original article

Prevalence of human papillomavirus in Jeddah, Saudi Arabia

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BACKGROUND: Human papillomaviruses (HPVs) are small, non-enveloped, double-stranded DNA viruses that consist of more than 200 genotypes. Low-risk genotypes are associated with warts or benign lesions, whereas high-risk genotypes are usually associated with malignancies and cancers including cervical cancer. However, the real prevalence and incidence of HPV in Saudi Arabia may be understated due to a lack of comprehensive data reporting.

OBJECTIVES: Determine the positivity rate of HPV in men and women in Jeddah, Saudi Arabia.

DESIGN: Cross-sectional.

SETTING: Tertiary care center in Jeddah.

SUBJECTS AND METHODS: Self-collected vaginal swab samples were obtained from females attending the gynecological clinic in the period between October 2017 and April 2018 at a tertiary care center, Jeddah, Saudi Arabia. PCR-positive HPV samples were sequenced to determine genotype. Additionally, serum samples were collected from healthy male and female blood donors and screened for HPV IgG antibodies by ELISA.

MAIN OUTCOME MEASURES: Molecular and serological positivity for HPV.

SAMPLE SIZE: 119 self-collected vaginal swabs from females at a gynecology clinic and 966 serum samples from healthy blood donors.

RESULTS: Of the 119 tested vaginal swabs, 7 samples (5.9%) were positive for HPV DNA. Several genotypes were identified. Most of the positive samples were from Saudi females in the age range of 31-50 years seeking care for infertility. Of the 966 serum samples, only 16 samples (1.7%) were positive for HPV IgG antibodies.

CONCLUSION: While the prevalence of HPV in men and women in our sample from the western region of Saudi Arabia was low, our data clearly show that it is not uncommon among high-risk groups and people are still exposed to the risk of HPV infection. Most importantly, these data provide valuable information that could aid in enhancing national awareness about HPV and in introducing an HPV vaccination program.

LIMITATIONS: Single hospital and a convenience sample

CONFLICT OF INTEREST: None.

Human papillomavirus (HPV) is a highly prevalent virus in sexually active men and women and has been associated with several types of cancers such as cervical, ovarian, vaginal, vulvar, anal, penile and oropharyngeal cancers.[1] HPV transmission during pregnancy and delivery from the mother to the fetus is also a medical concern, but the process of transmission is still unresolved. The more than 200 HPV genotypes are classified as either cutaneous or mucosal depending on their tropism. Cutaneous types target the skin of the hands and feet causing warts, whereas mucosal types infect the lining cells in mucosal or mucocutaneous tissues of the respiratory tract, mouth, throat or anogenital epithelium. HPV genotypes are further categorized as low-risk HPV (LR-HPV) or high-risk HPV (HR-HPV) based on their tendency to cause cancer or precursor lesions. LR-HPV types include types 6, 11, 42, 43, and 44, while HR-HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Types 16 and 18 are responsible for about 70% to 80% of all cervical cancer cases worldwide. They are the most common sexually transmitted pathogen.

HPV is maintained in humans because it usually causes productive infection rather than cancer. The invasion by the virus causes a local and often self-limited infection that usually regresses within 18 months. HPV persistence within the squamous cells increases the risk of malignancy.[2,3] HR-HPV types can persist for many years, causing cell proliferation in basal and parabasal sites that lead to different cancer types. The causal relationship between HPV and cancer development was recognized after observations from epidemiological and molecular studies.[4-7] This causal relationship has been evaluated through all the proposed sets of casualty and consequently, and was endorsed by the scientific community as well as major review institutes. Having been the first ever identified cause of human cancer, there is a clear need for undertaking an intensive study on HPV to establish control measures and implement vaccination.[6]

Cervical cancer is the fourth most prevalent form of cancer in women globally.[8,9] Although a national screening program is lacking in Saudi Arabia, the incidence of cervical cancer is reported to be comparatively low as it ranks at the twelfth position among other types of cancer affecting women and accounting for about 2.4% of the new cancer cases.[8] Thus, a true national picture cannot be created unless a national study covering all regions is undertaken.[9] Furthermore, most Saudi women infected with HPV visit hospitals at points where the infection is already at an advanced stage, which would require serious interventions such as chemotherapy, surgery and radiotherapy. The situation is further complicated by the fact that cytological examination of cervical samples is not common in Saudi Arabia, which is a sign of the lack of awareness of the serious nature of the infection among most Saudi women.

