



Laboratory investigations of herpes simplex virus-1 and -2 clinical samples in Korea

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ABSTRACT

Objectives: Herpes simplex virus (HSV) infections have been reported in 60% to 95% of the adult population worldwide, making them one of the most common infectious conditions globally. HSV-1 and HSV-2 cause severe disease in immunocompromised patients. Therefore, the aim of this study was to provide information that could be used to reduce the incidence of genital herpes caused by HSV-1 and HSV-2.

Methods: From September 2018 to December 2020, 59,381 specimens were collected from outpatients across primary and secondary hospitals in Korea who requested U2Bio (Korea) to conduct molecular biological testing of their samples for sexually transmitted infections. In this study, the positivity rates of HSV-1 and HSV-2 were analyzed according to age, sex, and specimen type.

Results: In the age-specific analysis of HSV-1, the highest positivity rate (0.58%) was observed in patients under 19 years of age, whereas the lowest positivity rate (0.08%) was observed in patients aged over 70 years. In the age-specific analysis of HSV-2, the highest positivity rate (2.53%) was likewise observed in patients under 19 years of age.

Conclusion: Our study identified differences in the infection rates of HSV-1 and HSV-2 depending on patients' sex and age. These differences will be useful for improving disease prevention and control measures for HSV-1 and HSV-2.

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Introduction

Herpes simplex virus (HSV) infections are among the most widespread infectious conditions worldwide, affecting 60% to 95% of the adult population [1]. HSV is the most common sexually transmitted infection (STI) in the United States [2]. According to Burn Aschner et al. [3], 67% of the worldwide population is infected with HSV-1 by 49 years of age; many individuals in

developing countries are infected early in childhood [3].

In the United States, between 2015 and 2016, the prevalence of HSV-1 and HSV-2 among people between 14 and 49 years of age was 47.8% and 11.9%, respectively [4]. During the period from October 2014 to March 2015 in Ghana, the prevalence rates of HSV-1 and HSV-2 among females were 99.2% and 78.4%, respectively [5]. These results indicate that the global spread of HSV-1 and HSV-2 is high and attention-worthy [6].

HSV-1 and HSV-2, which are double-stranded DNA viruses that typically infect humans [7], are related to many diseases and can cause rare but severe after-effects, such as meningitis and neonatal herpes [8]. Moreover, HSV infection during pregnancy is related to miscarriage, premature birth, and congenital and neonatal herpes, and it can cause severe infections in newborns owing to intrauterine vertical metastasis. In many cases, contact with HSV-infected genital secretions can lead to a high risk of mortality and neurodevelopmental disorders during vaginal delivery [1].

HSV infection can also function as a co-factor for the acquisition and transmission of other sexually contagious infections, including human immunodeficiency virus, syphilis, hepatitis C virus, and human papillomavirus [9]. HSV-1 and HSV-2 also cause blindness and severe infectious diseases in immunocompromised patients [10]. In the United States, the proportion of individuals co-infected with HSV-1 and HSV-2 decreased from 14.6% in 1988–1994 to 10.5% in 1999–2004 [11]. Co-infection with HSV-1 and HSV-2 severely affects the dynamics of patients with clinical genital herpes compared with infection with HSV-2 only [12].

Vaccines that prevent genital herpes infections affect public health [13]. However, vaccines intended to prevent HSV-2 genital herpes have not been successful to date [13]. Therefore, the aim of this study was to provide information to support the development of vaccines that would be useful for protection against genital herpes caused by HSV-1 and HSV-2 and to provide information that could be used to safeguard public health and reduce the incidence of genital herpes caused by HSV-1 and HSV-2.

Materials and Methods

Materials

From September 2018 to December 2020, 59,381 specimens were collected from outpatients across primary and secondary hospitals in Korea who requested U2Bio (Seoul, Korea) to conduct molecular biological testing of their samples for STIs. The specimens were classified into swab, urine, and other (catheter, pus, and tissue) samples. The study protocol was approved by the Institutional Review Board of Dankook University (IRB No. 2021-04-002).

Nucleic Acid Extraction

The collected clinical specimens were stored at -70°C until DNA isolation for multiplex polymerase chain reaction (mPCR). DNA for the mPCR assay was extracted using an ExiPrep Dx Bacteria Genomic DNA kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The concentrations of the extracted DNA samples were measured using the AccuPower STI8B-Plex Real-Time PCR kit (Bioneer).

Real-Time PCR Analysis

Real-time PCR analysis was performed using the AccuPower STI8B-Plex Real-Time PCR kit with an Exicycler 96 Real-Time Quantitative Thermal Block (Exicycler 96; Bioneer), according to the manufacturer's protocol. The amplification protocol comprised 1 cycle at 95°C for 5 minutes and 45 cycles at 95°C for 5 seconds and 55°C for 5 seconds. The threshold cycle was determined according to the manufacturer's instructions. Four pathogens—*Trichomonas vaginalis*, *Mycoplasma hominis*, HSV-1, and HSV-2—were evaluated. In this study, we analyzed HSV-1 and HSV-2. The real-time PCR target genes used and their product sizes are listed in Table 1.

