RESEARCH COMMUNICATION

High Prevalence of Human Papillomavirus DNA Detected in Cervical Swabs from Women in Southern Selangor, Malaysia

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Abstract

Persistent high-risk human papillomavirus (HPV) infection is known to play an important role in the genesis of cervical cancer. Since new screening and prevention strategies, namely improved HPV testing and HPV vaccination have been aggressively promoted recently, it is crucial to investigate the HPV distribution in Malaysia in order to maximize their cost-effectiveness. This study was therefore conducted to assess the HPV type distribution in the most populous region, the state of Selangor. A total of 200 cervical swab samples were collected in two health-screening campaigns, and also from women attending obstetrics and gynecology clinics in several hospitals in Selangor. DNA extraction was performed and HPV DNA was detected via nested PCR using MY09/MY11 as outer primers and GP5+/GP6+ as inner primers which target the L1 gene of the viral genome. The purified PCR products were subjected to automated DNA sequencing to determine the HPV genotype. Out of 180 β-globin positive samples, 84 (46.7%) were positive for HPV DNA. The most common HPV type found was high-risk oncogenic type 16 (40%), followed by HPV type 18 (3.3%), HPV 33 (1.7%), HPV 31 (0.6%), and low-risk HPV 87 (0.6%). Our study confirmed that nested PCR method is highly sensitive in detecting HPV DNA even in low risk patients. Since a relatively high prevalence rate of HPV infection was found in this population, prompt healthcare policy changes to bring about implementation of early HPV vaccination program is desirable to prevent a high incidence of cervical cancer.

Keywords: Human papillomavirus (HPV) - cervical cancer - Malaysian women - nested PCR

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Introduction

Cervical cancer is a common malignancy in women and a main cause of high mortality rates due to cancer (Castellsague et al., 2006). In Asia, approximately 265,855 cervical cancer cases were reported and approximately 142,734 of the patients died from cervical cancer (Ferlay et al., 2004). In Malaysia, cervical cancer is the second most common cancer in females, comprising about 12.9%, or approximately 2000-3000 hospital admissions due to cervical cancer per year (Lim et al., 2004).

Although cytological screening using the Pap smear test is readily available in developed countries and contributes towards effective control of cervical cancer, in middle and low-income countries, cervical cancer is still the second most common cancer among women. Wide-coverage population-based Pap screening has contributed towards reduction in cervical cancer incidence and mortality in many developed nations. However, in Malaysia, the lack of awareness, resources and infrastructure especially in rural areas have led to low rates of screening and hence increase in cervical cancer incidence. It was estimated that only about 6% of women in Malaysia are screened (Wong and Sam, 2007). Cultural inhibitions and misperceptions regarding the disease as well as the screening procedure have also contributed to the resistance towards Pap tests.

Human papillomavirus (HPV) had been identified to be the most important etiologic factor for cervical cancer. Over 100 types of HPV have been identified and were subdivided into two groups namely the oncogenic HPV types and non-oncogenic HPV types. HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are considered as high-risk or oncogenic types (Munoz et al., 2003; Bell et al., 2007). The low-risk HPV types including types 6, 11, 42, 43 and 44 can cause papillary condylomas or classic genital warts (Muñoz et al., 2003). HPV 16 and HPV 18 are the most frequently detected types in populations with and without cervical cancer (Castellsague et al., 2006).

Globally, there is a wide range of HPV type distribution

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across different geographical areas and different ethnic populations (Clifford et al., 2005; Lizano et al., 2005; Bell et al., 2007; Sowjanya et al., 2007; Bao et al., 2008; Bardin et al., 2008). Recently, two different prophylactic HPV vaccines have been developed to prevent infections with some of the low and high-risk HPV types, with the goal of diminishing worldwide incidence of cervical cancer. These HPV vaccines, one a bivalent (types 16, 18) and the other a quadrivalent (types 6, 11, 16 and 18) vaccine were touted to have tremendous potential to reduce morbidity and mortality associated with cervical cancer (Schiffman et al., 1991; Kahn, 2005). It was estimated that the implementation of first generation HPV 16/18 vaccines will protect against almost 67% of invasive cervical cancer for women in Asia and might improve cost-effectiveness (Bao et al., 2008). Nonetheless, there is very limited data on distribution of HPV types among women in South-East Asia including Malaysia. Malaysia has a multi-ethnic population comprising of Malays (53.3%), Chinese (26%), Indians (7.7%) and indigenous non-Malays (11.8%), and others (1.2%). In this study, we decided to focus on Selangor, the most populous and developed state in Malaysia.