The World Health Organization reported that about 6.5 million women older than 15 years of age in Saudi Arabia are at risk of developing cervical cancer.[10] It is estimated that about 55 women die in Saudi Arabia annually due to HPV-related cancers.[9] HPV-16, HPV-18 and HPV-45 are the most common genotypes in Saudi Arabia and are responsible for about 70% of all cervical cancer cases in the country.[11] The topic of prevalence and national awareness is thus one of great concern considering the gravity of the potential impacts associated with HPV. In this study, we tried to determine HPV prevalence in men and women in the western region of Saudi Arabia using serological and molecular methods in an effort to highlight the importance of awareness and to promote positive strides towards control and eradication.

PATIENTS AND METHODS

A convenience sample of self-collected vaginal swab samples were obtained from female participants attending the gynecological clinic in the period between October 2017 and April 2018 at a tertiary care center, Jeddah, Saudi Arabia. The collection method and instructions were explained to all participants. Married females older than the age of 18 years, who had not undergone hysterectomy or cervical resection, were included in this study. Women with a previous history of receiving antibiotics in the last two months or HPV vaccine were excluded from the study as previously described.[12] All swabs were tested for HPV by polymerase chain reaction (PCR) and positive samples were then used to determine circulating genotypes by sequencing. Additionally, archived serum samples that were randomly collected from healthy male and female blood donors (394 females and 572 males) in the years 2012, 2013 and 2017, were screened for HPV IgG by ELISA. All serum samples had been stored at -80°C. Informed written consent was sought from all participants before proceeding; anyone who refused to provide consent was excluded from the study. Protection of privacy was guaranteed to all participants, and no information was shared with third parties. All participants answered a self-applied questionnaire after obtaining ethical approval from Unit of Biomedical Ethics Research committee at King Abdulaziz University Hospital in Jeddah.

Viral nucleic acid extraction and PCR amplification and sequencing

Vaginal swabs were suspended in virus transport medium (VTM) upon collection and stored at -80°C until nucleic acid extraction. Nucleic acid was extracted from 200 µL of each sample using PureLink Viral RNA/DNA Mini Kit (Thermo Fisher Scientific Inc., USA) according to manufacturer's instructions. Viral nucleic acid was eluted with 50 µL of sterile DNase/RNase-free water and stored at -80°C until testing. Extracted nucleic acid was tested for HPV by nested PCR using a MY09/MY11 consensus primer pair, which amplifies many HPV types with a PCR product of about 450 base pairs (bp) followed by GP5+/GP6+ primers with about 150 bp PCR products.[13,14] The first PCR reaction was done in a volume of 50 µL using 500 ng of nucleic acid. The cycling profile included a 5 minute initial denaturation at 94°C, then 40 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minutes and elongation at 72°C for 1 minute, followed by a 10-min final extension at 72°C. The same conditions were used in the second PCR except for the annealing step, which was done at 40°C for 2 minutes as previously described.[14] The quality of the extracted nucleic acid was tested by amplifying the beta-globulin housekeeping gene using GH20/GH21 primers as previously described.[15] Positive (known positive HPV sample) and negative (known negative HPV sample or no template control) control samples were included in each PCR run. PCR products were visualized on 2% agarose gel stained with ethidium bromide. Samples with PCR product of about 150 bp were considered positive. Primers sequences are shown in **Table 1**.

Positive PCR products (about 150 bp) were gel purified using a DNA gel extraction kit (Norgen Biotek Corp., Thorold, ON) according to manufacturer's instructions, and used for sequencing and genotype determination. Extracted fragments were sequenced directly by Sanger sequencing on an ABI 3500 Automatic Sequencer (Applied Biosystems, USA) using the internal primers from the nested PCR and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to manufacturer's instructions. Obtained sequences were aligned using Geneious Prime 2019.1.1 software (*https://www.geneious.com*) and assembled based on similarity between the product of the forward and reverse primers to generate the contig for each sample. Contigs were then blasted and compared with those of known genotypes available in the GenBank database using BLASTn as previously described.[16]

ELISA

Serum samples were tested using DRG HPV IgG ELISA kit (DRG International Inc., Springfield, NJ) according to the manufacturer's instructions. Briefly, 100 µL of 1:100 diluted serum samples as well as negative and positive controls were tested in duplicate in a 96 microwell plates coated with recombinant virus-like particles derived from HPV types 6, 11, 16 and 18. Plates were incubated for 60 minutes at 37°C. After washing for 5 times, 100 µL of enzyme conjugate were added to each well, and incubated for 60 minutes at 37°C. Plates were washed again, and 100 µL of chromogen/substrate mixture were added to each well and incubated at room temperature for 20 minutes. Next, colorimetric reaction was stopped with a 100 µL of sulphuric acid, and the absorbance was measured at 450 nm. Samples were considered positive when their absorbance exceeded the cut-off of the assay.