Statistical Analysis

SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses, including descriptive statistical analysis and frequency analysis. Statistical significance was set at $p < 0.05$.

Results

In total, 59,381 specimens were tested for HSV-1 and HSV-2 infections between August 2018 and December 2020, and 1,429 (2.4%) of them tested positive.

In the age-specific analysis of HSV-1, the highest positivity rate (0.58%, 6/1,026) was observed in patients under 19 years of age, followed by those aged 20 to 29 years (0.50%, 80/16,076) (Figure 1). The lowest HSV-1 positivity rate (0.08%, 2/2,370) was observed in patients aged over 70 years (Figure 1). In the age-specific analysis of HSV-2, the highest positivity rate (2.53%, 26/1,026) was observed in patients under 19 years of age, followed by those aged 20 to 29 years (2.33%, 375/16,076). The lowest HSV-2-positivity rate (1.76%, 196/11,119) was observed in patients aged 40 to 49 years (Figure 1). The positivity rate

Table 1. Target genes and their product sizes for real-time polymerase chain reaction

Pathogen	Target gene	Product size (bp)
Herpes simplex virus type 1	<i>us4</i>	111
Herpes simplex virus type 2	<i>Gg</i>	86

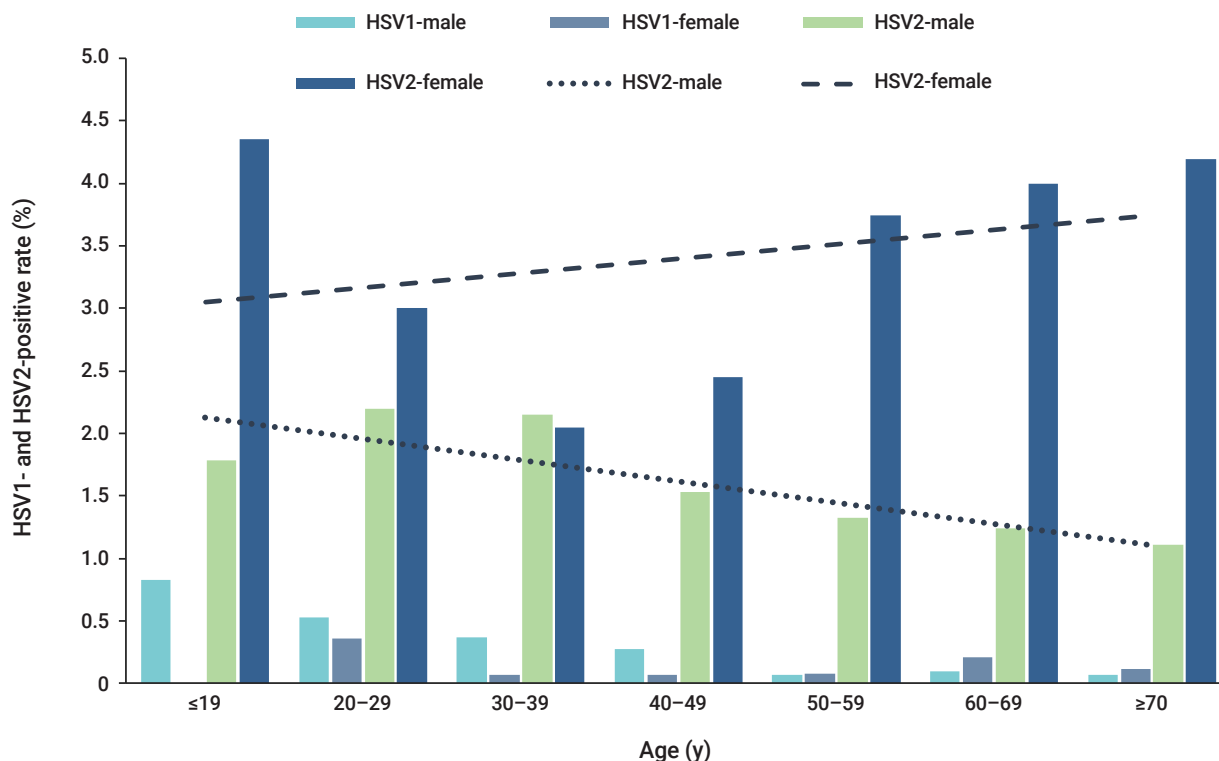


Figure 1. Herpes simplex virus (HSV)-1 and HSV-2 positivity rates according to age and sex. The HSV-2 positivity rate was higher than the HSV-1 positivity rate. The positivity rate of HSV was higher in females than in males.

of HSV-1 showed a significant association with age ($p=0.007$) (Table 2). Similarly, the positivity rate of HSV-2 was also significantly associated with age ($p=0.026$) (Table 2). Distinct patterns of infection were observed in different age groups.

