Iranian J Publ Health, Vol. 37, No.3, 2008, pp.101-106

Original Article

Prevalence of HPV Infection and Its Association with Cytological Abnormalities of Pap Smears in Tehran

*M Jamali Zavarei[1], *R Hamkar[2], V Ghobadi Dana[3], M Delforoosh[4], MH Shojamoradi[5], M Modarresi Gilani[1]*

[1]*Imam Khomeini Hospital, Tehran University of Medical Sciences, Iran*
[2]*School of Public Health, Tehran University of Medical Sciences, Iran*
[3]*Children's Medical Center, Tehran University of Medical Sciences, Iran*
[4]*School of Science, Islamic Azad University/ Qom branch, Iran*
[5]*Legal Medicine Organization of Iran*

(Received 25 Oct 2007 accepted 5 Jul 2008)

Abstract

Background: Human papillomavirus infection is one of the most common genital infections. More than 100 types of this virus have been identified, and most of them are capable of infecting the genital mucosa. Human papillomavirus is in association with cancerous and precancerous lesions of the cervix; some types like HPV 16 and 18 are highly carcinogenic, some types like HPV 31 and 33 are moderately and some types like HPV 6 and 11 are mildly carcinogenic. In this research, the relationship between cytological changes of the squamous epithelial cells and the presence of HPV infections in our cases has been assessed.

Methods: In this prospective study, we collected 681 samples from women admitted to different hospitals and private gynecological clinics in Tehran, during the years 2003-2005. Two specimens were collected from each patient; one for a Pap smear study and the other for PCR assay in order to detect HPV.

Results: Out of our 681 samples, 600 specimens were suitable for PCR assay, and 34 cases were HPV positive in PCR assay. This means that 5.7 percent of our patients were infected with HPV.

Conclusion: HPV infection is common in Iran and is nearly identical to European countries such as Germany, and Spain. Also, we found that using PCR assay in order to detect the presence of HPV viruses in vaginal discharges can be very helpful.

Keywords: *Pap smear, Human Papillomavirus, PCR, Iran*

Introduction

Human papillomavirus (HPV) infection is one of the most common genital infections. More than 100 types of this virus have been identified, and most of them are capable of infecting the genital mucosa. Human Papillomavirus is in association with cancerous and precancerous lesions of the cervix; some types like HPV 16 and 18 are highly carcinogenic, some types like HPV 31 and 33 are moderately and some types like HPV 6 and 11 are mildly carcinogenic (1, 2).

HPV has a tendency for infecting the squamous epithelium of different organs like the skin, anogenital region, lungs, esophagus, and the larynx. The life cycle of the virus starts with its entrance into the basal layer of the epithelium. The DNA of the virus has a limited replication in the basal layer, but the virus particles are not completely produced. Slowly, with the development of the epithelial cells and their keratinization, the virus can be completely replicated and the virions gather in the cell nuclei. When the cells die, the virions exit the cells (3, 4).

HPV infection is one of the most common and mostly contagious genital infections which are mostly sexually transmitted. In cases with erosion of the cervical mucosa because of trauma, vaginal delivery, or other reasons, the virus di-

***Corresponding Author:** Tel: +98 21 8896 2343, Fax: +98 21 8895 0595, E-mail: rhamkar@sina.tums.ac.ir

rectly invades the basal layer. The infection might remain as a hidden infection or present as a local papilloma called Condiloma Accuminata after a latent phase of six to eight weeks. The pathology of a condiloma is acanthosis, elongation of the reteridge, papillomatosis, proliferation of the submucosal capillaries, and the presence of koilocytes in the squamous mucosa. These lesions are more severe in pregnant women, diabetics, and in women using OCPs. Exophytic genital warts are highly contagious and more than 75% of the sexual partners of these patients present with this infection. Condilomas are often surrounded with vast regions of sub-clinical infection, so the eradication of the virus is nearly impossible, however removing exophytic lesions can result in relative relief. HPV types 6 and 11 are the most common infections which also have a high rate of lesion regression and low rate of persistent infection. HPV types 35, 33, 31, 18, and 16 are usually associated with persistent infections and cancerous lesions. These HPV types could be detected in about 95% of cervical cancers (5, 6).

Cytological methods, especially Pap smear, have been widely accepted as the primary methods for screening cervical lesions. The usage of these methods, has effectively controlled and reduced cervical cancer, but has not been able to eradicate it, because many of the results turn out to be false negative cases (5-8).

In this prospective double-blind research, we have assessed the association of some cytological features with the presence of HPV infection.

