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# Human papillomavirus related diseases in Malaysians

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*Abstract*

The surge of information on the aetiological association of the human papillomavirus (HPV) with some epithelial tumours emanating from various centres has prompted the initiation of a large-scale retrospective study at the Department of Pathology, University Hospital Kuala Lumpur to determine the prevalence and importance of this virus in some epithelial tumours of Malaysian patients. A retrospective analysis of 100 cases of large cell non-keratinising carcinoma of the uterine cervix by in-situ hybridisation on archival formalin-fixed, paraffin-embedded tissue has revealed the presence of HPV type 16 in 47% and type 18 in 41% of cases. This gives an overall detection rate of 88% of the two HPV types most commonly encountered in cervical carcinomas. Except for the unusually high frequency of HPV 18 detected in the cases, the overall prevalence is comparable to that reported in studies from most other centres. Although this higher frequency of HPV 18 may be due to geographical variation, the selection of the large cell non-keratinising type of squamous cell cervical carcinoma for study remains a possible reason for this phenomenon. In comparison to cervical carcinomas, HPV appears to be uncommon in penile carcinomas and HPV 6 was detected in only 1 of 23 cases studied.

*Key words:* Human papillomavirus, retrospective study, in-situ hybridisation, carcinoma of the cervix, carcinoma of the penis.

## INTRODUCTION

Human papillomavirus (HPV) is being increasingly alluded to as the aetiological agent in certain epithelial tumours, in particular cancers of the anogenital region. HPV has also been detected in some upper aerodigestive tract neoplasms and skin tumours.[1] While the presence of HPV in various tumours has been widely reported in the West, there has been no published data available in Malaysians. In an attempt to ascertain the status of HPV in some epithelial tumours of Malaysian patients and its likely implications we have embarked on a large-scale retrospective prevalence study based on in-situ DNA hybridisation. To date, preliminary studies on carcinoma of the cervix and squamous cell carcinoma of the penis have been carried out.

## Carcinoma of the cervix

In the 10-year-period between January 1983 and December 1992, 513 cases of cervical intraepithelial neoplasia (CIN) were histologically diagnosed at the Department of Pathology, University of Malaya. Of these, CIN 3 constituted 58% of the cases, CIN 1 26% and CIN 2 16%. Although, it may appear that CIN 3 was seen more commonly than the milder degrees of intraepithelial neoplasia, these figures should be interpreted in the light that a large number of cases with lesser grades of CIN were not subjected to biopsy examination. 75 cases of microinvasive and 681 cases of invasive carcinoma were diagnosed in this same period. Large cell non-keratinising squamous cell (LNK) carcinoma was the most common histological type encountered and constituted 61% of the cases. Using a non-isotopic DNA in-situ hybridisation technique, 100 cases of the large cell non-keratinising variety of squamous cell carcinoma were studied for the presence of HPV types 16 and 18. All cases were retrieved from the files of the Department of Pathology, University Hospital, Kuala Lumpur and re-confirmed histologically before admission into the study. The cases were selected for study according to the sequence of retrieval of the paraffin blocks from the store.

### *In-situ hybridisation technique*

In brief, the in-situ hybridisation method employed entailed unmasking of tissue nuclear DNA prior to hybridisation, by treatment with 0.02N HCl and Triton X-100 and proteinase K digestion.[2] DNA was denatured by heating in distilled water at 95°C for 40 mins. The digoxigenin-labelled genomic HPV probes were

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also denatured at 95%. The denatured probes diluted in hybridisation buffer, were applied to the tissue sections and the DNA of the tissues and probes were denatured a second time at 90°C for 10 mins.[3] Hybridisation was allowed to proceed at 42°C for 16 hours. High stringency washing conditions were used post-hybridisation to minimise non-specific annealing of probe and target DNA. Following the stringency washes, alkaline phosphatase linked anti-digoxigenin was added. Anti-digoxigenin binds to the digoxigenin-labelled probe hybridised to HPV DNA in the tissue. This reaction was visualised as bluish purple intranuclear dots via a colorimetric reaction between alkaline phosphatase and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Positive and negative controls were run with each batch of tests. CaSki and HeLa cells (ATCC) grown and processed into cell blocks served as positive controls for HPV 16 and 18 respectively. A case of normal cervix from a hysterectomy performed for a leiomyoma served as negative control. In addition, digoxigenin-labelled pBR 328 was substituted for probe and run as a negativecontrol for each run to ensure against non-specific staining.

HPV 16 was detected in 47 of the 100 cases of LNK studied while HPV 18 was detected in 41 of the cases, making an overall detection rate of 88%

**Squamous cell carcinoma of the penis**

In addition to HPV 16 and 18, HPV 6 and 11 were also included in the panel of probes used in the study of cases of squamous cell carcinoma of the penis. Using the in-situ HPV DNA hybridisation technique described previously, 1 of 23 cases of squamous cell carcinoma of the penis diagnosed between May 1980 and April 1989 demonstrated positivity for HPV 6. The rest of the cases were negative for all 4 HPV types. Cases of laryngeal papilloma, previously shown to be positive for HPV 6 and 11, served as positive controls.

## DISCUSSION

Briefly, HPV is a closed, circular, double-stranded DNA virus with a genomic length of about 8 kb pairs. On a recent count, no less than 70 types and many subtypes of HPV have been identified.[4] HPV is classified into types based on the degree of nucleotide sequence homology. The designation of a new isolate into a different type requires that there be less than 50% homology with known types. A new isolate with more than 50% homology but, nevertheless a different restriction enzyme cleavage pattern from a known type is categorised as a subtype. Clinically, the importance of typing lies in the observation that different HPV types are known to be associated with tumours from different sites and also at a particular location with tumours exhibiting a benign or malignant behaviour. In the uterine cervix, HPV 16 and 18 constitute the two types most commonly associated with malignant neoplasms whereas types 6 and 11 are more often detected in benign condylomatous lesions. The overall detection rate of HPV in our cases of carcinoma of the cervix of 88% is comparable with that reported from a earlier study conducted in Singapore based on dot blot hybridisation and Southern blotting in which the authors reported a detection rate of 72%; HPV 16 being detected in 52% and 18 in 15% of their cases.[5] The overall detection rate however rose to 96% when the same cases were subjected to polymerase chain reaction (PCR) amplification. The overall rate of detection of HPV in our study also parallels those reported from several other centres which range from 60-90%.[6-8] However, it has to be cautioned that the rate of detection is very much dependent on the sensitivity of the detection system employed, as can be appreciated from the Singapore experience. Notwithstanding the above, HPV appears to be encountered frequently in carcinoma of the cervix of Malaysian patients and at a rate similar to that found in women from other parts of the world. The reason for the higher frequency of HPV 18 is however unclear. It may entirely be due to geographical variation. Nonetheless, it has been reported that HPV 18 is more frequently associated with adenocarcinomas.[9] The possibility remains that large cell non-keratinising carcinoma resemble adenocarcinomas mare than conventional squamous cell carcinomas in their HPV type association. This would not be altogether surprising since as a histological group large cell non-keratinising carcinoma straddles between classical keratinising squamous cell carcinomas and adenocarcinonias.

In comparison with carcinoma of the cervix, HPV appears not to be commonly associated with squamous cell carcinoma of the penis in Malaysian patients. We however cannot be sure whether a true low prevalence of HPV prevails or whether penile carcinomas are associated with HPV types that have not been tested in our centre. Also, HPV may occur in such low copy numbers in penile carcinomas that detection by

in-situ hybridisation is not possible without amplification procedures.

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