It is important to have relevant data on HPV distribution in specific areas in order to gauge the efficiency of the new vaccines since there is evidence of not only a wide variation (nearly 20 times) in overall HPV prevalence but also regional variation in distribution of HPV types across different areas of the world (Clifford et al., 2005). The present pilot study was undertaken to assess the prevalence of HPV DNA in women without cervical cancer in South Selangor, Malaysia using the nested PCR-direct sequencing method.

Materials and Methods

A total of 200 samples were collected from March 2007 until September 2007 from women who underwent Pap smear screening. The majority (n=148) of the samples were collected from the Gynaecology and Obstetrics Clinics in several hospitals in Southern Selangor The participating hospitals included Hospital Kajang, Hospital Serdang, and the Britannia Women and Children Specialist Centre. The remaining samples were collected from volunteers who responded to a Health Screening Campaign organized by the MAKNA Cancer Research Laboratory, Institute of Bioscience, UPM.

Ethics approval was obtained from Institute of Medical Research (IMR), Ministry of Health, Malaysia and also from Faculty of Medicine and Health Sciences Medical Research Ethics Committee, University Putra Malaysia. Informed consent was obtained from each woman, who had to undergo pelvic examination for collection of cervical cells and also answer a self-administered questionnaire regarding socio-demographic factors and sexual lifestyle. Inclusion criteria were Malaysian citizens with no history of hysterectomy and cervical cancer, were sexually active and were not currently pregnant. The age groups vary from 19 to more than 60 years old, but women in the youngest age group are under-represented as virgins were not included in the study due to cultural reasons.

Pelvic examination was done by a gynaecologist or a trained gynaecologic nurse. Two swabs were obtained from each woman, in which one was sent for cytology examination by certified cytopathologists whereas the other was placed in phosphate buffered saline (PBS buffer, pH 7) for HPV DNA testing.

Sample Preparation and DNA extraction

Cells were collected from the ectocervix and endocervix with a cotton swab and also with cytobrushes and placed into a urine container containing 5ml Phosphate Buffered Saline (PBS) solution and stored on ice. The samples were transported to the laboratory; where the containers were vortexed to dislodge any cells that adhered to the cytobrushes, and then transferred into falcon tubes and centrifuged for 20 minutes at 6000 rpm. The supernatant was discarded and 200 μl of PBS was added to resuspend the pellet and the resulting cell suspension were either processed immediately or stored at -30°C. DNA extraction was performed using QIAamp DNA Blood Minikit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Finally, 50 μl of AE Buffer (provided in the kit) was used to elute the extracted DNA.

Nested Polymerase Chain Reaction (PCR)