Material and Methods

We collected an ordinary vaginal smear and a cytobrush sample for PCR assay, from married women between the ages of 15 to 55 yr old, admitted to different hospitals and private gynecological clinics, throughout the years 2003-2005, who were not in their menstrual period, had not had sexual intercourse during the 24 h before sample taking, and had not used any vaginal medications. All the samples were assessed for cytological features after Papanicola staining and also observed for HPV infection with PCR assay.

DNA was extracted using DNA extracting kits (Bioneer™, South Korea). For quality control of DNA extraction from specimens β-globin gene PCR was done using PCO3/PCO4 primers which amplify 110 bp segments of the β-globin gene, according to previously described procedures (5, 6). Positive specimens were identified as suitable samples for HPV-PCR. From the total of 681 samples taken, 600 samples were suitable regarding this criterion.

The detection of HPV in the specimens was done the with GP5+ and GP6+ primers in a PCR assay. The PCR process was done in 40 cycles, starting with Hot Start according to previously described procedures (5, 6).

Results

Endocervical cells and squamous metaplasia were found in 31%, severe exudates in 38.8%, red blood cells in 7.7%, benign reactive changes (BCR) in 13.3%, dysplasia 0.5%, and invasive squamous carcinoma in 0.5% (Table 1). Anucleated squamous cells were found in 8.5% of the specimens (Fig.1). Moniliasis (Candidiasis) was found to be seen in 6.7% of the patients and also 0.5% and 3.7% of patients were found to be infected with *Trichomonus* and *Hemophilus* respectively (Table 2 and 3).

The HPV genome was identified in 5.7% of the cases with PCR assay (Table 2). HPV infection was mostly seen in women who were 35-44 yr old, which accounted for 6.7% of the patients. As indicated in Table 2, anucleated squamous cells were found in all of the HPV positive vaginal smears (Fig. 1). This association is statistically significant (P= 0.000). Out of the 34 patients infected with HPV, the smears of 22 patients (64%) were reported to have severe exudates. Moderate and mild exudates were found in the rest of the HPV positive specimens (34%). HPV infection was not detected in any of the NIL specimens. This finding was also

statistically significant (P= 0.009). In the specimens infected with *Candida* and *Trichomona*, HPV virus DNA was not detected (Table 3).

The presence of koilocytes is one of the cytological findings of the HPV positive vaginal smears. These cells are completely typical, with large reactive and halo nuclei in the depth of the cytoplasm. Some expert cytologists believe that the detection of these cells is a definite indicator of HPV infection. Out of the 34 HPV positive specimens, koilocytes were only seen in 3 patients (8.9%). The presence of dyskaryocytes is another cytological finding of HPV positive vaginal smears, but it is not specific for HPV infection and we only found it in 3 of our HPV positive patients.

Correctly taken Pap smears, which contain endocervical cells help us make a better diagnosis, and has an important role in an exact cytological diagnosis. Our findings also approve this statement.

Table 1: Distribution of specimens by cytological suggestions

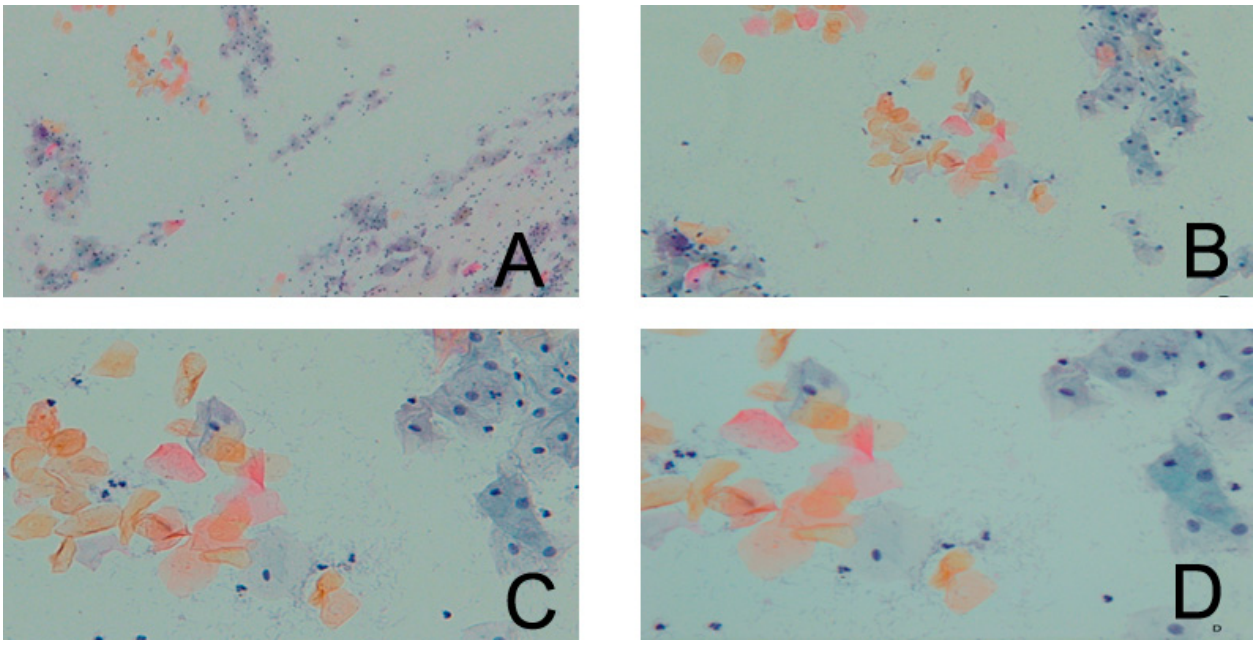
Cytological suggestions	Positive		Negative		Total
	#	%	#	%	
BRC	80	13.3	520	86.7	600
Dysplasia	3	0.5	597	99.5	600
SCC	3	0.5	597	99.5	600
Total	86	14	514	86	600