The results of the specimen-specific positivity rate analysis showed that in males, the highest HSV-1-positivity rate was found in urine specimens (0.66%, 5/760), and the highest HSV-2-positivity rate was found in swab specimens (5.92%, 45/760) (Table 3). In females, the highest HSV-1-positivity rate was found in swab specimens (0.23%, 15/6,665), and the highest HSV-2-positivity rate was found in swab specimens (3.81%, 254/6,665) (Table 3). To summarize, the highest positivity rate was observed in swab specimens of both males and females. The HSV-2-positivity rate was higher than that of HSV-1 during the study period. HSV-1 and HSV-2 co-infection ($n=5/59,381$) was observed.

Discussion

The study analyzed the positivity rates of HSV-1 and HSV-2 infections according to age, sex, and specimen type from September 2018 to December 2020. The highest positivity rates for both HSV-1 and HSV-2 were observed in the specimens of patients under 19 years of age. The HSV-2 positivity rate

Table 2. Age distribution of the positivity rate of HSV-1 and HSV-2 and correlation between age and the positivity rate of HSV-1 and HSV-2 between 2018 and 2020

Age (y)	HSV-1	HSV-2
≤ 19 ($n=1,026$)	6 (0.58)	26 (2.53)
20-29 ($n=16,076$)	80 (0.50)	375 (2.33)
30-39 ($n=15,644$)	49 (0.31)	333 (2.13)
40-49 ($n=11,119$)	25 (0.22)	196 (1.76)
50-59 ($n=8,474$)	6 (0.07)	175 (2.07)
60-69 ($n=4,672$)	6 (0.13)	98 (2.10)
≥ 70 ($n=2,370$)	2 (0.08)	52 (2.19)
p	0.007*	0.026*

Data are presented as n (%).

HSV, herpes simplex virus.

* $p < 0.05$.

was higher in females than in males.

HSV-1 prevalence tends to increase with age, and most incident cases occur in childhood and adolescence. Moreover, the shapes of age-specific HSV-2 and HSV-1 prevalence graphs differ primarily in terms of whether they show the expected patterns of an STI (HSV-2) rather than a non-STI (HSV-1) [8]. The prevalence of HSV-2 was previously reported to range from 34% to 79% among clinic- and community-based 15-

Table 3. Positivity rates of HSV-1 and HSV-2 according to the specimen type and sex between 2018 and 2020

Specimen	Male			Female		
	Total (n)	HSV-1	HSV-2	Total (n)	HSV-1	HSV-2
Swab	760	5 (0.66)	45 (5.92)	6,665	15 (0.23)	254 (3.81)
Urine	38,905	127 (0.33)	699 (1.80)	5,986	5 (0.08)	145 (2.42)
Other	6,168	22 (0.36)	99 (1.61)	897	0	13 (1.45)
Total	45,833	154 (0.34)	843 (1.84)	13,548	20 (0.15)	412 (3.04)

Data are presented as n (%).
HSV, herpes simplex virus.

to 24-year-old individuals in Eastern Africa and from 46% to 66% in high-risk populations [14,15].

Sexually acquired genital HSV-1 infections are common in young females and males with active sex lives [16]. According to Issakwisa et al. [16], the infection rates are higher in males than in females [16]. Our study also showed that HSV-1 infection was more common in males than in females. The age-specific HSV-2 prevalence is usually higher in females than in males, as well as in individuals with high-risk sexual behaviors [14,17]. This result appears to be consistent across multiple geographic sites, and females seem to show a higher risk of HSV-2 acquisition than males [8].

Severe HSV-1 and HSV-2 co-infections, with high mortality and morbidity rates, have been observed in newborns [18]. HSV-1 and HSV-2 co-infection were observed in this study; however, further research is needed to verify this finding.

This study has some limitations. First, it was conducted in the tests performed by a single organization, and it did not represent all regions in Korea. Additionally, the 3-year study period was relatively short, making it difficult to identify trends in HSV-1 and HSV-2 infections. Second, the sample data were anonymous, and the residence of the patients was not specified. Third, it was a retrospective study rather than a prospective study, and the results were not analyzed according to patients' clinical characteristics.

The prevalence of HSV-2 and HSV-1 is highly variable, and it depends on many factors, including country, area of residence, population subgroup, sex, and age [10]. Therefore, age-specific or age-adjusted prevalence among similar populations should be analyzed to compare the prevalence of HSV infections across geographic areas or between countries. Despite these limitations, we found distinct infection patterns in different age groups. We expect that this information will assist in the development and implementation of targeted behavioral interventions to reduce genital herpes infections caused by HSV-1 and HSV-2.

Conclusion

Our study identified differences in the infection rates of HSV-

1 and HSV-2 according to sex and age. The characterization of these differences will be useful for describing the dynamics and risk factors of HSV-1 and HSV-2, especially for improving disease prevention and control measures. We also expect that this study will provide information that can be used to safeguard public health and reduce the incidence of genital herpes caused by HSV-1 and HSV-2.

Notes

Ethics Approval

This study was approved by the Dankook University Institutional Review Board (IRB No. 2021-04-002).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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None.

Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: all authors; Data curation: EJO; Formal analysis: YSY; Investigation: all authors; Methodology: all authors; Project administration: JKK; Resources: all authors; Software: all authors; Supervision: JKK; Validation: all authors; Visualization: all author; Writing—original draft: EJO, JKK; Writing—review & editing: all authors.

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