Nested PCR was performed using a combination of MY09/MY11 as outer primers and GP5+/GP6+ as inner primers which targeted the L1 gene of the viral genome. The primers used for the first round of PCR were MY09 (5-CGTCCMARRGGAWACTGATC-3') and MY11 (5'-GCMCAGGGWCATAAYAATGG-3') while the primers for the second round were GP5+ (5'-TTTGTTACTGTGGTAGATACTAC-3') and GP6+ (5'-GAAAAATAAACTGTAAATCATATTC-3') (Jacob et al., 1997). To avoid false negative results due to poor DNA quality or low DNA yield, β-globin PCR was performed which targeted the human β-globin gene. PC03 forward (5'-ACACAACTGTGTTCACTAGC-3') and PC05 reverse (5'-GCTAGTGAACACAGTTGTGT-3') primers were used for β-globin PCR. PCR mixtures with total volumes of 25μl contained 3μl of template DNA, 3.0 mM of MgCl2 (Promega, USA), 200mM of dNTPs (Promega, USA), 10 pmol of each primer (OperonV, Germany) and 10X PCR buffer (Promega, USA). The PCR mixtures were set up in a laminar flow workstation (Holten LaminAir) using aerosol-resistant micropipette tips, at an area distinct from the DNA extraction work area. HPV 18-positive HeLa cell line was used as positive control while for the no template control (NTC) which served as a negative control, sterile distilled ultra-pure water was used to substitute for the DNA samples. PCR amplification was performed using MJ research PTC-200 thermal cycler with initial denaturation at 95°C for 3 min, denaturation at 95°C for 30 sec, annealing at 55°C 30 sec, extension at 72°C for 45sec and final extension at 72°C for 5 min. The PCR reaction was carried out for 35 cycles. The PCR products were loaded onto 1.5% w/v agarose gel and viewed under ultraviolet light using Alpha Innotech Alphaimager®HP to verify the size of the bands. Genotyping of HPV for the nested-PCR positive samples from second round nested PCR was achieved via automated sequencing. PCR

products were purified from the gel using the QIAamp Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's instruction manual and the purified amplicons were subjected to automated DNA sequencing. Both the antisense and sense strands were sequenced separately for each amplicon and the aligned nucleotide sequences were analysed using BLASTn software (http://www.ncbi.nlm.nih.gov/blast/html).

Results

Prevalence of HPV infection in South Selangor women

Based on the meta-analysis of HPV type distribution in Asian populations, it was estimated that the overall prevalence of HPV across Asia is about 14%. Taking into account this prevalence rate, the minimum sample size for this present study was determined using the Cochran's formula (1977) and it was found to be 46. Despite various limiting factors such as logistics and financial constraints, as well as cultural inhibitions, we succeeded to collect 200 samples. Out of the 200 women who consented to participate in this study, the cervical cells from 180 participants were of adequate quality (β-globin positive) to proceed with PCR analysis. The mean age of women who participated in this study was 39 (SD = 9.58). The range of age in our study was 19 to 69 years old.

The women in this study were sexually active with no previous cytopathological diagnosis or treatment related to cervical neoplasia, and were undergoing cervical cancer screening. A total of 84 (46.7%) out of the 180 β-globin positive samples were positive for HPV DNA. The prevalence of HPV infection was found to be the highest in the 31-40 age group (61.3%) as compared to the 19-30 (45.9%) and 41-50 age groups (34.5%) as illustrated in Table 2. Seventy six out of 168 married women in the study were found to be infected with HPV whereas 8 out of 12 unmarried women consisting of single and divorced women were positive for HPV infection. Among the three ethnic groups, Malays accounted for about 51.6% of the HPV-positive specimens followed by Chinese (46.3%) and Indians (25%), (Table 2).

Table 1. Frequency of HPV Infection in Women in Southern Selangor According to Age Group, Marital Status and Ethnicity

		Number of Patients	Percentage (%)
Age group	19-30	17/37	45.9
	31-40	38/62	61.3
	41-50	20/58	34.5
	>50	9/23	39.1
Marital status	Married	76/168	45.2
	Unmarried	8/12	66.7
Ethic group	Malay	48/94	51.6
	Chinese	31/66	46.3
	Indian	5/20	25

Table 2. Frequency of HPV Infection and Distribution of HPV Genotypes in Women in Southern Selangor

		Number of Patients	Percentage (%)
High- Risk Oncogenic HPV type	16	72	85.7
	18	6	7.1
	31	1	1.2
	33	3	3.6
	58	1	1.2
Low to Intermediate Risk HPV type	87	1	1.2

