

Prevalance Of Human Papilloma Virus In Squamous Cell Carcinoma Of Uterine Cervix

Pralhad Kushtagi, Subrato Sarkar,

Department of Gynecology, Kasturba Medical College, Manipal - 576 119. India

Summary: Human Papilloma virus (HPV) assay in 53 formalin fixed paraffin embedded biopsy specimens of squamous cell carcinoma of uterine cervix was carried out by in situ deoxyribonucleic acid (DNA) hybridisation using Digene HPV tissues hybridisation kit (Vira Type in situ HPV Probe Set : Catalogue number 42060100. Digene Diagnostics Inc., USA). Seven types of HPV were used as DNA probes. Eleven of the samples (20.8 percent) were positive for HPV. Types 16 and 18 present in 10 samples (18.9 percent) types 6 and 11 in 3 (5.7 percent) and types 31,33 and 38 in 1 (1.9 per cent). Three samples had multiple infection. Follow up outcome as related to the disease status in 11 HPV positive cases when compared to the matching (age, parity, stage, and degree of differentiation) controls was higher (27.3 percent to 9 percent) at 6 months, but showed no difference thereafter throughout the followup till 36 months of planned radiotherapy.

It is concluded that the low incidence of HPV in carcinoma cervix could be due to regional variations in occurance and that no opinion can be drawn regarding the influence of HPV genotype on clinical outcome.

Introduction

Human papilloma virus (HPV) induced lesions in the female genital tract are frequently associated with cervical intraepithelial neoplasia and invasive squamous cell carcinoma (Meisels and Fortin, 1976). Currently, 60 different types of HPVs have been detected. HPV 16 and 18 types are associated with the majority of cervical carcinoma and high grade dysplastic lesions. But, variable rates of presence of HPV have been reported in invasive carcinoma of uterine cervix (Durst et al, 1983; Walker et al 1989 ; Ichimura et al, 1989 and Labeit et al, 1992). Presence of a HPV genotype is said to influence the outcome of cervical cancer independent of other prognostic factors (Walker et al, 1989).

The present study was carried out to find the prevalence of HPV genotypes in specimens from women with squamous cell carcinoma of the uterine cervix and to examine the course of disease in patients whose tissues showed HPV genotypes.

Material and Methods

Case files of women with carcinoma cervix admitted at Kasturba Hospital, Manipal, from January through December 1991 were studied. The cases who had at least 6 months of followup after the optimal radiotherapy were short listed. Formalin fixed paraffin embedded blocks of cervical biopsy of these cases were retrieved from the

Department of Pathology.

Single 5-10 mm section cut from the blocks were obtained. The section cuts were tested for HPV antigens by the in situ deoxyribonucleic acid (DNA) hybridisation using Digne HPV Tissue Hybridisation Kit (Vira Type in situ HPV Probe set; catalogue number 42060100. DIGENE DIAGNOSTICS INC, USA) with the cooperation of Medical College of Ohio at Toledo, USA. The assay employs relatively low stringency conditions.

All the sections were viewed to determine if the HPV type present within a positive specimen belongs to group HPV 6 and 11, HPV 16 and 18 or HPV 33, 31 and 35.

A drop of Detection Agent* was added to the tissue section and incubated at 37° C for 20 minutes. Slides were then washed in three 200 ml changes of buffer 3* for 3 minutes each and were later reincubated at 37° C for 60 minutes. Washed slides in 3 changes of deionised water were conterstained by immersion in Nuclear Fast Red Stain* for 30-60 seconds, again washed in 3 changes of deionised water and then the sections were dehydrated in 95 % ethanol for a minute followed by absolute ethanol for 1 minute. These sections were then cleared for xylene for 1 minute, mounted with permanent mounting medium and read with standard light microscope.

[(*) provided with the kit]

The presence of HPV-DNA was visualised as purplish

blue colour and the tissues which did not contain HPV-DNA was pink as a result of counterstain. These colour changes were read against the positive and negative DNA control probes, respectively.

HPV type was determined based on the probe group generating the strongest and most abundant signal. If signal was equally strong for two of the HPV probe groups and was found in nonoverlapping areas of the epithelial regions, it was considered as true multiple infection.

In an attempt to explore the role of HPV positivity as prognostic indicator in carcinoma cervix, follow-up disease status was studied. For the HPV positive cases, controls were drawn from HPV negative cases matching in age and parity of the patient, FIGO stage and histological differentiation of the disease. The followup survival with or without the disease was studied in these two groups. The case was considered to be diseasefree, if there was no evidence of local disease (or disease at metastatic sites) clinically, cytologically on pap smear examination and histopathologically on biopsy.

