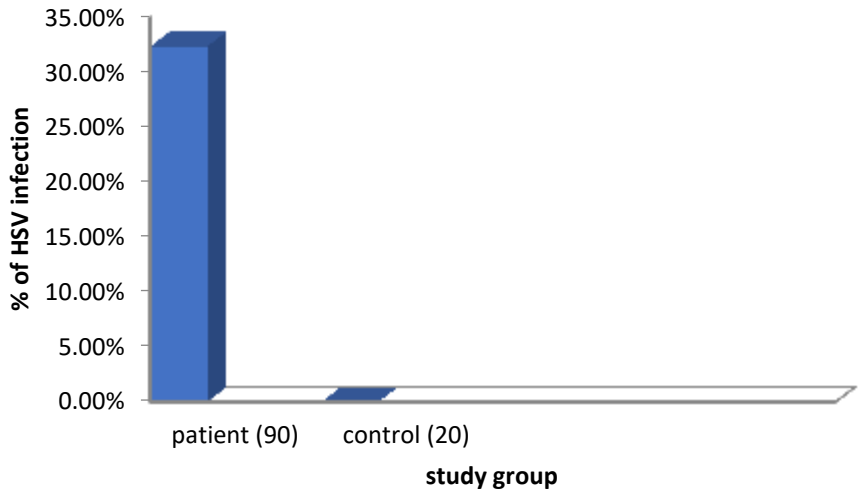

Figure 2: Detection of anti-HSV IgG in tears of patients and controls by ELISA.

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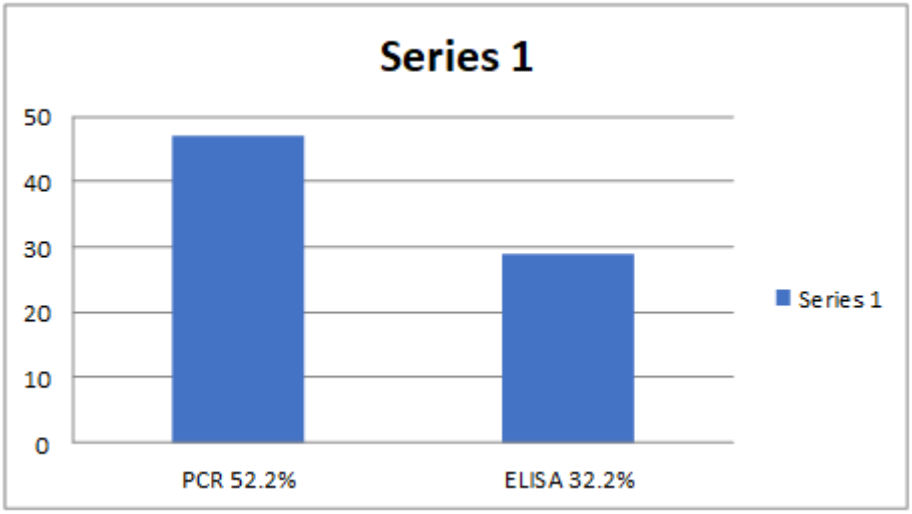

Figure 3: Rate of comparison between PCR and ELISA techniques used to detect HSV 1 infection.

The highest percentages of positive results for total HSV infection were obtained using qPCR, followed by ELISA, with 52.2% and 32.2%, respectively, as shown in Figure 3. The current study found that the qPCR assay is a highly specific method for detecting HSV-1 in patients with acute conjunctivitis infections and is a reliable tool for detecting HSV-1 infections early in the laboratory. This is particularly true in light of the new therapeutic possibilities that are currently being developed. However, the study discovered that using an ELISA assay to diagnose herpes virus infection may be useful for diagnosing conjunctivitis illness in high-risk populations of immune-compromised people. The presence of HSV infection revealed the association between mild and moderate clinical disease. There are a different of medical applications for the PCR technique, in the diagnosis of CML,[23] Adenocarcinoma, [24]detection of dangerous of microorganisms such *Brucella melitensis* , [25]methicillin-resistant *Staphylococcus aureus* ,[26,27] genotyping of *Clostridium perfringens* toxins, [28] *Proteus vulgaris*, [29] toxoplasmosis,[30,31] and SARS-Cov-2.[32]

Conclusion

The findings of this study reveal that real-time PCR is more accurate and reliable than ELISA for the identification of HSV type 1. HSV type 1 infection is statistically significantly more prevalent among Iraqi conjunctivitis patients than controls, according to qPCR and anti-HSV IgG immune responses obtained by ELISA technique. Using qPCR of tear swabs, HSV was discovered in 52.2% of the patients expressing conjunctivitis symptoms. In 32.3% of the individuals with conjunctivitis signs of tears, HSV was detected using ELISA.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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