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Cervical co-infection with high-risk Human Papillomavirus and Herpes simplex

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ABSTRACT

Background: Herpes Simplex Virus (HSV) is a common sexually transmitted virus that infects millions of individuals worldwide. The current study sought to determine the prevalence of Herpes simplex and Human Papillomavirus (HPV) coinfection in Saudi women. Methodology: From May 2020 to May 2021, 300 women's cervical smears were collected and sent to a cytopathology laboratory. Because of gynecologic concerns, the women in the study were referred for Pap smears. Cervical materials were then evaluated for the presence of HSV using Polymerase Chain Reaction (PCR) molecular techniques. Results: HSV was detected in 2% of patients (66.7% HSV-1 and 33.3% HSV-2). HSV-1 and HPV co-infection was found in 50% of patients, including HPV subtypes 16 and 52. HSV-2 and HPV infection, including HPV subtype 16, was found in 50% of the cases. Conclusion: The prevalence of HSV is minimal among Saudi women seeking gynecologic care. Coinfections of HSV and HPV, particularly HPV subtype 16, are prevalent.

Keywords: Herpes simplex virus, Human Papillomavirus, Saudi Arabia, cytology, cervical smear

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INTRODUCTION

Herpes simplex virus (HSV) is among the most prevalent sexually transmitted viruses. Infection treatment is not therapeutic, although it can alleviate symptoms and improve quality of life [1]. HSV type 1 or HSV type 2 usually causes genital infection, which can be primary or recurrent. On a regular basis, the virus replicates in epithelial cells and produces latency in sensory neurons before reactivating as a local lesion [2].

HSV is transferred through direct touch, resulting in chronic recurring and painful clinical symptoms. Symptoms include genital infection, cold sores, encephalitis, keratitis, meningitis, and blepharitis. Cervical cancer and a variety of sexually transmitted diseases can be exacerbated by visible illness [3].

The most common sexually transmitted infection in the world is human papillomavirus (HPV). More than 99% of cervical cancer cases are caused by high-risk HPV subtypes [4]. HSV and HPV coinfection has been reported in gynecologic and colposcopic medical procedures. HSV and high-risk HPV infections have been linked to a history of sexual behavior [5]. HSV type 2 and high-risk HPV coinfection were found to enhance the risk of invasive cervical cancer [6]. In this study, we looked at Herpes simplex and Human Papillomavirus coinfection in Saudi women.

MATERIALS AND METHODS

About 300 women were referred to the cytopathology laboratory of the maternity hospital in Al-Madinah, Saudi Arabia, between May 2020 and May 2021. The women in the research were referred for Pap smears due to gynecologic issues. A Pap smear is requested as part of the mandatory investigations in combination with the clinical assessment. Prior to sample collection, each patient was asked to sign a documented ethical consent form.

Cytological samples were obtained by scraping the ectocervix's transition zone. The components were evenly dispersed on a clean glass slide and were promptly fixed in 95% ethyl alcohol while still wet. Following fixation, the smears were stained using the Papanicolaou method (Pap. Method), as follows: the dehydrated smears were soaked in decreasing ethyl

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alcohol concentrations (90%, 70%, 50%, and finally DW), for two minutes at a time. After five minutes, the smears were stained with Harris' hematoxylin (nuclear stain), rinsed in DW, differentiated in 0.5% aqueous hydrochloric acid for ten seconds (to eliminate remaining stain atoms), and immediately rinsed in DW to end decolorization. The smears were then blued for five seconds in alkaline water before being dehydrated in ethyl alcohol concentrations of 50%, 70%, 90%, and 95% for two minutes each. The smears were then stained for two minutes with Pap. OrangeG6 (cytoplasmic stain), rinsed in 95% ethyl alcohol, and dyed in EA50 (cytoplasmic stain) for three minutes. The smears were then treated with 95% and 100% ethyl alcohol, cleaned in Xylene, and mounted in Distrene Polystyrene Xylene (DPX).

Cervical materials were further tested for the presence of HSV and HPV using molecular methods, such as polymerase chain reaction (PCR), as described by Scoular et al. [7] (See Table 1&2).

