Prevalence of genital human papillomavirus among Lebanese women

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Summary

Purpose: The purpose of this study was to determine the prevalence of human papillomavirus (HPV), and more specifically of HPV 16, in a group of Lebanese women.

Materials and Methods: Type-specific prevalence of cervical HPV and the presence of cytological abnormalities were determined in a cohort of Lebanese women. The population included 1,026 women, 18-76 years, seeking routine gynecological care at a tertiary care center. Demographic and behavioral data were collected. HPV DNA was detected in cervical scrapes by polymerase chain reaction using consensus primers. Cervical cytological abnormalities were identified by Papanicoleau (Pap) smears.

Results: The mean age of our population was 40 ± 11.3 years. General HPV DNA was detected in 50 patients (4.9%). The high-risk HPV type 16 DNA was detected in 31 patients (3%). Patients with HPV 16 were more likely to have an abnormal pap smear than those with negative tests (6.6% vs 1.6%, $p < 0.05$), and more likely, but not significantly, to be smokers (21.4% vs 18.4%, $p = 0.5$). The age-specific prevalence of HPV increased with age and peaked at 60-69 years.

Conclusions: The prevalence of HPV in this small group of Lebanese women is similar to its prevalence in the Mediterranean countries. The presence of HPV, its known association with the development of cervical neoplasia, and the lack of a universal screening program for cervical cancer in our country should be used to enforce implementation of proper screening programs.

Key words: Human papillomavirus (HPV); Pap smear; Cervical dysplasia; Cervical cancer; PCR; Lebanon.

Introduction

Epidemiologic studies have shown that the association of human papillomavirus (HPV) with cervical neoplasia is strong and consistent in several countries [1]. There are several risk factors that might be related to the development of cervical cancer including, but not limited to, parity, the number of sexual partners, the age at the first sexual intercourse, and smoking [2]. In contrast, precise risk factors for HPV infection and its incidence and duration are not well known.

Cervical cancer represents the second most common cancer in women worldwide. Crude incidence rates vary from about 10 per 100,000 per year in many industrialized nations to more than 40 per 100,000 in some developing countries [3]. The lowest reported incidence rates are from countries in the Middle East, such as Saudi Arabia where cancer of the cervix ranks 9th in the frequency of cancers among Saudi females [4]. In Lebanon, the national tumor registry is non-functioning. An independent recent survey of the cancer incidence in 1993 and 1998 revealed a crude incidence rate of 4.6 and 4.8 per 100,000, respectively (Shamsseddine *et al.*, personal communications). Substantial environmental and lifestyle factors contributing to the etiology of cancer of the cervix may be operating differently in different countries. Moreover, because of widespread differences in the availability of screening programs and risk factors, there is marked variation in the relative frequency of cervical cancer. In Lebanon, as in many developing countries, the diagnosis of cervical cancer is usually made in advanced stages because of inadequate screening. In addition, there are no prior studies on the prevalence and role of HPV and other risk factors in the etiology of cervical cancer in our country.

This pilot study was undertaken to investigate the prevalence of HPV infection, and specifically of the high risk HPV 16, amongst a group of women coming for gynecological care at a tertiary care center in Beirut, Lebanon.

Materials and Methods

Women between the ages of 18-70 years who visited the private clinics at the American University Medical Center (AUMC) and received a pelvic examination as part of routine gynecologic care were eligible for enrollment. Women who were pregnant or had previous hysterectomy were excluded from the study. Each woman was informed about the study and those who had any reservations were excluded. The study protocol was approved by the Ethics Committee of the Chronic Care Center as well as by the Institutional Review Board of the American University Medical Center.

Reproductive history, results of previous cytology smears, history of genital warts or cervical cancer were elicited and also the use of tobacco was queried. Following visualization of the cervix, an Ayre spatula was rotated 360 degrees over the ectocervix for collection of cells from the endocervix and the transformation zone and swept across a glass slide. Similar rotation in the endocervical canal by a cytobrush or a wet Q-tip was done and swept across the same slide that was then immediately fixed with ethanol. The cytobrush or the Q-tip was then dipped in a sterile tube containing 2 ml of serum-free phosphate buffer saline (PBS) and kept in the refrigerator for subsequent DNA extraction and PCR amplification.

