RESEARCH ARTICLE

Distribution of HPV Genotypes in Cervical Cancer in Multi-ethnic Malaysia

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Abstract

Background: Cervical cancer is the third commonest type of cancer among women in Malaysia. Our aim was to determine the distribution of human papilloma virus (HPV) genotypes in cervical cancer in our multi-ethnic population. Materials and Methods: This was a multicentre study with a total of 280 cases of cervical cancer from 4 referral centres in Malaysia, studied using real-time polymerase chain reaction (qPCR) detection of 12 high risk-HPV genotypes. Results: Overall HPV was detected in 92.5% of cases, in 95.9% of squamous cell carcinomas and 84.3% of adenocarcinomas. The five most prevalent high-risk HPV genotypes were HPV 16 (68.2%), 18 (40%), 58 (10.7%), 33 (10.4%) and 52 (10.4%). Multiple HPV infections were more prevalent (55.7%) than single HPV infections (36.8%). The percentage of HPV positive cases in Chinese, Malays and Indians were 95.5%, 91.9% and 80.0%, respectively. HPV 16 and 18 genotypes were the commonest in all ethnic groups. We found that the percentage of HPV 16 infection was significantly higher in Chinese (75.9%) compared to Malays (63.7%) and Indians (52.0%) ($p<0.05$), while HPV 18 was significantly higher in Malays (52.6%) compared to Chinese (25.0%) and Indians (28%) ($p<0.05$). Meanwhile, HPV 33 (17.9%) and 52 (15.2%) were also more commonly detected in the Chinese ($p<0.05$). Conclusions: This study showed that the distribution of HPV genotype in Malaysia is similar to other Asian countries. Importantly, we found that different ethnic groups in Malaysia have different HPV genotype infection rates, which is a point to consider during the implementation of HPV vaccination.

Keywords: Cervical cancer - distribution of HPV - human papillomavirus - HPV genotyping - Malaysia

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Introduction

Cervical cancer is the second commonest cancer in women worldwide; with more than 490,000 diagnosed each year (Ferlay et al., 2002). About 265,885 (54%) cases occur in Asia, posing a major health problem for women in worldwide (Ferlay et al., 2002). In Asia, it is the second commonest cancer in Vietnam and Thailand, and the third commonest cancer in Indonesia and Malaysia (Ferlay et al., 2008; Zainal et al., 2011). In comparison to Western countries and other South East Asian countries like Indonesia, Philippines, Vietnam, Brunei and Singapore, Malaysia has a higher incidence of cervical cancer (Ferlay et al., 2008).

The geographical preferential differences in human papillomavirus (HPV) genotypes have been reported, where different genotypes exist in different countries, and even in different areas of the same country (Munoz et al., 2003; Wheele et al., 2006; Smith et al., 2007). The distribution of HPV genotypes in Asia is heterogeneous, probably because of the broad geographical and cultural diversity of Asian populations (Clifford et al., 2006). For example, a high prevalence of HPV 58 (12.5%) previously identified in normal cervical cytology samples of women in Taiwan which was also observed in the East Asian populations (9.7%). However in India, the prevalence rate was lower (1.2%) (Liaw et al., 1997; Clifford et al., 2005).

Human papillomavirus (HPV) has been established as a precursor of cervical cancer. HPV prophylactic

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vaccination hold great promise in reducing the global burden of cervical cancer with several countries already implementing this programme in school children including Malaysia (Harper et al., 2004). Thus, the detection of HPV genotypes has become the main focus of cervical cancer prevention strategy (Schiffman et al., 2005). Previous studies of multi-centre and meta-analyses provided information about HPV type distribution in Asia. However, results from the Asian region were limited in terms of geographic coverage and status of the cervical lesion (Clifford et al., 2003; Munoz et al., 2003).