Data analysis

Categorical data are reported as frequency and percentage (%). Continuous data are reported as mean and standard deviation (SD). The statistical package SPSS Version 21 was used for analysis. Chi-square or Fisher exact tests were used for comparison of categorical data. A $P<.05$ values (two-sided test) was accepted as statistically significant.

Table 1. Primers for polymerase chain reaction.

Name	Description	Oligonucleotide Sequence (5'-3')
GH 20	β-globin forward primer	CAACTTCATCCACGTTCACC
GH 21	β-globin reverse primer	GAAGAGCCAAGGACAGGTAC
MY09	HPV external forward primer	CGTCCMARRGGAWACTGATC
MY11	HPV external reverse primer	GCMCAGGGWCATAAYAATGG
GP5	HPV internal forward primer	TTTGTTACTGTGGTAGATACTAC
GP6	HPV internal reverse primer	GAAAAATAAACTGTAAATCATATT

RESULTS

Detection and genotyping of human papillomavirus in females

Between October 2017 and April 2018, 119 self-collected vaginal swab samples were obtained from females attending the gynecological clinic at a tertiary care center in Jeddah (34 samples were from 2017 and 85 from 2018). The mean age of the tested females was 31.8 (6.5) years (median age 31 years, range 18–50 years). Infertility was the most frequent visit reason in 74.8% (89/119) of the females, followed by visits for antenatal care (9.2%, 11/119) or for regular checkups (9.2%, 11/119). Other visit reasons included fibroids (2.5%, 3/119), recurrent abortions (1.7%, 2/119), urinary tract infection (1.7%, 2/119) and endometrial polyp (0.8%, 1/119). Out of the 119 swabs, 7 vaginal swabs were positive for HPV DNA by nested PCR, representing 5.9% of the tested females (**Figure 1a**). Out of the 7 detected positive samples, 6 were from Saudi females and one from a non-Saudi woman (**Table 2**). The detected viruses were in women in the age range of 31-50 years visiting for infertility (4.5%, 4/89), regular checkup (9.1%, 1/11) or other reasons such as recurrent abortions or endometrial polyp (25.0%, 2/8) (**Table 2**). As shown in **Figure 1b**, all samples had positive β-globulin PCR products confirming that there was no PCR inhibition and DNA was properly extracted from all collected swabs. Importantly, it shows that use of self-collected vaginal swabs could be a valid procedure for HPV screening in females. Alignment of obtained sequences showed a diversity in detected HPV types except for samples 1 and 7, which had identical sequences (**Figure 1c**). Blasting of these sequences showed that they belong to HPV genotypes 10, 11, 58, 62, 66 and 67 in which two samples were positive for genotype 67.

Seroprevalence of human papillomavirus in healthy blood donors

Between 2012 and 2017, 966 serum samples were obtained from healthy blood donors. The mean age of the tested individuals was 28.5 (8.4) (median age 26 years, range 17–67 years). Males represented 59.2% (572/966) with a mean age of 30.5 (8.0) (median age 30 years, range 17–67 years). Females comprised 40.8% (394/966) with a mean age of 25.5 (8.1) (median age 22 years, range 17–67 years). Of the total samples, 182 (18.9%) were collected in 2012, 238 (24.6%) in 2013, and 546 (56.5%) in 2017. Most of the samples were collected from Saudis (541, 56.0%) compared to non-Saudis (425, 44.0%). Out of the total tested serum samples, 16 samples were positive (1.7%) for HPV IgG antibodies against genotypes 6, 11, 16 and 18. Among the positive samples, 5 (0.9%) and 11 (2.8%) were from males and females, respectively. Nine of the positive samples were from Saudi donors and the rest (7 samples) were from different nationalities including 4 from Yemen, and one individual each from Jordan, Philippines and Palestinian (**Table 3**). Most of the positive samples were found in individuals younger than 40 years as shown in **Table 3**.