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Table 1. — *HPV primers and probes.*

Name	Target DNA	Orientation	Position	Nucleotide sequence (5'-3')	Size
MY 09	HPVs L1 gene	Antisense	6722-6742	CGT CCM ARR GGA WAC TGA TC	450 bp
MY 11	(consensus)	Sense	7170-7150	GCM CAG GGW CAT AAY AAT GG	
GP5	HPVs L1 gene	Sense	6764-6784	TTTGTTACTGTGGTAGATAC	139 bp
GP6	(consensus)	Antisense	6904-6884	GAAAAATAAACTGTAAATCA	
16F194	HPV16, E1 gene	Sense	194-217	GTGTACTGCCAAGCAACATTACTG	357 bp
16R551		Antisense	551-528	GGGTTTCTCTACGTGTTCTTGATG	
16FL416X	HPV16, E1 gene	Sense	416-442	AACTGTCAAAAGCCACTGTGTCCTGAA	
16LC444p		Sense	444-470	AAAAGCAAAGACATCTGGACAAAAAGC	
βF270	β-globin gene	Sense	270-288	CCTGTGGAGCCACACCCTA	476
βR746		Antisense	746-727	GCCATGAGCCTTCACCTTAG	

M = A+C, R= A+G, W = A+T, Y = C+T.

Nucleic acid extraction

The viral DNA was then extracted using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH) according to the protocol recommended by the manufacturer. The DNA was eluted in 50 μl of the kit's elution buffer and stored at –20°C until use.

HPV Detection

The presence of HPV DNA was assessed by polymerase chain reaction (PCR) in three different ways (Table 1).

Single-step PCR: HPV DNA amplification was performed using 2 uls of each sample and the consensus primers for the L1 region MY 09 and MY 11 [5, 6]. In a separate reaction and prior to HPV amplification, a 476 bp fragment of the β-globin gene was amplified using the beta globin specific primers, βf270 and βr746. A successful amplification of the β-globin fragment indicated that the sample was adequate for HPV analysis.

Two-step (nested) PCR): The My11-MY09 primer pair was used to amplify 2 μl of each sample in a final volume of 20 μl. After 25 amplification cycles, 1 μl aliquots of each amplified sample were transferred to fresh PCR tubs for a second round of amplification using GP5 and GP6 primers [5, 6].

HPV 16 Genotype specific PCR using hybridization probes:

Real time flurogenic PCR analysis for HPV 16 detection was performed with the LightCycler (LC) instrument (Roche Diagnostics GmbH). Primers were designed to target the region between nucleotide 194 and 551 of HPV 16 genome, (16F194 and 16r 551) in the E6 gene [7]. For product detection, HPV genotype specific hybridization probes, 16FL416X and 16LC444p were used.

Amplification data was collected and analyzed by the LC software.

Contamination prevention:

Separate areas were used for DNA amplification and sample preparations. Using the LightCycler where the amplified DNA sample is detected without the need to open the tube as well as the use of uracil N-glycosylase added an additional level of contamination prevention. Multiple negative controls were included with every set of samples.

Statistical analysis:

The data was analyzed using the SPSS version 9.0. The Student's *t*-test was used to compare continuous variables, while the Chi-square was used to compare categorical variables; $p < 0.05$ was considered significant.