Information on the HPV genotype status in Malaysia is required for the government in public health to decide which HPV genotype to be considered in the prophylactic vaccine. The development of new second-generation HPV prophylactic vaccines will likely to include additional high risk genotype, in addition to HPV 16 and 18 (Domingo et al., 2008). The aim of this study was to determine the prevalence and distribution of high-risk HPV (HR-HPV) genotypes in cervical cancer in our multi-ethnic population in Malaysia. This finding will enable us to decide which HPV vaccine is most suitable against cervical cancer in our population.

Materials and Methods

Sample collection

This is a multi-centre, retrospective study using paraffin-embedded tissue biopsies of 280 patients diagnosed with cervical cancer (ICC) from Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Hospital Kuala Lumpur (HKL), Hospital Tengku Ampuan Rahimah (HTAR), Hospital Alor Setar (HAS) and Hospital Kota Bharu (HKB) for a period of 9 years. All cases were histopathologically confirmed cervical carcinoma, consisting of 197 squamous cell carcinoma (SCC) and 83 adenocarcinoma (ADC). Relevant clinical data were retrieved from the laboratory information system and hospital records of the respective hospitals.

Sample processing

Eight micrometer (μm) thick sections were cut from the paraffin embedded tissue block. Gloves and blades were changed during sectioning to avoid cross contamination between samples. DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Germany, Catalog No. 69506). Tissue sections were deparaffinised using xylene and alcohol according to the manufacturer's protocol. Samples were lysed using proteinase K. Lysates were loaded into DNeasy spin columns. After two washings, pure DNA was eluted in low salt buffer. Presence of DNA was determined by 1% agarose gel electrophoresis. The optical density (OD) was measured using ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA) to determine the purity and concentration of DNA within a ratio of 1.7 to 2.0. The extracted DNA was stored in -20 °C until further analysis using quantitative real-time polymerase chain reaction (RT-PCR).

High-risk HPV genotyping

HPV genotyping was carried out using SACACE HPV High Risk Typing Real-™ kit (SACACE, Italy, Catalog No. TV26-100FRT). This kit is an *in vitro* Real-time amplification test for qualitative detection and genotyping of 12 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) which contained the E7 target region. It contains four PCR-mix tubes each of which contain primers directed against regions of three HPV genotypes and the α-Globin gene as an internal control. Multiplex amplification reaction was performed in a volume of 13 µl containing 8 µl of reaction mix (PCR-mix-1, PCR-buffer FRT, Hot Start DNA Polymerase) and 5 µl of DNA sample. The reaction was carried out for 45 cycles, under the following conditions: 15min at 95ºC, 20 sec at 95ºC and 60 sec at 60ºC using Real-Time PCR STRATAGENE MX3000P (Stratagene, La Jolla, CA). Data obtained from Real-time PCR was then analysed by a software (Microsoft® Excel HPV Typing Real-Time MX Results Matrix.xls) provided by the SACACE kit.

Statistical analysis

Chi-square (χ^2) test were performed using the SPSS statistical software package version 12 to determine the relationship between HPV genotype and other parameters (age, ethnic group and histological type). The p value of <0.05 is considered as statistically significance.

Results

A total of 280 cases of cervical cancer (197 SCC and 83 ADC) were analysed. HPV was detected in 92.5% of the cases (95.9% in SCC and 84.3% in ADC) ($p<0.05$). The mean age of patients was 55.8 years and the age range was 24 to 88 years. The highest number of HPV positive cases was noted to be in the ≤39 years age group (95.2%). The prevalence of HPV was highest in the Chinese (95.5%), followed by Malays (91.9%) and Indian (80%) (Table 1). HPV 16 (68.2%) was the commonest genotype detected followed by HPV 18 (40.0%), 58 (10.7%), 52 (10.4%), 33 (10.4%), 45 (9.6%), 39 (7.5%), 56 (7.1%), 59 (5.7%), 51 (2.5%), 35 (1.4%) and 31 (0.7%) (Figure 1).