Distribution of HPV Types Among the Positive Samples

Nested PCR products from the HPV-positive specimens were gel-purified separately and subjected to automated sequencing using the GP5+ and GP6+ primers for determining the sequences for both strands. The nucleotide sequences obtained from automated sequencing were compared for homology with the established gene sequences in the GenBank database. A Blastn result which corresponds to more than 94% of maximum identities to the available HPV types in GenBank was considered as significant. Remarkably, an alarmingly high proportion (98.8%) of the women was infected with high-risk oncogenic HPV types. As shown in Table 2, HPV 16 is the most predominant HPV type found in this study at 85.7% among all HPV-positive patients, followed by HPV 18 (7.1%), HPV 33 (3.6%), HPV 31 (1.2%), HPV 58 (1.2%), and HPV 87 (1.2%), respectively. HPV 16, 18, 31, 33, and 58 are high-risk HPV types. HPV 87, a rarely encountered HPV type which was considered to be of low to intermediate risk for development of cervical carcinoma, was detected only in 1 woman.

Table 3 shows that the identity of the gene in GenBank with the highest hit for each sample was distributed into several different isolates or variant subtypes within the same HPV type. For instance, there were 11 variant subtypes of HPV 16, 3 variant subtypes of HPV 18 and 3 variant subtypes of HPV 33 dentified. Thirty-two samples were identified as HPV type 16 isolate 114/K, followed by 18 samples which were HPV type 16 isolate CNE-q, 5 samples which were Type 16 isolate CNE-f, 2 samples which were Type 16 clone 17, 7 samples which were found to be HPV type 16 late major capsid protein L1 gene, 2 samples which were Type 16 isolate IR-ESO-HPV-16/15, and 1 sample each which was Type 16 isolate IR-ESO-HPV-16/14, Type 16 isolate 2, and Type 16 isolate PT

Table 3. Distribution of HPV Variant Subtypes Among the HPV-Positive Samples

Accession Number	Types Of HPV	Number of Samples	Identities		
			100%	>90%	>80%
gbEU118173.1	Type 16 114/K, complete genome.	31	31		
gb|EU430688.1	Type 16 isolate CNE-q L1 protein gene, complete cds.	18	14	4	
gb|EU430680.1	Type 16 isolate CNE-f L1 protein gene, complete cds	5	5		
gb|EF140826.1|	Type 18 isolate CC major capsid protein L1 gene, complete cds	4	4		
gb|DQ422774.1|	Type 16 clone 17 L1 protein gene, partial cds	2	2		
gb|AF548834.1|	Type 16 isolate 19 L1 gene, partial cds	1	1		
gb|DQ218253.1|	Type 16 isolate PVPOA3 major capsid protein L1-like gene, partial sequence	1	1		
gb|DQ448196.1|	Type 16 isolate IR-ESO-HPV-16/15 L1 gene, partial cds	2	2		
gb|DQ448195.1|	Type 16 isolate IR-ESO-HPV-16/14 L1 gene, partial cds	1	1		
gb|AF548817.1|	Type 16 isolate 2 L1 gene, partial cds	1	1		
gb|EF133498.1|	Type 16 isolate PT 62-05 L1 protein gene, partial cds	1		1	
gb|AF393502.1|	Type 16 late major capsid protein L1 gene, complete cds	7	7		
gb|AF043287.1|	Type 16 Phil late major capsid protein L1 gene ç complete cds	1	1		
gb|DQ448204.1|	Type 18 isolate IR-ESO-HPV-18/3 L1 gene, partial cds	1		1	
gb|EF140829.1|	Type 18 isolate N15 major capsid protein L1 gene partial cds	1	1		
gb|AF548851.1|	Type 31 isolate 5 L1 gene, partial cds	1			1
gb|AF548853.1|	Type 33 isolate 1	1		1	
gb|AF548855.1|	Type 33 isolate 3 L1 gene partial cds	1	1		
gb|DQ448214.1|	Type 33 isolate IR-ESO-HPV-33/2 L1 gene partial cds	1	1		
gb|DQ057326.1|	Type 58 isolate HK2178 L1 protein gene, partial cds	1	1		
emb|AJ400628.2|HPA400628	Type 87 (candidate)complete genome	1		1	
gb|AF190174.2|HHP2423S2	Homo sapiens/HPV type 16 strain 2423 3' junction sequence	1			1**

