Human Papillomavirus Genotype as a Major Determinant of the Course of Cervical Cancer

Mohammad Niakan, Ph.D.[☆ ♦], *Zahra Eftekhar, Ph.D.*[★], *Mohammad Jamali Zavareh, Ph.D.*[★], *Faranak Golalipour, Ph.D.*[★]
Soghrat Faghihzadeh, Ph.D.[★], *Mohammad Reza Jalali, Ph.D.*[☆]

☆ *Microbiology and Pathology Department, Faculty of Medicine, Shahed University*

★ *Obstetrics Gynecology and Pathology Department, Faculty of Medicine, Tehran University*

★ *Medical Science Department, Tarbiat Modarres University*

♦ **P.O.Box: 14155-7435, Microbiology and Pathology Department, Faculty of Medicine Shahed University, Tehran, Iran**

Abstract

Received 19/Nov/2002, Accepted 7/Nov/2003

Introduction: Certain types of human papillomavrus (HPV) are associated with cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC). The aim of the observations reported here was to determine whether the prognosis for invasive cancers of the uterine cervix is related to the type of human papillomavirus asociated with the tumor.

Material and Methods: Twenty Patients with invasive cervical cancer were prospectively registered from 2000 to 2001. HPV typing was performed by insitu hybridization (ISH) on DNA extracted from frozen, formalin-fixed, paraffin-embedded tumor specimens. The specimens mostly represented classifications SCC Stage 1 and Stage 2 of the International Federation of Gynecology and Obstetrics (Table 1). HPV- DNA was detected by insitu hybridization, using three different DNA Probes: types 6/11, 16/18 and 31/33/51.

Results: HPV DNA was detected in the nuclei of SCC tumor cells in 13(65%) of 20 cases. Of the 13 HPV-DNA positive cases three reacted only with the HPV 31/33/51 probe, two reacted only with the 16/18 probe, three showed strong hybridization for both 31/33/51 and 6/11 probes, four showed 6/11 and 16/18 genotypes and one case reacted with 31/33/51,6/11and16/18 probes.

Conclusion: The prognosis for invasive cancers of the uterine cervix is dependent on the oncogenic potential of the associated HPV type. HPV typing may provide a prognostic indicator for individual patients and is of potential use in defining specific therapies against HPV harboring tumor cells. These findings are consistent with the hypothesis that HPV infection is the primary cause of cervical neoplasia. Furthermore, they support HPV vaccine research to prevent cervical cancer and efforts to develop HPV DNA diagnostic tests.

Key words: Human Papillomavirus, Insitu Hybridization, HPV typing, Squamous Cell Carcinoma, Genotype

Introduction

Cancer of the uterine cervix is the second most common cancer in women worldwide (1, 4). Early observations indicated that a major risk factor for this tumor was a venereally transmissible oncogenic agent (2), later identified as human papillomavirus (HPV) (3). Virological analysis of skin and genital lesions has disclosed the great plurality of HPVs (4) and the high frequency of association of specific viral types with invasive cancer (5, 9) and cervical intraepithelial neoplasia (CIN) (6). HPV DNA sequences are found in more than 90% of invasive cervical cancers , (2-7) and their role in the development of cervical neoplasia is well established (8). Experimental finding have shown that the E6 and E7 viral oncoproteins, co-expressed in the majority of HPV-associated carcinomas, (9) are the main determinant in the induction and maintenance of the malignant phenotype (10, 12). Immunotherapy targeted to these proteins may provide an opportunity to prevent or treat HPV-associated malignancies (11). However, the diversity of HPVs may be a limiting factor in this approach as more than twenty different HPV genotypes have been associated with cervical carcinoma, with the HPV 16 and 18 types the most frequently detected (7). HPV have been classified into three groups according to oncogenic potential (12). The low risk HPVs (6/11, 42, 43, 44) are commonly present in low-grade CIN but rarely in invasive cancers; the intermediate risk HPVs (31, 33, 51, 52 and 58) are more prevalent in CIN than in invasive cancers; the high risk HPVs (18, 45 and 56) are found more frequently in invasive cancers than in CIN.

