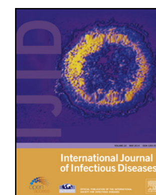
International Journal of Infectious Diseases 46 (2016) 94–99

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid



Sociodemographic characteristics and sexual behavior as risk factors for human papillomavirus infection in Saudi Arabia

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ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 31 March 2016
Accepted 3 April 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Human papillomavirus
HPV
Epidemiology
Sociodemographic characteristics
Saudi Arabia

SUMMARY

Objectives: To determine the prevalence and the sociodemographic characteristics and sexual behavior risk factors for human papillomavirus (HPV) infection in a hospital-based cohort of women in Saudi Arabia.

Methods: Cervical specimens and questionnaire data were collected from women attending clinics in Riyadh, Saudi Arabia. Cervical specimens were examined for abnormal cytology using a standard Pap test and for the presence of HPV-DNA using PCR and reverse line blot hybridization tests.

Results: Approximately 73% of the 400 women tested were Saudi nationals. Nearly 50% were under 40 years old (range 22–80 years, mean $\pm$ standard deviation 41.20 $\pm$ 10.43 years). Approximately 17% of the women were HPV-positive. The most commonly detected HPV types were HPV-18 (34%) and HPV-16 (19%), with multiple infections detected in 10% of positive specimens. Multivariate analyses revealed that smoking and multiple partners were significant risk factors for HPV infection ($p < 0.01$).

Conclusions: Because of societal challenges and an unsubstantiated assumption of low HPV prevalence, few studies have examined sociodemographic characteristics or sexual behaviors associated with HPV in Saudi women. However, a high prevalence of HPV infection was found, with smoking and multiple partners as significant risk factors, in this hospital-based cohort of predominantly Saudi women.

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1. Introduction

The presence of human papillomavirus (HPV) infection, a key etiological factor in carcinoma of the cervix and other mucosal epithelia, is associated with approximately 4.8% of all human cancers,[1] posing a significant morbidity and mortality risk worldwide. Papillomaviruses belong to a family of small, non-enveloped viruses with a double-stranded DNA genome of approximately 7.9 kb.[2] HPV establishes productive infections only in keratinocytes of the skin or mucous membranes. More than 180 genotypes of HPV have been sequenced, and these are classified as either low-risk HPV (LR-HPV) or high-risk HPV (HR-HPV), depending on their oncological potential for transforming cells. Benign hyperproliferative lesions or genital warts are caused by LR-HPVs, while HR-HPVs are strongly associated with premalignant and malignant cervical lesions that lead to different types of cancers such as cervical, vulvar, vaginal, penile, oropharyngeal, and anal cancers.[3,4]

Cervical cancer is the eighth most frequent cancer among women in Saudi Arabia aged 15 to 44 years,[5] and according to the World Health Organization, 6.51 million Saudi women 15 years and older are at risk of developing cervical cancer.[6] An estimated 152 Saudi women are diagnosed with cervical cancer and 55 die from the disease every year. By contrast, the prevalence of HPV in Saudi Arabia and many developing countries remains unknown or controversial. However, approximately 2.2% of women living in

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http://dx.doi.org/10.1016/j.ijid.2016.04.004
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Western Asia, which includes Saudi Arabia, harbor cervical HPV infection, with approximately 68.5% of invasive cervical cancers in Asia attributable to HPV-16 or HPV-18.[6] In Saudi Arabia, the prevalence of HPV infection is under debate. Some researchers have suggested that Saudi Arabia has the lowest HPV infection rate in the world (e.g., 1.9 cases/100 000 women),[7] but others claim a much higher rate.[8–12]

Cervical cancer is preventable, and with an early diagnosis, it may also be curable. Unfortunately, most women in developing countries, including Saudi Arabia, remain undiagnosed until advanced stages, which decreases survival rates.[13] Therefore, the present study was conducted to report the prevalence of HPV infection in a hospital-based cohort of women in Saudi Arabia and to establish a sociodemographic profile and sexual health behavior as risk factors for HPV infection.