Results

In all 53 adequate formalin fixed, paraffin embedded tissue sections could be retrieved and subjected to in situ DNA hybridisation. Eleven of these samples (20.8 percent) were positive for HPV. HPV types 16 and 18 were present in 10 samples (18.0 percent) types 6 and 11, and the lone samples positive for types 31, 33, and 38, also

showed positivity for types 16 and 18.

Detailed information regarding follow up was available in 50 of 53 HPV reported cases. The mean values for the 50 cases were for age 52.6 (SD 2.2). Twelve of the 50 women (24 percent) were premenopausal and 4 of them (8 percent) were less than 40 years of age.

All the 50 tissue biopsies were from squamous cell carcinoma (early stage 3, stage IIB 9, Stage III 35 and Stage IV 3), 3 of them histologically well differentiated, 40 moderately differentiated and 7 with poor differentiation.

Followup outcome as related to the disease status in 11 HPV positive cases when compared to the matching HPV negative controls was higher (27.3 per cent HPV positive and 9 per cent HPV negative) at 6 months and showed no difference thereafter till the last reported followup at 36 months (Table I).

Discussion

Carcinoma of the uterine cervix is a most common malignancy affecting Indian women (National Cancer Registry 1988). But the prevalence of HPV-DNA in 53 tumours analysed by in situ hybridisation in this study was low viz 20.8 percent. In contrast, Walker et al (1989) using Southern blot hybridisation, found HPV-DNA in 64 of 100 tumors studied. Whereas, Ip et al (1992) could detect DNA of HPV types 16 and 18 in 37 of 45 (82 percent) young

Table I

Carcinoma Cervix Disease Status at Followup and Human Papilloma Virus (HPV) Positivity

Groups/ Folowup (in months N=	HPV Positive				HPV Negative			
	Disease Free		With disease		Disease free		With disease	
	N	(%)	N	(%)	N	(%)	N	(%)
6* N=11	8	(72.7)	3	(27.3)	10	(91.0)	1	(19.0)
12 N = 10	7	(70.0)	3	(30.0)	6	(60.0)	4	(40.0)
24 N = 10	10	(100)	-	-	(10)	(100)	-	-
36 N = 10	8	(80)	2	(20.0)	7	(70.0)	2	(20.0)
					(one death not due to disease)			

*One patient did not present for followup after a visit at 6 months of radiotherapy.

patients under 45 years of age by polymerase chain reaction. Durst et al (1983) noted HPV-DNA sequences in 61.1 percent of 18 German patients and only 34.8 percent of 23 cancer biopsy samples from Brazil and Kenya. These divergent prevalence figures note in different series could be due to different techniques used to analyse the presence of HPV. It could also be due to geographical variations. It is also possible that HPV may not be the major etiological factor for the malignant transformation in this geographical region. However, other known social, environmental and sex behavioural factors have not been controlled in this study. And, more cases need to be studied to arrive at any conclusion in this regard.

As in other studies, HPV types 16 and 18 were the predominant group (10 of 11), when present, in tissues of carcinoma cervix. Though, the followup at 6 months showed higher persistent disease in HPV positive cases, subsequently, with timely adjuvant/additional treatment there was no difference with respect to persistent and/or recurrent diseases status in HPV positive and negative patients (Table I). This observation is similar to that made by Ip et al (1991), who could not demonstrate the prognostic influence for HPV after careful statistical analysis in their study. But, Walker et al (1989) are of the opinion that presence of HPV genotype can influence the clinical outcome of women with cervical cancer. With only 11 HPV positive cases in the present study, any attempt to

deduce regarding the influence of HPV on disease outcome will be premature.

Epidemiological studies are required: for compiling prevalence of HPV genotypes in cancer cervix in different regions, using a standard technique; for eliciting casual relationship of HPV, controlling known influencing factors for the disease; and for studying the course of disease in HPV positive cases.

References:

1. Durst M, Gissmann L, Ikenberg H, Zur Hausen H. Proc Natl Acad Sci USA 80 : 3812, 1983.
2. Ichimura H, Yamasaki M, Tamura I, Kurimura O, Kurimura T, Gan No Rinscho 35(10) : 1133, 1989 (Japanese publication. Abstracted in English on Medline)
3. Ip EW, Collins RJ, Srivastava G, Cheung AN, Wong LC. Int J Gyn Pathol 11: 258, 1992.
4. Labeit D, Baek W, von Weizsacker F, Herman P, Melchert F, Leonard K, Labeit S. J Med Virol 36: 131, 1992.
5. Meisels A, Fortin R. Acta Cytol 20: 505, 1976.
6. National Cancer Registry. Annual Report. Indian Council of Medical research. New Delhi, p 4-20, 1988.
7. Walker J, Bloss JD, Liao S-Y, Burman M, Bergen S, Wilczynski SP. Obst Gyn 74: 781, 1989.