Human papillomavirus infection among women attending health facilities in the Kingdom of Bahrain

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ABSTRACT

Objective: To investigate the occurrence of human papillomavirus (HPV) infection and the associated risk factors in Bahrain's female population.

Methods: This study was carried out between March to December 2004, which includes cervical scrapings for Pap smear and HPV-DNA testing using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, obtained from 100 women attending the Gynecology Clinic at Salmaniya Medical Center and Sheikh Sabah Health Center in the Kingdom of Bahrain. We distributed questionnaires that include the sociodemographic data as well as information on risk factors such as smoking, parity, and the contraceptive used.

Results: Eleven women (11%) with normal cytology were HPV-positive. The RFLP analysis detected HPV-types 16, 18, 45, 62 and 53. Positive women were significantly older (43.3 ± 10.1 years) than negatives (36.5 ± 9.9 years; p=0.04), however, there was no difference in age of first sexual contact (positive: 18.1 ± 5.7 years versus negative: 20.6 ± 4.4 years). Polygamy, smoking and hormonal contraception was not identified as risk factors, but positive women showed higher parity.

Conclusion: In this study on HPV infection in Bahrain, the 11% positivity with high risk HPV types, in the presence of normal cytology suggests that in addition to the cervical cancer screening program, offer of HPV testing deserves consideration.

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Human papillomavirus (HPV) is one of the most common causes of sexually transmitted infections (STI) with estimates of the population prevalence around the world ranging from 2-44%.[1,2] The link between genital HPV infection and cervical cancer (Ca cervix) was first demonstrated in the 1980s[3] and HPV types are grouped into high and low risk types on the basis of their association with Ca cervix and its precursor lesions. Factors such as onset of sexual activity at an early age, cigarette smoking, hormonal contraceptive usage, high parity, number of lifetime sexual partners, male partner sexual behavior, immune status and concomitant infection with other sexually transmitted pathogens appear to act in concert with

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persistent infection to stimulate oncogenesis.[4-7] The presence of HIV/AIDS increases the risk of HPV acquisition and development of neoplasia.[8]

In the Kingdom of Bahrain, Ca cervix is the 6th most common malignancy in women with age standardized incidence rate in 2002 being 4.9/100,000 women[9] and although free Pap smears for screening are provided in the National Healthcare system, the prevalence of HPV infection remains unknown. The American Cancer Society and The American College of Obstetricians and Gynecologists recommend combined HPV testing and Pap smear for Ca cervix screening in women aged 30 years or older.[10] This multimodal screening approach has been shown to be more sensitive and cost-effective compared to Pap smear alone.[11-13] For the introduction of HPV testing for screening, an understanding of the occurrence of HPV infection in the target population is needed. To consider the applicability of a multimodal screening approach in Bahrain, this study was designed to assess the occurrence of HPV infection and associated risk factors among women and determine the HPV types present.

Methods. ***Subjects and setting.*** Between March-December 2004, 100 women attending the Gynecology Clinic at Salmaniya Medical Center (SMC) and Sheikh Sabah Health Center (SSHC) were recruited for the study. Salmaniya Medical Center is the national secondary and tertiary referral center for specialist care, laboratory diagnosis and admissions while SSHC is a primary healthcare facility with a catchment population of 38,426. All women attending the 2 facilities were eligible for recruitment with the exception of pregnant women, those menstruating on the day of presentation and those with history of cervical dysplasia or neoplasia. Institutional ethical approval was obtained and eligible women were enrolled in sequential order of presentation after giving verbal or signed informed consent. Endocervical scrapings for HPV DNA detection and Pap smear were collected using wooden spatulas. Samples for HPV DNA analysis were immediately placed in sterile TEN buffer (10 mM Tris HCl, 1mM EDTA, 0.1 M NaCl pH 8.0), transferred to the laboratory within 2 hours and stored at -80^{O}C until processing. A standardized pre-tested questionnaire designed to collect sociodemographic data as well as information on risk factors such as smoking, parity, contraceptive use and STI was administered by the attending doctor.