Results

Out of 1,026 Pap smears, general HPV DNA was detected in 50 patients (4.9%) using single step PCR amplification, and HPV type 16 DNA was detected in 31 patients (3%). One sample was positive for HPV 16 DNA and negative for the HPV general DNA. This is certainly due to the more sensitive fluorescent-based technique used for the detection of HPV 16. This also explains the fact that among the general HPV DNA positive cases, more than half were positive for HPV 16. When a second round of amplification (nested PCR) was used to increase the HPV DNA detection sensitivity, 17.8% of the samples were positive for HPV DNA. Due to the high sensitivity of this technique, a higher number of false positives could be generated which may partly explain the significant increase of HPV positive samples. The mean age of the participants was 40.0 ± 11.3 years (range 18-79 years). The mean age, gravity, parity, the rate of abnormal pap smears and smoking habits are listed in Table 2. Although the overall prevalence of positive smears was low in our population, this proportion was significantly higher in patients with HPV 16 than in those with negative tests (6.6% vs 1.6%, $p < 0.05$). This was not true for patients with general HPV DNA only. Patients with positive HPV 16 were more frequently smokers than those with negative HPV 16 but the difference did not reach statistical significance (data on smoking was available on half of the population only).

Table 2. — *HPV16 prevalence in Lebanese women: demographic data.*

	n	Age (yrs)	Gravida	Parity	Abnl.pap	Smoking
General Pop.	1026	41.15 ± 10.75	2.86 ± 1.91	2.27 ± 1.45	1.75%	27.80%
HPV (+) Pop.	31 (3%)	40.0 + 9.8	2.8 + 1.9	2.2 + 1.3	6.60%	21.40%
HPV (–) Pop.	995 (97%)	40.3 + 11.3	2.8 + 1.8	2.3 + 1.5	1.60%	18.40%

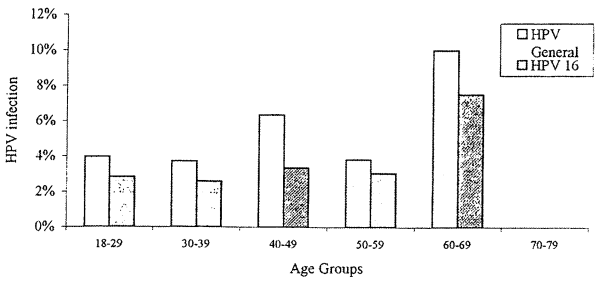

Figure 1. — Distribution of HPV infection among different age groups.

The mean parity and gravity in the whole population was 2.8 ± 1.8 and 2.2 ± 1.5 with no significant difference among the various groups. The age distribution among the various HPV categories is shown in Figure 1. The rate of positive HPV (general) increased with age from < 5% to around 10% at 60-69 years and the percentage of HPV 16 also increased from less than 3% to 7% at 60-69 years.

Discussion

HPV appears to represent the most common sexually transmitted agent studied to date. In some populations, cross-sectional studies of cytologically normal women reported an incidence of 20-40% detectable HPV infection amongst sexually active women and the prevalence decreases with age [8-11]. More than 100 HPV types have been identified and in most studies, HPV 16 has been found to be the most prevalent HPV type (around 50%) in cytologically normal women, women with cervical intraepithelial neoplasia (CIN) and women with cervical cancer [3]. Based on a report by Menczer *et al.*, HPV DNA was found in more than 93% of cervical cancer specimens except for one study from Israel which showed a much lower prevalence [12]. However, the significance of sub-clinical HPV 16 infection with regard to the frequency and rate of neoplastic progression is uncertain. Ho *et al.* followed 608 college women every six months for three years. HPV DNA by PCR and Southern blot on cervical smears showed a cumulative 36-month incidence of 43%. The risk increased with younger age; increased number of sexual partners, sexual habits, and alcohol consumption. The median duration of new infection was eight months (7-10 months). The risk of abnormal Pap smear increased by 37-fold in the presence of high-risk HPV [13].