** AF190174.2 Homo sapiens/human papillomavirus type 16 strain 2423 3' junction sequence-- 87% identity

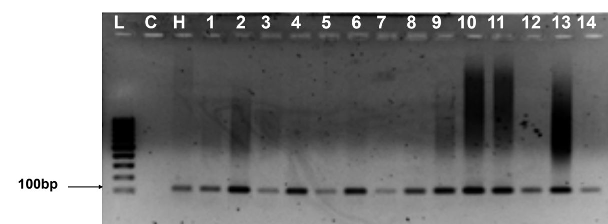

Figure 1. Representative Gel Electrophoresis Result of β-globin PCR. All samples which had undergone β-globin gene amplification gave positive results. The expected size of the band is 110bp. L - 100bp DNA ladder marker; C - nontemplate negative control; H - positive control (HeLa cell DNA); 1-14b - cervical swab samples

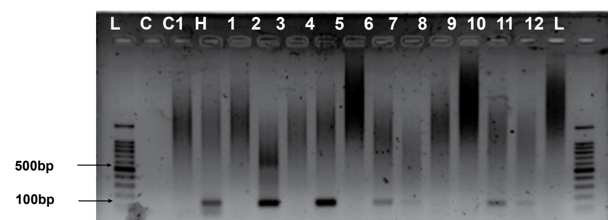

Figure 2. Representative Gel Electrophoresis Result of Second Round Nested PCR Using GP5+/6+ Primers. After the second round nested PCR, eight samples were identified as HPV-positive. The bands generated were between 100bp and 200bp, with the expected size of the amplicon being 140bp. L - 100bp DNA ladder marker; C - negative nontemplate control; C1 - negative control using the post-PCR mixture from C in the first round as the template; H - positive control (HeLa cell DNA); 1-12 - cervical swabs

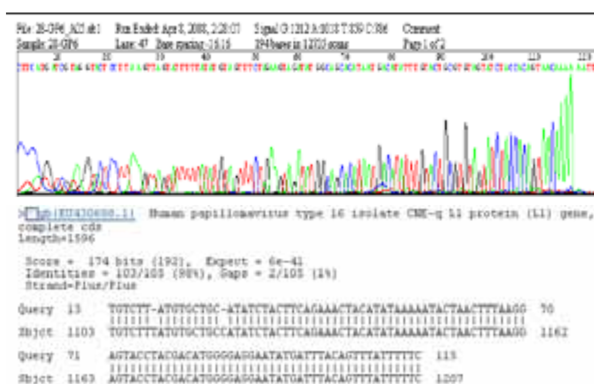

Figure 3. Representative Sequencing Chromatogram. The result from DNA sequencing and BLASTn analysis below revealed that sample no.2 contained HPV type 16

62-05. A total of 6 samples were found to be HPV 18. The most predominant isolate or subtype of HPV 18 was HPV type 18 isolate CC with 4 samples.

All 32 HPV type 16 114/K samples were 100% identical with the gene sequence in Genbank database. There were 14 samples of HPV type 16 isolate CNE-q L1 protein gene that displayed 100% identity to the GenBank entry whereas another 4 samples were >90 percent identical to the Genbank sequence. Most of the results were 100 percent identical and 7 samples were >90 percent identical. Only 2 samples had identities of <90% but >80%. These 2 samples were HPV type 31 isolate 5 (80% identity), and Homo sapiens/HPV type 16 strain

2423 3' junction sequence (87% identity) respectively.

Interestingly, 4 of the specimens were histopathologically classified as low grade squamous intraepithelial lesion (LSIL) or cervical intraepithelial neoplasia (CIN) stage 1; whereas 1 specimen was classified as CIN 3. All 5 CIN cases were positive for HPV, with the CIN3 case having highest similarity to HPV type 18 (isolate CC). Two of the CIN1 cases had highest similarity to HPV type 16 clone 114K, 1 was most similar to type 16 isolate IR-ESO-HPV-16/15; while 2 of the CIN1 cases had the highest homology to type 18 isolate N15.