Molecular diagnostic methods for the identification of HPV DNA include insitu hybridization, hybrid capture test and polymerase chain reaction. The selection of method is influenced by considerations such as characteristics of the sample, sensitivity required and the cost. HPV DNA testing would be a clinically useful diagnostic method, when used in conjunction with the PAP smear in population screening or in conjunction with cytology and colposcopy to identify women infected with high-risk HPVs or women who have equivocal cervical lesions. The in situ hybridization (ISH) technique has detected HPV DNA in 50% to 100% of cases of squamous cell carcinomas of the cervical canal (2, 6). Investigations should be directed at more accurately delineating its role in human health care (2, 10).

General characteristics of histopathology of genital HPV infections are the typical findings of condyloma accuminta showing exophytic growth which can be seen with the naked eye, flat condyloma seen through colposcopy, microscopic koilocytosis showing pre nuclear halo in the epithelial cells and dyskaryosis with nuclear pyknosis.

The aim of the observations reported here was to determine whether the prognosis for invasive cancers of the uterine cervix is related to the type of human papilloma virus associated with the tumor.

Material and Methods

The study group consisted of 20 patients with primary invasive carcinoma of the cervix treated at the Department of Pathology and Gynecology, Imam and Mirza Medical Center, Tehran, Iran between October 2000 and December 2001. This group, which represented 42% of the women treated for a cervical cancer during the same period, was composed of all patients from whom a biopsy sample of tumor tissue for HPV typing had been taken before treatment. Patient details, medical history and histopathology reports were available from the patients files. For 14 patients (70%), the tumor size was less than 4cm. Histological assessment of formalin-fixed, paraffin-embedded tissue blocks showed that 13 tumors (65%) were squamous cell carcinomas (SCC) and 7 (35%) were adenocarcinoas (AC). The patients ranged in age from 22 years to 71 years (mean 45.4 years, standard deviation 14.8 years). At the time of diagnosis, hematoxylin and eosin-stained tissue section were examined to identify areas of the tissue blocks with a high proportion of tumor cells.

* *Insitu Hybridization*

Insitu hybridization was performed according to the manufacturer s protocol, using three different biotin-labelled DNA probes that recognize HPV types 6/11, 16/18, and 31/33/51 (Dako Inc). A positive control

probe for human genomic DNA and a negative control for unrelated DNA sequences were included in each hybridization assay. Briefly, the method includes incubation of deparaffinized tissuesections in digestion reagent at 37°C for 2 hours. Following hybridization the sections were reacted with an alkalinephosphatase-antibiotin antibody conjugate, developed using nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate, and light counter stained with nuclear fast red staining (Figure 1).

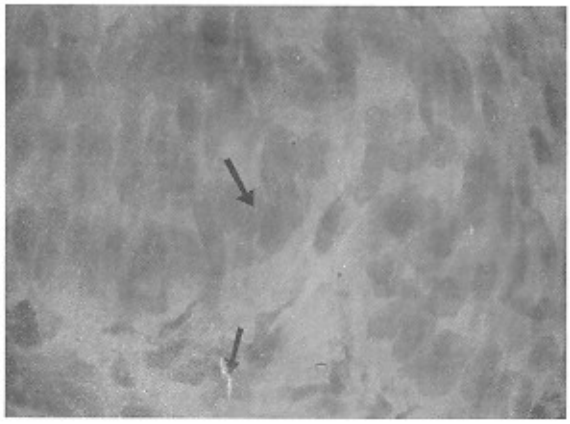

Figure 1: Positive Hybridization of a tumor tissue section (Red nucleus).

* *Statistical Analysis*

The statistical significance of the prevalence of HPV, as detected by ISH, relative to patient age, sex, histologic type, and grade of tumor was estimated using both a two samples t-test and Fisher sexact test.

Results

PV-DNA was detected in the nuclei of tumor cells in 13 of 20 patients. Three cases reacted only with the 31/33/51 probe, two reacted only with the 16/18 probe, three showed strong hybridization for both 31/33/51 and 6/11 probes, four showed 6/11 and 16/18 genotypes and one case reacted with 31/33/51, 6/11and16/18 probes (Table 2).