DNA extraction

Proteinase K degrades proteins as well as potentially hazardous enzymes such as nucleases. A buffer containing the denaturing agent sodium dodecyl sulfate (SDS) was added to facilitate digestion. Nucleic acids were isolated from tissue lysate using high-speed centrifugation and buffer-saturated phenol. After that, RNase A was added to the phenol extractions to remove any tainted RNA. Following RNase A incubation, further phenol extractions were performed to remove any remaining enzyme. After adding sodium acetate and isopropanol to precipitate the DNA, high-speed centrifugation was used to pellet the DNA and make it easier to extract the isopropanol. After washing the DNA with 70% ethanol to remove excess salts, it was centrifuged to re-pellet it. DNA is quantified, resuspended in distilled water, and stored at 20 °C. Later, purified DNA was utilized in downstream applications of PCR.

DNA quantification

To assess DNA quantity after DNA extraction, we used a Nano-Drop spectrophotometer to analyze DNA.

Amplification of HPV

Type-specific primers (primer for HPV 31, HPV 33, HPV 35, HPV 39, and HPV 45) were used to detect HPV31, 33, 35, 39, and 45 DNA in cervical lesions using the American Joint Committee on Cancer (AJCC) TNM classification and the International Federation of Gynecology and Obstetrics (FIGO) staging system. The HPV31, 33, 35, 39, and 45 amplification kit from Sacace-Biotechnologies S.r.l. Caserta, Italy was utilized. The total volume of the final reaction was 40 l, which included 10 l of mix-2, 20 l of mix-1 (contained in PCR tubes), and 20 l of extracted DNA (sample). Negative control, positive HPV31,33,35,39, and 45 DNA tubes received 10 l of DNA buffer and 10 l of HPV31,33,35,39, and 45.

Gene Amp PCR system 9700 was used to amp up samples and controls.

Gel-electrophoresis

The PCR products were realized in a 2% Agarose gel with 0.5 g/ml Ethidium bromide. Ten microliters of PCR product and a 100 bp DNA ladder were placed onto the gel. Gel electrophoresis was performed at 120 V and 36 mA for 60 minutes. The photographs were taken with a Gel documentation system (Gel mega, digital camera and computer software).

Table 1. HSV genotype

HSV Genotype	Sequence (5'–3')	Amplification (bp)
HSV-1	CAGACAGCAAAAATCCCCTGAG ACGAGGGAAAACAATAAGG	196
HSV-2	CACCGTCGCCCTATACAGCTT ATCGACGGGATGTGCCAGTTT	210

Table 2. PCR steps. Cycle's time temp PCR steps

	Steps	Temperature	Time	Cycles
0		94°C		Pause
	1	94°C	5 min	1
	2	94°C	30 sec	25
		48°C	30 sec	
		72°C	25 sec	
	3	72°C	1 min	1
4		4°C		Storage

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Statistical Analysis

Data were first organized in an Excel spreadsheet before being moved to SPSS software for analysis to obtain frequencies, percentages, means, cross-tabulations, and the chi-square test. P-values of 0.05 are deemed statistically significant when the 95% confidence interval is used.

Ethical Consent: Before the interview, participants were requested to sign a written ethical consent form. The proposal for the current study was authorized by the Ethical Committee at the College of Medicine, University Ha'il, Saudi Arabia. 00130/CM-UOH.04/20 HREC.

RESULTS

Three hundred samples from women were examined for the presence of HPV and HSV. The women's ages ranged from 20 to 70. As shown in Fig 1, the majority of women were between the ages of 31 and 40, with 41-50, 51-60, 20-30, and >60 years constituting 27.7%, 26.7%, 24.3%, 10.7%, and 10.7%, respectively.