Table 2: Distribution of specimens by occurrence of Anucleated squamous cells, Exudates and HPV

	Anucleated squamous cells					Exudates							
	Positive		Negative		Total	NIL		Light		Moderate		Severe		Total
HPV	#	%	#	%		#	%	#	%	#	%	#	%	
Positive	34	100	0	0	34	0	0	6	18	6	18	22	64	34
Negative	77	14	489	86	566	39	6.9	124	21.9	192	33.9	211	37.3	566
Total	111	18.5	489	81.5	600	39	6.5	130	21.7	198	33	233	38.8	600

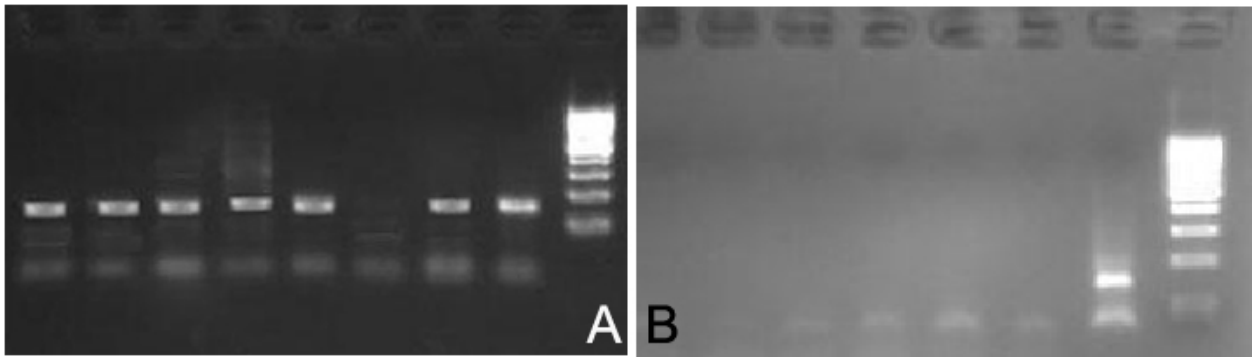
Table 3: Distribution of specimens by occurrence of Microorganisms and HPV

HPV→	Positive		Negative		Total
Microorganisms↓	#	%	#	%	
Negative	17	7.5	208	92.5	225
Monilia	0	0	40	100	40
Bacilli	6	3	194	97	200
Coccoid	11	10	99	90	110
Hemophilus	0	0	22	100	22
Trichomonus	0	0	3	100	3
Total	34	5.7	566	94.3	600



A= 10X × 4 B= 10X × 10 C= 10X × 25 D= 10X × 40

Fig. 1: Anucleated squamous or keratinized cells



A= Some HPV positive samples B= Some HPV negative samples

Fig. 2: Gel electrophoresis of HPV PCR products

Discussion

The effective usage of cytological tests in detecting invasive cervical cancers and precancerous lesions has lead to a significant decline in the mortality rate of this cancer. However, cervical cancer is still one of the main causes of death among women (1, 2, 5, 6). The cytological screening programs using the Pap smear test for detecting atypical cells within the cervical epithelium, has reduced the mortality rate of cervical cancer but has not yet lead to the eradication of this cancer (5- 9). Many reports have marked this test unsuccessful in exactly predicting the incidence of cancer, and more than 50% of these tests have been reported to be false negative (9). In this detection system, errors can occur both in taking the smears and in analyzing the specimen (8, 10, 11). Regarding that some types of HPV are known as the main causes of cervical cancer, detecting HPV

along with routine Pap smears can be helpful in identifying cases, which would most probably proceed to cancer.