Discussion

Malaysia is a multi-ethnic country with the largest group being the Malays whose ancestry can be traced to the surrounding Indo-Malaysian archipelago. This is followed by Chinese and Indians, many of them second or third generation descendants whose ancestors had emigrated from their original countries during British-colonised era. There are also the Non-Malay indigenous groups which comprise more than half of the populations in East Malaysia. Geographically, the country is separated by the South China Sea into two parts, namely peninsula Malaysia which is south of Thailand, and East Malaysia situated on Borneo island which comprises of Sabah and Sarawak. In this study, we decided to focus on Selangor, the most populous and developed state in Malaysia. Although the incidence rate of cervical CA in Malaysia is estimated to be about 13%; the actual figure may be higher, because many Malaysians choose to seek non-Western treatments such as herbal medicine, traditional Chinese medicine and ayurveda treatments when they are diagnosed with cancer. Education level and knowledge about HPV infection and its association with cervical cancer and genital warts is still very low in this country and the acceptance of the HPV vaccines still remains to be seen among the young adults (Wong and Sam, 2007).

An astoundingly high prevalence of HPV infection was discovered among the women that was screened in this study (46.7%), with a vast predominance of high risk HPV types 16 and 18 (combined incidence of 92%). To rule out the possibility of false-positive results, stringent controls were performed in the sampling and experimental procedures. These included the use of sterile disposable specula, separate swabs for cytological screening versus for HPV DNA detection, and use of sterile transport medium transported on ice to the laboratory. Each PCR reaction was performed in triplicates in a UV-exposed, alcohol wiped-down laminar flow using sterile aerosol-resistant filter tips, and the DNA extraction area was separated from the PCR work area as well as the gel electrophoresis area. The crucial steps in DNA extraction were also carried out using aerosol-resistant filter tips to avoid cross-contamination between samples.

Comparatively, a meta-analysis of HPV type distribution in Asian women (Bao et al., 2008) with normal histoloty/cytology found a lower overall prevalence of approximately 14%. It is worth noting that the Malaysian study quoted in that analysis was done more than 10 years ago and was limited to cervical carcinoma cases. Elsewhere in the world, Clifford and coauthors reported an overall HPV prevalence of 24.7% for Nigeria, 15.5% for Argentina, and 14.6% for Columbia (2005).

One postulate for the discrepancy between our prevalence rate with those reported elsewhere is the different sensitivity level of the methods used for HPV detection and typing. Most of the other studies employed a single round GP5+/6+ PCR, some with a subsequent probe hybridization or enzyme immunoassay for HPV typing. In our study, we used the nested PCR method with two rounds of PCR firstly with MY09/11 primers followed by GP5+/6+ in the second round. It was observed that for the HPV-positive samples, upon the first round PCR using MY09/11 primers, no PCR products could be detected visually through gel electrophoresis, indicating the low abundance of the viral copy number. Only a few samples had a slightly visible band after the first round PCR, and these were later revealed to be CIN1/3 samples through cytological screening. The fact that the HPV DNA was only detected in the second round nested PCR using GP5+/6+ primers strongly imply that there was low viral copy number in the cytologically normal samples possibly due to transient infection. Normally, HPV enters a quiescence period of 2-12 months upon infection, and in immunocompetent women with absence of triggers, the cell-mediated immunity is able to clear the infection. One study had emphasized the importance of continued HPV testing, whereby it was shown that the risk for developing cervical cancer precursor was 14 times higher for women with at least 3 times positive HPV test results compared with women who had negative HPV test results (Moscicki et al., 1998). Hence, the women who were tested HPV-positive in the present study were counseled regarding their relative risks of eventually developing cervical carcinoma, and were advised to return to the respective hospitals for more regular half-yearly to quarterly PAP screening and PCR for constant monitoring.