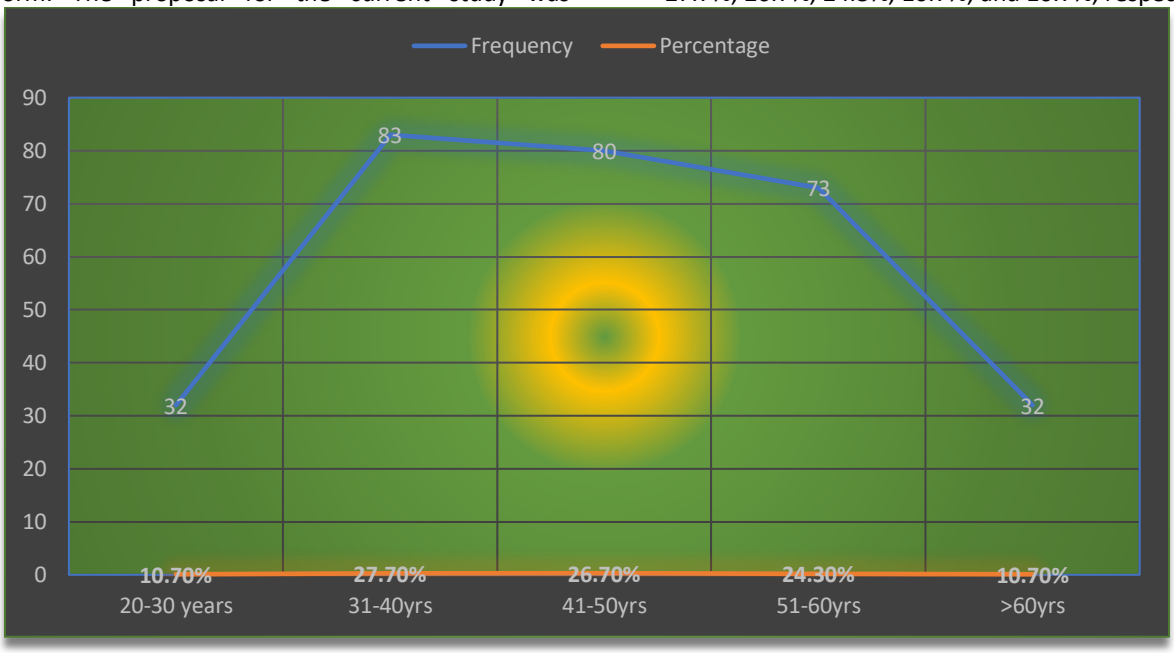

Figure 1. Description of the study subjects by age

HSV was discovered positive in 6/300 (2%) of the patients in this series (4/6(66.7%) HSV-1 and 2/6(33.3%) HSV-2). HSV-1 and HPV co-infection was found in 2/4 (50%) of the patients, which comprised HPV subtypes 16 and 52. HSV-2 and HPV coinfection was found in 12 (50%) of the cases, including HPV subtype 16, as shown in Table 1 and Figure 2

Table 1. Correlation between HSV and HPV

Variable	Herpes simples					Total
	HSV-1		TOTAL		HSV-2	
	POSITIVE	NEGATIVE		POSITIVE	NEGATIVE	
HPV						
Positive	2	12	14	1	13	14
Negative	2	284	286	1	285	286
Total	4	296	300	2	298	300
HPV-subtypes						
HPV-16	1	5	6	1	5	6
HPV-18	0	1	1	0	1	1
HPV-52	1	2	3	0	3	3
HPV-58	0	2	2	0	2	2
HPV-33	0	2	2	0	2	2
Total	2	12	14	1	13	14

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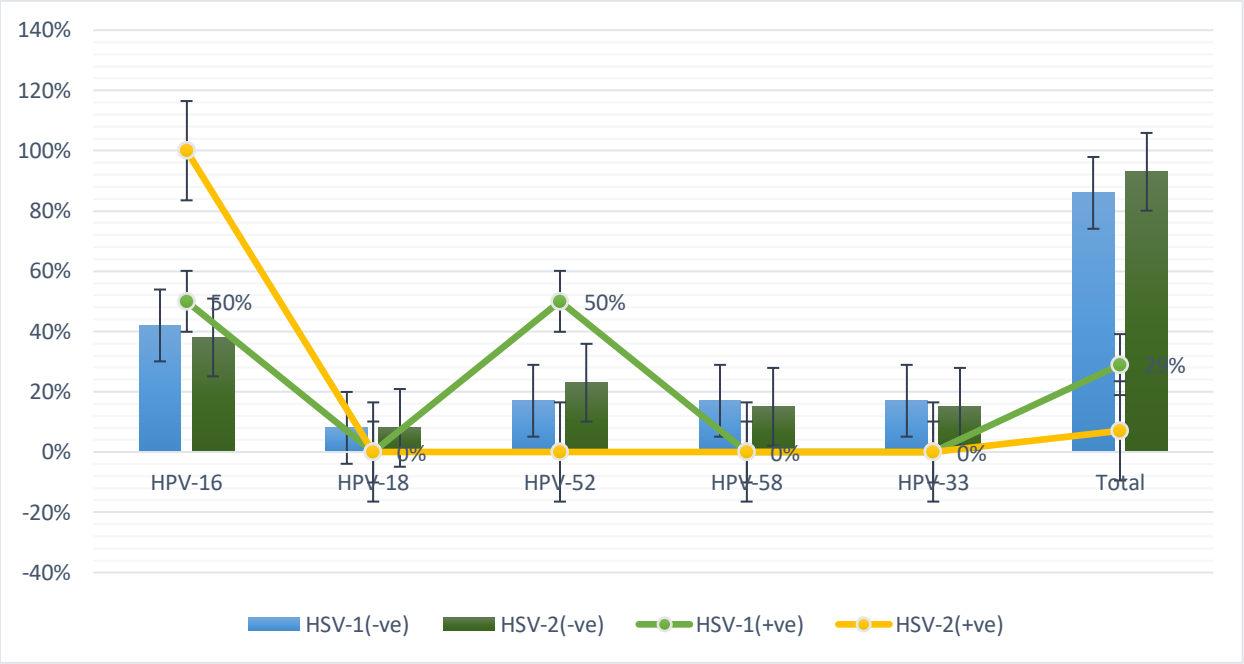

