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BRIEF REPORT

Human papillomavirus (HPV) detection in biopsies from cervical cancer patients; A population–based study from Iran

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ABSTRACT

Background: Human papillomavirus (HPV) is associated with various benign and malignant lesions including genital condyloma and anogenital cancer. The presence of HPV-DNA was studied in archival biopsies of high- and intermediate-risk lesions for cervical carcinoma in women referred to Mirza hospital in Tehran.

Patients and methods: Totally, 105 Iranian archived biopsies collected during February and November 2006. HPV-DNA was isolated from the biopsies and detected by means of consensus polymerase chain reaction (PCR) detecting a broad spectrum of genital HPV types.

Results: Totally, 26 samples (24.7%) were positive for oncogenic HPV-DNA. Risk of HPV infection was significantly higher in biopsies obtained from patients with confirmed squamous cell carcinoma (SCC) who started sexual activity more recently (≤4 years ago) when compared with those who started earlier (≥10 years ago). HPV-16 was more frequently detected in biopsies of younger women.

Conclusion: HPV was more frequently detected in young women. Our data confirm the usefulness of this method for detection of HPV in archival biopsies.

Keywords: *Human papillomavirus (HPV), Cervical cancer, Archival biopsy samples, Polymerase chain reaction (PCR).*

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INTRODUCTION

Cervical carcinoma (CC) is the second most common malignancy among women in both incidence and mortality (1). Although much is known about the etiology and treatment of CC, the role of genetic alternations in the multistep pathway of cervical tumor genesis is largely unknown. Converging points of evidence implicate infection by high- risk human papillomavirus (HPV) types as a critical etiologic factor (2,3).

The development of CC is preceded by distinct morphological changes from normal epithelium to carcinoma through low-grade and high-grade squamous intraepithelial lesion (SILs), which represent mild to severe dysplasia. A molecular study on early and invasive CC has identified several structural and functional alterations in oncogenes (4). Recently, it has become possible to detect the HPV genome by means of polymerase chain reaction (PCR) in DNA extracted from cervical biopsies. In fresh cervical swabs, an

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increased prevalence of HPV has been found with increasing severity of intra-epithelial lesions (5). Correspondingly, HPV has more frequently been found in archival biopsies with cytological changes than in archival samples with normal cytology (6,7).

The aim of the present study was to detect HPV-DNA in archival biopsies of high- and intermediate-risk lesions for cervical carcinoma in patients presenting to a referral teaching hospital in Tehran.

PATIENTS and METHODS

Totally, 105 formaldehyde-fixed and paraffin-embedded CC tissue fragment from patients attended at Mirza Khochakkhan hospital in Tehran were collected during February and November 2006. Mirza Khochak khan is a referral teaching hospital in Tehran that welcomes female patients from all around Iran. In brief, random samples were drawn from the computerized registry system of oncology department of the hospital.

The biopsies of females with invasive squamous cell carcinoma (SCC) were analyzed to detect HPV- DNA genotypes.

Clinical and epidemiological data were recovered from hospital files or an interview, whenever available, and include information on sexual habits, sexually transmitted diseases (STDs) and smoking status.

Then, paraffin blocks of archival cervical biopsies were cut in 5-µm section using a new blade for each specimen to minimize block-to-block contamination. Tissue sections were, then, deparaffinized by treatment with xylem and washed with ethanol. The proteins were digested by proteinase k (200µg/ml), overnight, at 55˚C, and the extracted DNA purified by a standard kit (Biotool Inc.Spain).

To evaluate the quality of the DNA isolation procedures, samples were submitted to polymerase chain reaction (PCR) of the β-globin human gene sequence, using a pair of primers named PCo3/PC04. DNA amplification for HPV detection was performed in two distinct genomic regions; E6-E7, using primers PUIM/PU2R, and L1, using primers GP5+/GP6+.

PCR reactions were performed in a final volume of 50µL, containing 10µL of extracted DNA, 20mM Tris HCL (PH:8.4), 50mM KCL, 3mM MgCl2, 250µM dATP, dCTP, dTTP and dGTP, 2.4 Pmol of each primer and 2.5u of Tag DNA polymerase. Forty cycles of amplification were conducted in an Techne research thermocycler (first cycle: 95˚C 5min, 40˚C 3 min , 72˚C 3min; second to 39th cycle; 95˚C 1min). Finally, PCR products were analyzed by electrophoresis on 1.5% agarose gels followed by 10mg/ml ethidium bromide staining.

Categorical variables were analyzed with chi square test. Risk factors for HPV-DNA detection in the cytological biopsies were analyzed. All variables were adjusted for potential confounders by mean of a multiple logistic regression analysis. Prevalence odd's ratios (PORs) as maximum likelihood estimates of the relative risk of HPV positively were computed with 95% confidence interval (CI).

RESULTS

Subjects were married with the mean age of 54 years (27-82 years). Table 1 represents age distribution, age at which sexual intercourse was commenced, sexual active years and previous pregnancy.

The histopathology diagnosis of squamous cell carcinoma (SCC) was as follow: 70% classified as well differentiated, 20% moderately and 10% as poorly –differentiated SCC. Oncogenic HPVs-DNA were isolated from 26 samples (24.7%) (table 2).

Risk of HPV infection was significantly higher in biopsies from women who started sexual activity more recently (respectively, ≤4 than ≤6 years ago)

when compared with those who started earlier (≥10 years ago).

HPV-16 was more frequently detected in biopsies from younger women than older subjects. Correspondingly, HPV was three times more likely detected in younger women.

Table 1. Selected characteristics of the studied patients

Age	Number	Percentage	P-value
Age group (yrs)			
20-24	21	20.0	
25-29	19	18.1	
30-34	31	29.5	
35-39	34	32.4	
Age at first sexual intercourse (year)			
≥15	54	51.4	
14-16	48	45.7	0.03
≤13	3	2.9	
Sexual active years			
≤4	3	2.9	
5-9	29	27.6	0.001
10-19	49	46.6	
≥20	24	22.9	
Pregnancy			
Never	21	20.0	0.02
Ever	84	80.0	

Table 2. Distribution of oncogenic human papillomavirus (HPV) types in archival biopsies

HPV type	Number	Percentage
HPV-16	10	9.5
HPV-18	3	2.9
HPV-31	4	3.8
HPV-33	0	0
HPV-58	2	1.9
Other types	2	1.9
Unidentified	5	4.8

DISCUSSION

Given the well-established strong relationship between cervical neoplasia and HPV infection, a high frequency of oncogenic HPV-DNA in cytologically abnormal biopsies should be expected. All of 26 abnormal archival biopsies in this study were HPV-DNA positive confirming the usefulness of the assay. The higher HPV detection rate in young women is consistent with several other studies of HPV in fresh cervical specimens (3,4,8).

The higher HPV risk in females aged >35 years is in line with the higher cervical cancer risk in this age group. Our data confirm the usefulness of this method for detection of HPV in archival biopsies as all dysplastic changes were HPV positive and the predictor of HPV presence. Thus, this method can be useful for large scale epidemiological studies of HPV-DNA in already samples material.

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