Koutsky and co-workers [14] performed serial Pap smears and dot blot hybridization of cervical HPV DNA on women attending a clinic for sexually transmitted diseases (STD). Among women who were cytologically normal at enrollment, the 2-year cumulative incidence of high grade CIN (2 and 3) was 28% among HPV infected women as compared to 3% of the women in whom infection was not detected. Joseffson *et al.* [15] demonstrated that women with the highest amount of HPV 16 DNA were at a 60-fold higher risk for developing cervical carcinoma in-situ than women negative for HPV 16. Moreover, nucleotide alterations of HPV 16 affect the potential for oncogenic transformation in vitro [16]. The risk for CIN 2-3 is not the same for all variants of HPV 16 and non-prototype like variants confer a greater risk compared with prototype variants [17].

The number of abnormal smears (1.6%) in the current study is lower than the numbers reported in most studies. This is mainly due to differences in the population screened. In 3,088 Saudi women, Altaf [4] reported an incidence of 3.14% abnormal smears. Recently, Manos *et al.* [18] found that 3.5% of 46,009 women who had routine Pap smears had ASCUS, 0.5% had AGUS, 0.9% had LSIL, and 0.3% had HSIL.

The importance of determining the prevalence of HPV is its high association with the development of cervical dysplasia and cancer.

The low prevalence of HPV DNA and HPV type 16 DNA of 4.9% and 3%, respectively, in our participants is in line with the low incidence of abnormal cytological smears in that same population. The prevalence of HPV varies dramatically depending on the population studied and the sensitivity of the technique used in the detection of the HPV DNA. In Spain, the prevalence of HPV in the general population as determined from case-control studies was 5% and between 13-20% in Thailand, the Philippines, Paraguay, Brazil and Colombia. HPV prevalence in Western Europe and the United States is generally less than 10% [3].

The significance of these subclinical infections with regard to the frequency and rate of neoplastic progression remains uncertain. In a population based study with cervical cytological studies to detect HPV, Syrjanen *et al.* [19] found HPV in 0.8% of 63,115 women aged 20-65 years, while De Villiers *et al.* [20] using Pap smears and filter in situ hybridization, found HPV in 9% of women aged 15-81 years. Other studies that used more sensitive techniques such as Southern blot or PCR found HPV rates of 32% and 14-60%, respectively. In this study we showed a significant increase from 4.9% to 17.8% when nested PCR was used as the HPV DNA detection method. This increase may be partly due to the false amplifications that are often associated with this technique as well as its increased sensitivity. The (two-step) nested PCR was reported to detect less than ten copies of the HPV genome in a sample [5, 6]. Of equal or greater significance than the detection technique used are the age and the recent sexual activity of the population. Syrjanen *et al.* [19] found HPV in 6.1% of women aged 20-29 but only 2.2% in women aged 30-39. De Villiers *et al.* [20] similarly found HPV in 10-13% of those aged 15-20 years, but only 2-5% among those older than 55 years. In this study, there was an increase of HPV (general) as well as HPV 16 detection with age. This marked deviation from the results reported elsewhere could be due to two factors. First the mean age of 40 years in the studied group is much older than the reported peak age incidence for HPV, hence the prevalence could be higher in younger, more sexually active women. Second, the sexual practices of this

population are markedly different from the populations studied in the other reports.

Since the development of cervical cancer and its precursors is epidemiologically associated with oncogenic, high-risk types of HPV genital infection, the availability of testing for HPV opens new dimensions for pathogenesis of cervical neoplasia in addition to the traditional cytological screening. The availability of HPV testing raises the question whether it should be used as a screening tool for cervical cancer. HPV testing may reduce costs by identifying low-risk groups for less frequent screening. However, there is no evidence that follow-up of patients needs to be changed if HPV infection is not demonstrable, because new infection may occur in the future. Detection of low-risk HPV subtypes warrants regular follow-up, as even low-risk HPV subtypes can be found in cervical cancer and future re-infection with high-risk subtypes is possible. Likewise, the detection of a high-risk subtype still requires regular follow-up and given the state of knowledge, this infection does not warrant aggressive treatment unless concomitant cytological abnormalities exist. Furthermore, HPV testing, in addition to its cost, may yield needless anxiety and overtreatment.

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