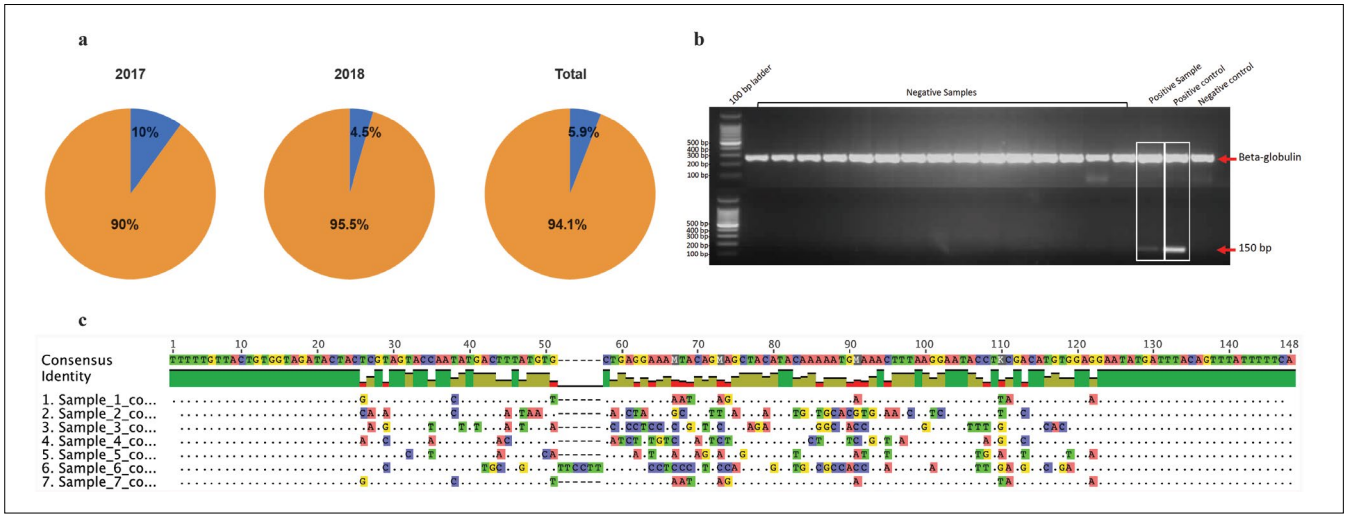

Figure 1. HPV detection in self-collected vaginal swabs. (a) Pie Chart showing positive HPV cases in females in the years 2017 and 2018. Thirty-four samples were tested from 2017 and 85 from 2018. (b) Representative gel of HPV nested PCR screening. Samples were tested by nested PCR and only one sample was positive with the expected size of ~150bp. Positive and negative controls were included. All samples were also used for β-globulin amplification as internal control. PCR products were run on 2% agarose gel stained with ethidium bromide and visualized by UV-trans-illumination. (c) Alignment of obtained sequences.

DISCUSSION

HPV is associated with several forms of cancer in sexually active men and women. Therefore, it becomes imperative to understand the prevalence and circulation of prevalent HPV genotypes in a given population. Previous studies from Saudi Arabia have reported differing results for HPV prevalence.[11,14,16-18] These discrepancies could be attributed to geographical, clinical, and demographic factors, as well as sample size and types, and the use of different detection techniques. Nonetheless, these reports have clearly established a correlation between HPV infection and cervical cancer and carcinoma wherein high prevalence of HR-HPV and LR-HPV, ranging from 42% to 95.5% have been found.

In the present study, 119 self-collected vaginal samples were collected from females who visited gynecological clinic mostly for infertility consultations, in addition to some other routine visits. Seven vaginal swabs (5.9%) were found positive for HPV-10, HPV-11, HPV-58, HP-62, HPV-66 and HPV-67. This is in contrast to most commonly detected HPV genotypes from previous studies which included HPV-16 (30%-71%), HPV-18 (3.4%-26.2%), HPV-45 (4.0%-7.1%), HPV-31 (2.2%-7.9%) and HPV-73 (2.3%-4.0%).[11,14,16-18] However, it is of note that these previous studies investigated HPV prevalence in cervical and ovarian cancer patients compared to our sample cohort which included females who had never been reported to be HPV positive or complained from HPV-related cancers. Interestingly, our data are consistent with a previous study from the same region which reported 5.6% HPV prevalence in females attending gynecological clinic for routine visits.[19] Both reports showed lower rate than those reported (9.8%-31.6%) in other regions by others.[20-22] Nonetheless, these studies clearly suggest that there is an apparent increase in the HPV infection and prevalence in Saudi women. Most importantly, they clearly show that HPV is not uncommon among high-risk groups such as adult women and that females in Saudi Arabia are still unprotected and are exposed to the risk of HPV infection.