Cytological analysis. Standard Pap smear processing was carried out at the SMC Pathology Department, Bahrain. Cytology reports by Pathologists were in compliance with the 2001 Bethesda Classification System.

HPV DNA detection. The DNA was extracted from cell suspensions using the Qiagen® amp DNA Mini Kit. All primers used were obtained from Thermo Electron Corporation, Germany. As a control for DNA adequacy in each sample, the primer set PC04 and GH20 was used to amplify the 260 bp fragment of the cellular ß-globin gene. After quantification, 100 ng of total DNA was used as a template for each PCR assay. HPV DNA amplification was carried out using the degenerate consensus primers MY09–MY11 targeting a 450 bp length region in the L1 ORF of the viral genome.[14] The PCR cycles included initial denaturation at 94^{O}C for 5 minutes followed by 35 cycles at 94^{O}C for 1 minute, 55^{O}C for 1 minute and 72^{O}C for 1 minute with a final elongation step of 6 minutes at 72^{O}C using the DNA thermal cycler (Perkin-Elmer 9700). HeLa cell DNA (harboring HPV type 18) was used as positive control. Electrophoresis of PCR products was carried out on 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

The HPV typing. Typing of HPV DNA positive samples was carried out using restriction fragment length polymorphism (RFLP) analysis. Briefly 10 μl of crude PCR products were digested with seven recommended restriction enzymes, BamHI, PstI, HaeIII, HinfI, DdeI, Sau3A, Rsa, in separate reactions.[15-17] Digestion products were separated by gel electrophoresis in 2.5% agarose gel and the profile was compared with published reference data.

Microsoft access software was used for the questionnaire data entry. Data were analyze using the SPSS version 12. Students' t test and Fisher's exact test were used to analyzed the statistical significance and p value <0.05 was taken as the level of significance.

Results. The demographic profile of the participants as obtained from the questionnaire is shown in **Table 1**. Out of the 100 women who were enrolled in this study, 80 were of Bahraini nationality. Eleven women (all Bahrainis) were positive for HPV DNA. Although the mean age of HPV DNA positive women was significantly higher, the mean age of first sexual intercourse was comparable in both groups. All HPV DNA positive women were in monogamous marriages compared to 77.3% in the negative group. Majority of women in both groups were non-smokers and although a trend of higher parity was observed among HPV DNA positive women this was not statistically significant (p=0.06). Eleven women had past history of STI (one HPV DNA positive and 10 HPV DNA

Table 1 - Profile of the study population stratified according to HPV DNA positivity and negativity.

Profile of participants	HPV DNA positive	HPV DNA negative
Number of women	11	89
Age		
Age Range	31-59 years	20-60 years
Mean ± SD*	43.3 ± 10.1	36.5 ± 9.9
Age at first sexual contact		
Range	13-27 years	13-35 years
Mean ± SD	18.1 ± 5.7	20.6 ± 4.4
Marital status†		
Married (N/total)	90.9% (10/11)	98.8% (84/85)
Divorced/widow (N/total)	9.1% (1/11)	1.2% (1/85)
Smoking‡		
Yes (N/total)	9.1% (1/11)	6% (5/84)
No (N/total)	90.9%(10/11)	94% (79/84)
Parity (Number of children)§ 5 and above	45.5% (5/11)	20.4% (17/ 83)

*p=0.04, †There were no singles (never married) among the women; Data on marital status was not available for 4 HPV negative women
‡Data on smoking history was not available for 5 HPV negative women
§Data on parity was not available for 6 HPV negative women

negative). There was no significant difference in the use of hormonal contraceptives between HPV negative and positive women (p=0.26).

Viral genotyping. In 6 of the HPV DNA positive women, RFLP analysis detected HPV types 16, 18, 45, 53 and 62 (2 women had type 53) while 2 other women were determined to have mixed infection the specific HPV types they harbored could not be resolved due to the presence of multiple bands on electrophoresis. HPV typing remained inconclusive in 3 other women due to very weak restriction patterns with indistinct bands.