Figure 2. HSV and HPV co-infection

Atypical cytological alterations were found in 4/6 (67%) of HSV-positive individuals, including 2/4 (50%) of HSV-1 cases and 2/2 (100%) of HSV-2 cases. In two cases, inflammatory cell infiltrates were observed: one (25%) HSV-1 and one (50%) HSV-2. As shown in Table 2 Fig 3, all Candida Albicans cases tested negative for HSV.

TABLE 2. Distribution of HSV by cervical cytopathology

VARIABLE	HERPES SIMPLES					
	HSV-1		TOTAL	HSV-2		TOTAL
	POSITIVE	NEGATIVE		POSITIVE	NEGATIVE	
CYTOLOGICAL ATYPIA						
NORMAL	2	265	267	1	266	267
MILD ATYPIA	0	10	10	1	10	11
MODERATE	0	11	11	0	10	10
SEVERE	2	10	12	0	12	12
TOTAL	4	296	300	2	298	300
INFLAMMATORY CHANGE						
YES	1	70	71	1	70	71
NO	3	226	229	1	228	229
TOTAL	4	296	300	2	298	300
CANDIDA ALBICANS						
YES	0	7	7	0	7	7
NO	4	289	293	2	291	293
TOTAL	4	296	300	2	298	300

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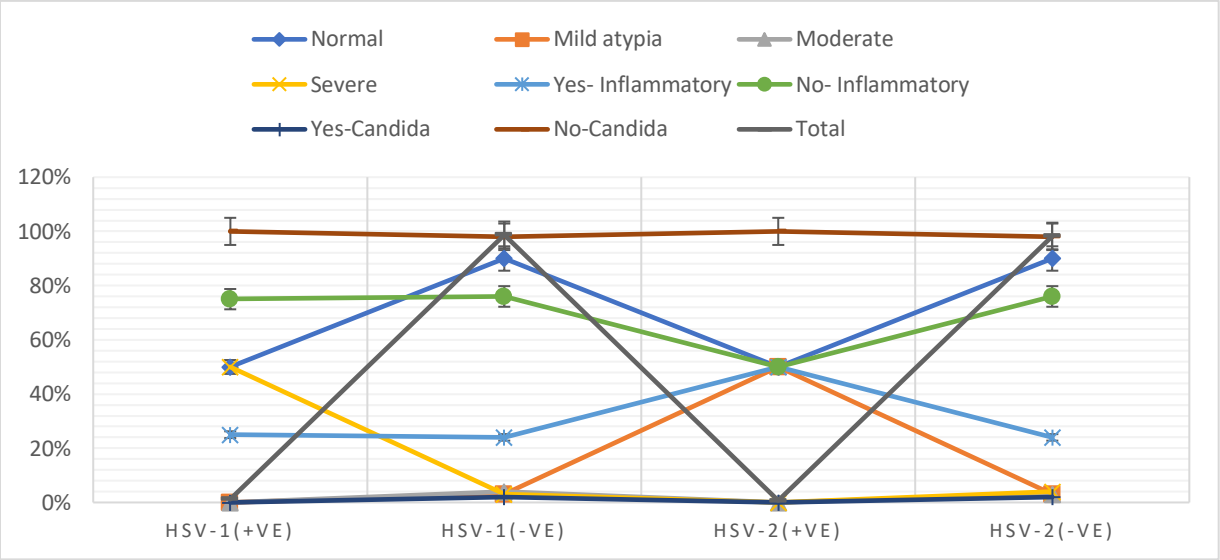

Figure 3. HSV by cervical cytopathology

As shown in Table 3, approximately 2/4 (50%) of the HSV-1 positive cases were seen in the age group >60 years, one (25%) in the age group 51-60 years, and one (25%) in the age group 41-50 years, whereas one (50%) case HSV-2 was seen in the age group 20-30 years and the remaining one in the age group 41-50 years.