The pathogenesis of cervical carcinoma through HPV infection has been reviewed extensively. Persistent HPV infection, whereby the virus either exists as episomal form or integrated form in the human genome would lead to development of precancerous lesions (CIN1-3) of the cervix (Pagliusi and Aguado, 2004). High-grade lesions may result in invasive carcinoma when left untreated. Nonetheless, even the majority of infections with high-risk HPV types generally clear within 2 years, without progressing to cervical cancer. This suggests that HPV infection alone is not a definitive factor for cervical cancer; rather, a synergistic interaction between high-risk HPV types and other host or environmental cofactors (e.g. smoking, oral contraceptive use) is required for disease progression. Certain host genetic factors, such as polymorphisms or variations in specific human leukocyte antigens (HLA) and cellular immunity impairment due to parasitic infection and malnutrition have been implicated in the natural history of HPV infection (Gravitt and Jamshidi, 2005).

In the literature reviewed, prevalence of HPV infection generally decreases with age while the highest incidence is usually detected in women between 20-35 years; which is the sexually most active period (Clifford et al., 2005;

Sapy et al., 2008). The results of this study also suggest a similar trend of highest HPV incidence among the sexually active group of 31-40 years of age, followed by 19-30 years of age. However, Bell and coauthors (2007) reported the highest HPV infection rate in younger women of American Indian descent in the lower than 24 years old age group. It has also been reported elsewhere that the highest incidence for HPV occurs in adolescent females of 15-19 years of age (Figueroa et al., 2005). In Malaysia, it is very uncommon, even taboo for virgins among certain ethnic groups to undergo a gynaecological examination, much less a Pap test, hence the study was limited to women who were sexually active. Moreover, it is rare for unmarried girls to engage in sexual relationships, therefore women from the 19-25 age group is somewhat under-represented in this study.

In terms of HPV type distribution, the present study reported the highest incidence rate for HPV16/18 by far compared to other reports. In one study, women in Europe had a proportion of 25.5% HPV16, whereas in Nigeria, there was low proportion of HPV16 (12.3%) (Clifford et al., 2005). We did not find any HPV35 among our population sampled, but in sub-Saharan Africa HPV35 incidence was almost equal to that of HPV16 (Clifford et al., 2005). However, a few cases of other oncogenic types of HPV such as HPV31, 33 and 58 were detected among the women in our study. HPV-type distribution is rather heterogeneous in Asia, probably due to the wide range of geographical and cultural factors. The fact that our study utilized the MY09/11 primers which can apparently identify more HPV types compared to other primers and methods, especially low-risk types; but yet our results showed a large majority in proportion of high-risk HPV16/18 leads us to conclude that our population is truly susceptible predominantly to these two high-risk HPV types. However, a limitation of our study is that the nested PCR-direct sequencing method adopted is unable to accurately identify co-infection with multiple HPV types, unlike the PCR-hybridization based methods used in some other studies.

Several reported findings have led to the proposal that intratypic variants of HPV type 16 and 18 possess different biological properties (Berumen et al., 2001; Chakrabarti et al., 2004; De Boer et al., 2005; Stoppler et al., 2006). A study on a Mexican population found a specific variant of HPV16 was present exclusively in cancer cases but not in precancerous lesions (Lizano et al., 2005). More sequencing analysis on the samples in the present study needs to be conducted in order to determine the HPV type variants and to correlate this to the long-term progressive outcome of the patients.

Based on the results of the present study, among the future investigative directions to be taken include i) to quantitate viral load and evaluate the importance of viral copy number to precancerous and cancerous lesions; and ii) to study the host genetic factors in patients with invasive cervical carcinoma.

In conclusion, this study has provided baseline data on the HPV type distribution in the population, the first-ever study in cytomorphologically normal individuals in this country. Thus, it provides information that is critical in order to ensure that a population-based vaccination program, if introduced in the future, is feasible and cost-effective. Information on the frequency of HPV types is also crucial for estimating the effectiveness of the currently available prophylactic vaccines in Malaysians and it may provide useful supporting information for healthcare policy makers in their deliberation on implementing mass-screening programmes.

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