Cytology. Cytological assessment was carried out for 92 women as samples were not sent to the Pathology Laboratory for 7 women and one sample was considered inadequate and deemed unsatisfactory for evaluation. All HPV DNA positive women had negative Pap smears, although one woman showed an inflammatory pattern due to *Candida* infection. One HPV DNA negative woman had mild dysplasia with koilocytic changes. Ninety per cent of HPV DNA positive women gave a history of having had a Pap smear screening prior to participation in this study compared to 52% HPV DNA negative.

Discussion. The complexity of conducting this study in a setting where STI is still perceived with a stigma limited the recruitment of subjects, hence the low sample size. However, data obtained from this study represent a significant baseline for describing the pattern of HPV infection in this population. Although the MY09/MY11 consensus primers we used have been shown to be more robust in detecting infections with multiple HPV types with relatively consistent sensitivity compared to other primer sets,[18] our finding of 11% HPV DNA detection is lower compared to the 13.9-64% detection rates among women with normal cytology described in other similar studies.[14,19-21] The RFLP analysis we used offers the advantage of enabling the typing of all known and novel HPV types, variants or sub-types and such degree of diversity in detection is difficult to achieve when using type-specific primers or hybrid capture probes.[14]

Despite quality assurance standards, it is now accepted that Pap smear cytology as a screening tool remain less than optimal and evidence for the clinical utility of HPV DNA testing has become very convincing.[11,13] In our study, all the HPV positive women had normal cervical cytology and 90% of them have had at least one Pap smear carried out prior to participation in this survey. This finding is in keeping with other studies, which have demonstrated normal cervical cytology on Pap smears in women with HPV infection.[14,20] Although it is generally difficult to ascertain whether the presence of HPV DNA in the absence of cytological abnormalities reflects a recent infection or a predictor of future cervical dysplastic or neoplastic abnormalities, it is now recommended that such women would benefit from closer follow up with regular or more frequent Pap smears.[4] Thus, by having shorter screening intervals in HPV DNA positive women and longer intervals for negative women, savings could be made on screening costs.[22,23]

Various factors have been suggested as acting in concert with HPV infection to induce oncogenesis and the questionnaire was designed to assess the significance of such factors. The mean age of HPV DNA positive women were significantly higher in this study group although other studies have reported a decline in HPV infection with age.[24-26] While it is difficult to decipher for how long a woman has been infected, factors such as older age, high risk HPV types and presence of multiple HPV types, which were demonstrated in this study, are indicators of persistent infections.[1,27,28] The mean age of first sexual intercourse was found to be comparable in the 2 groups and reflects the facts that in this sociocultural setting, onset of sexual intercourse takes place within the context of marriage which tends to occur at an

early age and a woman having multiple partners is generally unusual. Indeed, over 90% of the women in this study are married. To assess the impact of multiple partners as a risk factor, polygamy which is culturally accepted in this setting was used as a measure of the effect of male sexual behavior. Although all HPV positive women were found to be in monogamous marriages, we were unable to assess any other pattern of male sexual behavior or determine if this finding is reflective of a true monogamous status, thus, the effect of this risk factor remains unclear. High parity has been suggested as a risk factor for HPV infection and Ca cervix[4] and the data indicate that this may be a pertinent risk factor in this population but a larger study is needed to confirm this finding.

The presence of high-risk HPV types with persistent infection constitutes significant determinants for onset of oncogenesis. It is therefore of concern that all the HPV types identified in this study population are associated with cervical intraepithelial neoplasia, with types 16, 18 and 45 being confirmed high-risk types. This finding therefore underscores the need for a larger study to define the prevalence of HPV infection and describe the HPV types circulating in this population. Nevertheless, the finding of HPV infection (with high risk HPV types) in the presence of normal cervical cytology among women accessing screening services suggests that in addition to the ongoing National Pap smear screening program, introduction of HPV testing should be considered.

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