Table 3. HSV infection status by age

Variable	Herpes simples					Total
	HSV-1		Total	HSV-2		
	Positive	Negative		Positive	Negative	
20-30 years	0	32	32	1	31	32
31-40	0	83	83	0	83	83
41-50	1	79	80	1	79	80
51-60	1	72	73	0	73	73
>60	2	30	32	0	32	32
TOTAL	4	296	300	2	298	300

Figure 4. PCR amplification of HSV-1 and HSV-2 in cervical samples.

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The products were separated on a 2% agarose gel and stained with an ethidium bromide 1000bp ladder (arrows show 100 and 200 bands), with HSV-1 positive in 196 bp. HSV-2 is not present

DISCUSSION

In this Saudi patient series, the overall HSV prevalence was 2% (with roughly 67% HSV type 1 and approximately 33% HSV type 2). Saudi Arabian epidemiological data on HSV are limited. A Saudi Arabian study assessed IgG and IgM antibodies in women and neonates in a group of patients with recurrent abortion. The presence of IgM in 1.3% of women indicated a recent HSV type 1 infection. The presence of IgG, observed in around 81% of the women, indicated past HSV exposure.

As a result, HSV infection is associated with infertility [8]. Another Saudi Arabian study included 4985 women who were screened for HSV. The total prevalence of HSV type 1 was 88.8%, while HSV type 2 was 1.26%, according to the study. The incidence was substantially higher among those who were married, divorced, or widowed. Other sexually transmitted diseases, such as Treponema pallidum and the Human Immunodeficiency Virus (HIV), have been found to be more common [9]. However, 491.5 persons were predicted to be infected with HSV type 2, accounting for 13.2% of the world's population aged 15-49. Furthermore, 3752 million people were found to be infected with HSV type 1, for a global prevalence of 66.6%. Infection patterns varied according to geographical area, sex, and age [10].

In the current study, almost half of the cases of HSV infection had HPV coinfection. Some investigations have assumed HSV and HPV virus co-infection, as well as other carcinogenic viruses [11,12]. Furthermore, some researchers believe that HVS infection may encourage subsequent HPV infection [13]. Coinfection occurred with a variety of HPV subtypes, with a higher prevalence of HPV subtype 16.

The current study found that HSV type 1 was linked to severe cytological atypia, whereas HSV type 2 was linked to mild cytological atypia. However, HSV replication in human epithelial cells causes structural alterations in the cell cytoplasmic organelles and nucleus, which eventually result in visible morphological abnormalities [14]. Infection with HSV type 1 disrupts the end of transcription of RNA polymerase II in host genes [15].

In the current investigation, inflammatory cells were found in two cases, one (25%) HSV-1 and one (50%) HSV-2.

HSV can activate both innate and adaptive immune responses in host cells, triggering a cascade of events that results in the release of inflammatory mediators that recruit a large number of inflammatory cells [16].

In the current study, HSV infections were more common in older adults (> 50 years old); the incidence of HSV by age varies according to a variety of factors, including geographical region, community sexual behavior, and population subgroups. Females are more likely than males to be infected with HSV type 2 [17].

The current analysis revealed some hidden HSV data in Saudi Arabia, but it has significant limitations, including a lack of previous immune globulins, which predicts previous exposure.

In conclusion: The frequency of HSV is low among Saudi women seeking gynecologic care. Coinfections of HSV and HPV, particularly HPV subtype 16, are prevalent.

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Authors contribution

SAM: Conceptual, consultation, funding, and approval of the final version

AMAA: Conceptual, data analysis, funding, and approval of the final version

EBSM: conceptual, manuscript drafting, and approval of the final version

AAM: conceptual, administration, funding, and approval of the final version

HGA: conceptual, administration, funding, and approval of the final version

FUNDING:

Self-funded.

DATA AVAILABILITY:

The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research supporting data is not available.

DISCLOSURE OF INTEREST

No interest to declare

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