Our serological data showed a low seroprevalence (1.7%) of HPV-6, HPV-11, HPV-16 and HPV-18 in the general population in the western region of Saudi Arabia. A higher seroprevalence was observed in females (2.8%) as compared to males (0.9%), wherein the most seropositive samples were from individuals younger than 40 years. This is markedly lower than seroprevalence rates reported from other countries such as England, Australia, Italy and Iran.[4,23-26] While this low seroprevalence rate may reflect the lack of vaccine availability in Saudi Arabia, these seropositive individuals most probably developed HPV IgG due to natural HPV infections

Table 2. Prevalence of human papillomavirus in a convenience sample of females attending a gynecological clinic in Jeddah.

	Tested samples	HPV positive	*P* value
Total number	119	7 (5.9)	
Nationality			
Saudi	100 (84.0)	6 (6.0)	NS
Non-Saudi[a]	19 (16.0)	1 (5.3)	
Age range[b]			
<20	6 (5.0)	0 (0.0)	0.022
21-30	46 (38.7)	0 (0.0)	
31-40	50 (42.0)	5 (10.0)	
41-50	8 (6.7)	2 (25.0)	
Visit reason			
Infertility	89 (74.8)	4 (4.5)	NS
Antenatal care	11 (9.2)	0 (0.0)	
Regular checkups	11 (9.2)	1 (9.1)	
Other reasons[c]	8 (6.7)	2 (25.0)[d]	

Data are number (%)

[a]Non-Saudi females were from Ethiopia (3), India (3), Philippines (3), Sudan (3), Yemen (2), Pakistan (2), Tunisia (1), Lebanon (1), Egypt (1). [b]Age was known for 110 females only out of the 119 participants. [c]Other visit reasons included fibroids (2.5%, 3/119), recurrent abortions (1.7%, 2/119), urinary tract infection (1.7%, 2/119) and endometrial polyp (0.8%, 1/119). [d]Positive samples were from females visiting for recurrent abortion or endometrial polyp.

Table 3. Seroprevalence of human papillomavirus in healthy blood donors (N=966).

	Tested samples	HPV positive	*P* value
Total	966	16 (1.7)	
Gender			
Male	572 (59.2)	5 (0.9)	.037
Female	394 (40.8)	11 (2.8)	
Nationality			
Saudi	541 (56.0)	9 (1.7)	NS
Non-Saudi	425 (44.0)	7 (1.7)	
Age			
<20	169 (17.5)	5 (3.0)	NS
21-30	466 (48.2)	7 (1.5)	
31-40	235 (24.3)	3 (1.3)	
41-50	83 (8.6)	1 (1.2)	
>50	13 (1.4)	0 (0.0)	

Data are number (%)

or exposure especially that HPV seropositivity is directly associated with increased sexual behavior in men and women.[25] Notably, HPV antibodies induced in response to natural infection may not always be protective and could be associated with an increased risk of genital HPV infection in men,[27,28] and abnormal cytology in HPV-positive women.[25]

One of the important key factors in designing strategies to combat HPV infection and related diseases is knowledge and awareness. Unfortunately, it was found that 95.7% of Saudi female students enrolled in healthcare institutions had poor knowledge of cervical cancer.[29] Another study showed that only 34.5% and 27.4% of females were aware of HPV infection and its relationship with cervical cancer, respectively.[30] Furthermore, there was a relatively low (32.3%) level of knowledge and awareness of HPV vaccine and its importance in the prevention of cervical cancer and other HPV-related disease.[30] Several factors were found to play a vital role in increased knowledge and awareness of HPV-infection and its related consequences among Saudi females. These factors included association with health profession, higher educational levels, older age, high monthly income, and previous experience with cervical cancer.[29,30] In these studies, 64.3% of females were willing to receive the HPV vaccine, whereas 35.7% refused the vaccine mostly due to fear of injections and/or vaccine side effects.[29,30]

While our study indicates a low prevalence of HPV in sexually active men and women in the western region compared to other regions in Saudi Arabia as well as other countries as indicated above, HPV is clearly not uncommon among adult women and men in Saudi Arabia. Such results highlight the importance of co-testing for HPV along with Papanicolaou (Pap) testing especially since routine Pap testing is not very common in Saudi Arabia and most Saudi women usually seek healthcare at advanced stages. Nonetheless, our data may not be reflective of the actual prevalence of HPV in the Saudi community. Therefore, more comprehensive studies and investigations are required to ascertain the actual prevalence of HPV in country. The ultimate objective should be to undertake a national program in all 13 provinces of the country to know the exact prevalence of HPV. Such studies would without doubt help to develop a strategic plan to initiate a vaccination program that includes young people, both male and female and help in preventing and safeguarding from HPV-infections and related diseases.

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