Significant correlation was found between the detection of HPV-DNA, patient age, histological type and grade of the tumor. The pattern of nuclear staining for insitu hybridization positive cases was not related to the histological type or grade of tumor.

Table1-Distribution of the reported Stage of SCC in the biopsy specimens

SCCS tage	No	Percent
Stage 1	4	30.7
Stage 2A	3	23.1
Stage 2B	5	38.5
Stage 3-4	1	7.7
Total	13	100

Table 2: The responses to HPV DNA probes of specimens from 13 cases of SCC

HPV DNA Probe(s)	31/33/51	16/18	31/33/51 6/11	6/11, 16/18	31/33/51, 6/11 16/18
Positive cases	3	2	3	4	1

Discussion

Cytology and colposcopy are still the most important tools in the diagnosis of cervical cancer and their results can be supported by cervical histo-pathology. The role of HPV typing is as an adjunct to cytology, colposcopy and histology in the early diagnosis of cervical cancer. At present the technical complexity of HPV DNA typing is a disincentive to its wider use. Nevertheless, it is believed that HPV typing will take a greater role in early detecting, diagnosing and prevention of cervical cancer. Our study was limited to the analysis of a small number of cases, precluding the realistic possibility of determining significant clinical correlation with the detection of HPV-DNA. As HPV infection of the cervix is generally regarded as a sexually transmitted disease, it seems reasonable to hypothesize that cervical carcinoma, because of it association with HPV, also has the epidemiologic features of a sexually transmitted disease. It is believed that HPV typing will take a greater role in early detecting, diagnosing and prevention of cervical cancer.

References

1. Smund Berner AA, Holm Ruth: HPV 16 Infection in a patient with two primary squamous cell carcinomas of the uterine cervix and the anal mucosa. APMIS 1997; 105: 207-212

2. Dybikowsk A, Licznerski Pawel, Poolhajska Anna: HPV detection in cervical cancer patients in northern Poland; Oncol Reports 2002; 9: 871-874

3. Bradley J, Guadi, Annue yang BA: Expression of the P53 homologue P63 in Early cervical neoplasia: Gynecologic

Oncol 2001; 80: 24-29
4. Svare EI, Kjaer SK, Smits HL: Risk factors for HPV detection in archival Pap smears. A population-based study from Greenland and Denmark. European Journal of Cancer 1998; 34: 1230-1234
5. Diana L: Schnider Rolando Herrero, Cervicography screening for cervical cancer among 8460 women in a high-risk population. Am. J Obstetrics and Gynecol 1999; 180: 290-298
6. Harnish DG, Belland LM: Evaluation of human papillomavirus consensus primers for HPV detection by the polymerase chain reaction. Mol Cell Probes 1999; 13: 9-21
7. Low Glass AG: Detection of human papilloma virus DNA in cytologically normal women and subsequent cervical squamous intraepithelical lesions. J Natl Cancer Inst 1999; 91: 954-960
8. Marc T, Goodman Katharine Mc Duffie: Gyp 1A1, GSTM1, and GSTT1 polymorphisms and the risk of cervical squamous intraepithelial lesion in a multiethnic population. Gynecol Oncol 2001; 81: 263-269
9. Octavian Lungu, Xiao Wel Sun, Wright TC: A polymerase chain reaction-Enzyme-Linked Immuno sorbent Assay Method for Detecting Human papillomavirus in cervical carcinomas and high-grade cervical cancer precursors. Obstetrics and Gynecol 1995; 85: 337-342
10. Finan RR, Irani-Hakime N, Tamim H: Detection of human papillomavirus (HPV) genotpes in cervico-vaginal scrapes of woman with normal and abnormal cytology. Clinical Microbiol and Infection (ESCMD) 2001; 7: 688-692
11. Kado S, Kawamata Y: Detection of human papillomaviruses in cervical neoplasia using mutiple sets of generic polymerase chain reaction primers. Gynecol 2000; 10: 47-52
12. Namkong SE: Clinical application of HPV typing in cervical cancer; Int. J Gynecol & obstetrics 1995; 49 suppl 457-459