2. Materials and methods

2.1. Participants, specimens, and data collection

Women who attended the Primary Care Clinic (namely, Family Medicine) or the Obstetrics and Gynecology Clinic at King Faisal Specialist Hospital and Research Centre (KFSHRC) in Riyadh, Saudi Arabia, for routine cervical examinations from November 2013 to November 2015 were included in this 2-year study. These clinics treat persons of all socioeconomic classes who are eligible for treatment in this hospital. The inclusion criteria for participation in the study were women who were married, divorced, or widowed, and the exclusion criteria were women who were pregnant or virgin. Given religious and cultural constraints, Saudi women who have never married could not be recruited for this study; therefore, all women who had never married were excluded from the study to avoid bias. Women who did not fill in the questionnaire in its entirety were also excluded from the study; thus, all survey questions were answered.

Cervical specimens were collected using a cytobrush. Two cytobrushes were used to collect the specimens: the first was transferred into a vial containing liquid-based cytology transport medium (PreservCyt; ThinPrep Pap Test Boxborough, MA, USA) for use in a routine Papanicolaou (Pap) test; the second cytobrush was transferred into a vial containing RNAlater stabilizing reagent (Qiagen, Valencia, CA, USA) for use in molecular detection experiments. The cervical specimens were examined for normal or abnormal cytology, and the stages of abnormal cytology were identified according to the Bethesda classification,[14] as follows: negative for intraepithelial lesion (NIEL), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LGSIL), high-grade squamous intraepithelial lesion (HGSIL), and cervical carcinoma.

Questionnaires were completed by the enrolled participants and were collected by a clinical coordinator during the participants' clinical visits. Women who agreed to participate in the study filled out the questionnaire in its entirety, leaving no question unanswered.

2.2. Ethics approval

This study was approved by the Office of Research Affairs (ORA) of King Faisal Specialist Hospital and Research Centre (RAC #2130 033), and written informed consent was obtained from each participant.

2.3. DNA extraction from cervical specimens

Cervical cells were collected using centrifugation, and total genomic DNA was extracted using a Gentra Puregene Cell Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The extracted DNA was eluted in 50 μl of RNase/DNase-free water. The quality and quantity of the extracted DNA were determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The quality of DNA extracted from cervical specimens was determined with β-globin primers. The amplified products were visualized using 1% agarose gels stained with ethidium bromide.

2.4. HPV detection

The MY09/MY11 and GP5+/GP6+ primer sets were used to target sequences located within the L1 region. The MY09/11 primer set targeted a 450-bp conserved sequence and was used for the first round of PCR. The GP5+/GP6+ primer set targeted a 150-bp sequence within the 450-bp product and was, therefore, used for nested PCR. The internal control was the β-globin gene, the positive controls were HeLa and SiHa cells, and the negative controls were UltraPure DNase/RNase-free water and HEK293 cells. Positive amplicons were sequenced for confirmation at the Sequencing Core Facility of KFSHRC using an ABI3730XL sequencer (Applied Biosystems, Foster City, CA, USA).

2.5. Genotyping by reverse line blot (RLB) hybridization

The oligoprobes (n = 23; C12 Aminolink) were synthesized as described previously.[15] The genotyping protocol followed has been published previously.[16] Briefly, oligoprobes were spotted on a carboxyl-coated nylon membrane (Biodyne C 0.45 μm; Pall Corporation, Pensacola, FL, USA). For hybridization, biotinylated PCR products were added to the membrane. Subsequently, the membrane was incubated with an anti-fluorescein peroxidase conjugate. HPV genotypes were detected using an enhanced chemiluminescence kit.

2.6. Data and statistical analyses

Demographic information and sexual behavior data were collected using a questionnaire provided to each participant. Previously published papers were used to identify the risk factor variables used in the present study.[17–20] The demographic variables included age, religion, marital status, education, nationality, smoking habit, and income. The sexual variables included age at first intercourse, number of sexual partners, number of children, contraceptive use, and duration and type of contraception used.

All data collected were stored and analyzed using IBM SPSS Statistics version 22 software (IBM Corp., Armonk, NY, USA). Univariate and descriptive statistics were used to estimate the proportions. Significant associations between HPV status and study variables were assessed using a Chi-square test. Logistic regression modeling was used to determine the adjusted odds ratios and 95% confidence intervals (95% CI) to estimate the relative odds for demographic and sexual behavior variables and smoking status. Variables that were significant in univariate analyses and variables that were considered relevant based on the previous research were evaluated in a multiple logistic regression model. The final model was created with the inclusion of some risk variables with potential biological significance, which were those that remained statistically significant after adjustment. All p-values reported were two-sided and were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. HPV prevalence and cytology results

Of the 400 women who were recruited and examined in this study, approximately 17% (n = 67) tested positive for HPV DNA. The

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