Our findings show that there is a significant correlation between the presence of anucleated squamous cells and HPV infection in vaginal smears (Table 2). As is shown in Table 2, all the specimens that have been proved to be infected with HPV by PCR assay, have shown to have anucleated squamous cells or anucleated-keratinized cells. This correlation is statistically significant (P= 0.000). Therefore, finding these cells in vaginal smears necessitates colposcopy and PCR for detecting HPV. However, not all of the patients been found to have Anucleated keratinized cells in their vaginal smears are infected with HPV. We also found that 18.5% of our patients had anucleated squamous cells in their vaginal smears, however only 5.7% were HPV positive. In other words, 14% of the patients showed anucleated squamous cells in their smear, without an HPV infection. Our findings show that the presence of these cells should be taken seriously and even a small number of them should be mentioned in cytology reports, and the case should be referred for colposcopy and PCR assay in order to detect HPV infection. Unfortunately, no attention is paid to mentioning the presence of anucleated squamous cells in vaginal smears, in cytology reports. In the currently used Bethesda cytology report forms, unidentified squamous cells that the cytologist does not recognize are named ASCUS, which are completely different from Anucleated squamous cells. The importance of reporting the presence of these cells becomes clearer, when we consider its association with 100 percent of the HPV positive smears. Regarding the causative role of some types of HPV in cervical cancer, reporting these cells in the cytology report can be especially helpful in preventing invasive cervical carcinoma.

Our findings are suggestive of a significant correlation between the presence and severity of exudates in the Pap smears and HPV infection in the patient (Table 2). In cases which exudates were not present, HPV was not detected either. Most of the HPV infections (64%) were in the smears which had been reported to have severe exudates. This correlation is statistically significant (P= 0.009). Therefore, in exudate positive smears, more detailed observation to find perhaps anucleated squamous cells and referring the patient for PCR assay in order to detect HPV is highly recommended.

As a conclusion, considering our results in this research, we recommend cytologists to pay more attention to the presence of anucleated squamous or keratinized cells, and also the presence of exudates in the smears.

Acknowledgments

The authors would like to kindly thank the Research Deputy of the Tehran University of Medical Sciences, which supported the funds for this research. We also thank Doctor Ghaffari, and Doctor Hojjati who kindly helped us in collecting our samples.

The authors declare that they have no conflict of interests.

References

1. Franco E (1991). Viral Etiology of Cervical Cancer: A Critique of the evidence. *Rev Infect Dis*, 13:1195-206.
2. IARC Working Group (2007). *Human Papillomaviruses, IARC monographs on the evaluation of carcinogenic risk to human*. IARC, Lyon, France, pp. 181-91.
3. Adam E, Berkova Z, Daxnerova Z, Icenogle J, Reeves WC, Kaufman RH (2000). Papillomavirus Detection: Demographic & Behavioral `Characteristics influencing the identification of cervical disease. *AJOG*, 182: 257-64.
4. Djamali M, Niakan M (2004). Human Papillomavirus genotype as a major determinant of the course of cervical cancer. *Yakhteh*, 5: 154-57.
5. Hamkar R, Mokhtari-Azad T, Mahmoodi M, Seyedirashti S, Severini A, Nategh

R, (2002). Prevalence of Various Types of Human Papilloma Virus Among Cervical Cancer and Normal Biopsy Specimens in the Mazandaran Province in Iran. *Eastern Mediterranean Health J*, 8(6):805-11.

6. Hamkar R, Mokhtari-Azad T, Mahmoodi M, Seyedirashti S, Severini A, Nategh R (2004). Prevalence of various types of HPV among cervical cancer and normal biopsy in north of Iran. *Iranian Journal of Infectious Diseases and Tropical Medicine*, 8(22): 9-16.
7. Walboomers JM, Roda Husman AM, Snijders PJ, Stel HV, Risse EK, Helmerhorst TJ, Voorhorst FJ, Meijer CJ (1995). Human Papillomaviruses in false negative archival cervical smears: implications for screening for cervical cancer. *J Clin Pathol*, 48:728-32.
8. Djamali M (1999). *Cytology of women genital system*, Arvich publisher, p.123.
9. Van der Graaf Y, Vooijs GP (1987). False negative rate in cervical cytology, *J Clin Pathol*, 40:438-42.
10. Djamali M (2006). Epidemiologic study of HPV infection and its association with cytologic abnormality of Pap smear in women referred to gynecologic clinic of Imam Khomeini hospital in 2004. *1st International congress of the Iranian society of gynecological oncology*. pp. 85-6.
11. Elfgren K, Kalantari M, Moberger B, Hagmar B, Dillner J (2000). A population based five year follow-up study of cervical human papillomavirus infection. AJOG, 183:561-67.