DISCUSSION

Cervical cancer is the second most common cancer in women worldwide. Although the Papanicolaou (PAP) test is the mainstay of cervical cancer screening, the examination needs an expert cytologists to justify the results and it is also difficult to predict the regression or progression of the patient's stage by once a test. However, the study that compared the results of cytological and histological examination with HPV infection revealed that in HPV positive samples diagnosed histologically as CIN2/3 lesions, 44% had negative cytology and 22% showed only borderline or mild cytological changes (Cuzick *et al*, 1995). This indicated that detection of HPV could at least identified a substantial number of cytology negative women as a high-grade diseases. This study used a sensitive PCR technique with L1 primers to detect HPV DNA in cervical samples with different cytological and histological abnormalities including LGSIL, HGSIL and invasive carcinomas as well as the cytological normal cervix. The HPV DNA was detected in only 5% of LGSIL cases and none in the cytological normal cervix. Although the detection rate here is quite low as compared to other studies, this could be due to several factors including the limited number of samples obtained in each group and the methods used for sample collection. The scraping method is to generally scrape at the exocervix, which may not relate directly to the lesions. In addition, the samples that are obtained by scraping, contain mostly superficial exfoliated cells, but not latently infected basal cells. Although the scraping method is not invasive, as compared to biopsy, this process might be a major drawback in the sensitivity of HPV detection in normal and preinvasive cervical samples obtained this way. Another explanation may be related to the clinical apparent of women whose samples were collected. Among the 60 samples diagnosed as LGSIL, 53 had cytological apparent inflammation and 7 indicated mild dysplasia. The HPV DNA were detected in 3 of those 7 dysplasia, but none were observed in the samples with inflammation. When compared to LGSIL, the HPV detected in HGSIL (29.5%) and invasive stages (73%) was higher and increased with the severity

of the disease. However, infection by HPV is believed to be a necessary precursor of cervical neoplasia. Thus, women who harbor HPV are at risk of developing cervical abnormalities that subsequently lead to malignant lesions. The results from a 5-year follow-up study of HPV infection in women with and without cytological abnormalities revealed that the progression of HPV positive women from normal cytology to CIN or cancer occurred at an annual frequency of 0.082%. This resulted in a lifetime risk of about 3.7% if an infected life span of 45 years was assumed (de Valliers *et al*, 1992).

In addition, different HPV types have differences in pathogenicity. Regarding their oncogenic potential, HPV types can be divided into 3 groups; high-risk , intermediate-risk and low-risk. Persistence and progression of cervical lesions have been reported to correlate with lesion severity and HPV-type infection. The high grade lesions such as HGSIL (CIN II, CIN III and CIS) and the high-risk HPV-type-infected lesions are more likely to persist or progress (Koutsky and Holmes, 1992; Nasiell *et al*, 1983; Nasiell *et al*, 1986). Whereas, the low grade lesions and low-risk HPV-type-infected lesions tend to regress over a period of time (Carmichael and Masken,1986). Thus, it is widely accepted at present that the typing of HPV from clinical samples may have some prognostic or therapeutic implication. For example, infection with high-risk HPV types might demand special attention with careful and more frequent examination, while infection with low-risk HPV types may safely follow the routine check-up schedule. This study used the PCR-based RFLP for the typing of HPV from cervical samples and found that HPV16 was predominate among the HPV types detected in both HGSIL and invasive carcinomas. The result of this study were also consistent with other reports (Torroella-Kouri M *et al*, 1998; van Muyden RCPA *et al*, 1999; Astori G *et al*, 1999; Sebbelov *et al*, 2000). It is interesting that HPV-18 and -35 were detected infrequently in HGSIL (6.6% each), but increasingly to 14.3% each in invasive carcinomas. This finding also agreed with the study by Kurman *et. al.* in 1988 that HPV18 was found in approximately 22% of invasive carcinomas and in less than 3% of CIN lesions (Kurman *et al*, 1988). These observations suggested that HPV-18 or -35 might associate with or rapidly progress to more aggressive forms of invasive cancer than other HPV types, including HPV16. This

suggestion was intensified by a multivariate analysis that patients with HPV18-associated tumors had a relative risk (RR) of death 2.4 times greater than that for patients with HPV16 and 4.4 times greater than that for patients with a tumor associated with a viral type different from HPV-16 and -18 (Lombard *et al*, 1998). Within the same study, the 5-year disease-free survival (DFS) rate of different HPV type infection was also analyzed; the DFS rate of patients with HPV18 containing tumors was 38% while the DFS rate of 58 and 100% was observed in patients with HPV16 and intermediate-risk HPV-associated tumors, respectively.

The over expression of E6 and E7 transcripts from the high-risk HPV type was necessary for malignant transformation *in vitro*. Several recent studies have revealed an active expression of high-risk HPV type E6/E7 ORFs in both preinvasive and invasive stages of cervical cancer. The active expression of E6/E7 transcripts in tumor cells are the consequence of the integration process of the viral genome. Upon integration, the viral E2 ORF is invariably deleted. In productive infection with an intact viral genome, the E2 protein inhibits the expression of E6/E7 ORF by suppressing the viral gene promotors, p97 or p105, located just downstream from the URR. So, loss of the E2 function can overexpress E6/E7 transcripts. In general, integration to the host genome is an essential process of the oncogenic viruses in the development of cancer.

The findings in this study also confirmed those previously reported. At least two subsets of the spliced E6/E7 transcripts corresponding to the E6*I and E6*II in HPV16 containing samples were detected. Moreover, in order to see whether the relative proportion between E6*I and E6*II associated with the tumor histology or whether it had any prognostic value in tumor development, the fluorescent labeled RT-PCR assay was used to analyze the relative amount of each transcript. The advantage of using this kind of assay was not only its high sensitivity, but also its quantitative capability in analysis. Here, it was found that in almost all HPV-16 positive samples, the E6*I, which putatively encoded the E7 protein, was synthesized to a higher amount than the E6*II that encoded the E6 protein. The relative proportion between E6*I and E6*II is ranging from 0.6 to 2.42. Although this study observed some differences in the relative proportion of E6*I and

E6*II transcripts among the groups of LGSIL, HGSIL and invasive carcinomas, it was not significantly different when statistically analyzed. It remains a possibility that the quantity of E6/E7 transcripts vary with tumor stages, particularly during the progression from low to high grade lesions and finally maintained at the invasive stage. Corresponding to the progression of those tumor stages is the gradual transition of the viral genome from the episomal to integrated form. This issue cannot be satisfactorily addressed at present, partly because most studies involved are limited to the number of samples with HPV containing low and high grade lesions, a short periods of follow-up and a sensitive quantitative assay that has not yet been widely adopted.

In the HPV18 containing samples, only one spliced transcript corresponding to E6*I was detected. This may be due to the nucleotide sequence of the E6 ORF, which was flanked between the primers used in the amplification process, containing only one splice acceptor site. This observation confirmed the earlier sequencing data that the HPV18 E6*I transcript was generated from splicing out the nucleotide between position 233 to 416. Those nucleotide positions represented the splice donor and splice acceptor sites, respectively. The difference in the splicing signals of these two high-risk HPV types might directly involve in the pathogenic potential of the viruses.

The results from this study, and those cited, support the belief that specific HPV type testing may be a helpful adjunct to routine cytology. Although the expression patterns, as well as the relative proportion of the E6 and E7 transcripts of the high-risk type HPV observed in this study, were found to be independent of the tumor histology, it is indicated that the E6 and E7 genes are transcriptionally active in those tumor stages. The hypothesis that E6/E7 proteins are necessary for the maintenance of the malignant phenotype was also confirmed.