Table 1. Prevalence of HPV Infection by Demographic Characteristics, (n=280)

Variable		Total no. of patients	No. of patients without HPV		No. of patients with HPV		p value†
			n	(%)	n	(%)	
Age	≤39	21	1	4.8	20	95.2	>0.05
	40-49	81	4	4.9	77	95.1	>0.05
	50-59	75	9	12.0	66	88.0	>0.05
	60-69	58	4	6.9	54	93.1	>0.05
	≥0	45	3	6.7	42	93.3	>0.05
Ethnic group							
Malay		135	11	13.0	124	91.9	<0.05
Chinese		112	5	7.5	107	95.5	<0.05
Indian		25	5	23.3	20	80.0	<0.05
Others		8	0	0.0	8	100.0	<0.05
Histological Type							
SCC		197	8	4.1	189	95.9	<0.001
ADC		83	13	21.2	70	84.3	<0.001

*HPV: Human papillomavirus; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma; †p value<0.05

In SCC, HPV 16 was the commonest (76.6%) genotype detected followed by HPV 18 (35.0%), 58 (13.7%), 52 (12.7%), 33 (11.0%), 45 (9.6%) and 39 (8.1%) (Table 2). In ADC, HPV 18 (51.8%) was the commonest genotype detected followed by HPV 16 (48.2%), 56 (12.0%), 45 (9.6%), 33 (8.4%), 39 (6.0%), 52 (4.8%) and 58 (3.6%). SCC showed a higher prevalence of HPV 16 (76.6%) compared to ADC (48.2%) ($p<0.001$), whereas ADC showed a higher prevalence of HPV 18 (51.8%) compared to SCC (35%), ($p<0.001$) (Figure 2).

Multiple infections with at least 2 HPV genotypes were noted in 55.8% (156 of 280) of the cases. Presence of only a single genotype was observed in 36.8% cases while 35.4%, 15.4%, 4.3% and 0.7% showed two, three, four and five genotypes respectively. Multiple HPV infections are more prevalent (55.8%) than single HPV infection (36.8%).

Interestingly, we found that the pattern of HPV distribution among the 3 major ethnic groups differs. Six most prevalent HPV genotypes are: 16, 18, 58, 56, 52 and 39 in the Malays: 16, 18, 33, 52, 45 and 58 in the Chinese; and 16, 18, 51, 45, 31 and 33 in the Indians (Figure 3). However, HPV 16 and 18 still remain the commonest genotypes in all ethnic groups. The percentage of HPV 16 infection was significantly higher in Chinese (75.9%) compared to Malays (63.7%) and Indian (52.0%) ($p<0.05$), while HPV 18 was significantly higher in Malays (52.6%) compared to Chinese (25.0%) and Indians (28%)

Table 2. Prevalence and Distribution of HPV Genotypes SCC and ADC, (n=280)

		SCC		ADC		Total (ICC)	
		n	(%)	n	(%)	n	(%)
HPV genotype	HPV 16*	151	76.6	40	48.2	191	68.2
	HPV 18*	69	35.0	43	51.8	112	40.0
	HPV 31	1	0.5	1	1.2	2	0.7
	HPV 45	19	9.6	8	9.6	27	9.6
	HPV 33	22	11.0	7	8.4	29	10.4
	HPV 35	3	1.5	1	1.2	4	1.4
	HPV 39	16	8.1	5	6.0	21	7.5
	HPV 51	6	3.0	1	1.2	7	2.5
	HPV 52*	25	12.7	4	4.8	29	10.4
	HPV 56	10	5.1	10	12.0	20	7.1
	HPV 58*	27	13.7	3	3.6	30	10.7
	HPV 59*	15	7.6	1	1.2	16	5.7
Total HPV genotypes**		364		124		488	
No. of cases with HPV infection		189	95.9	70	84.3	259	92.5
No. of cases without HPV infection		8	4.1	13	15.7	21	7.5
Total no. of cases		197		83		280	

*p value <0.05; **Samples with one or more HPV genotype; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma

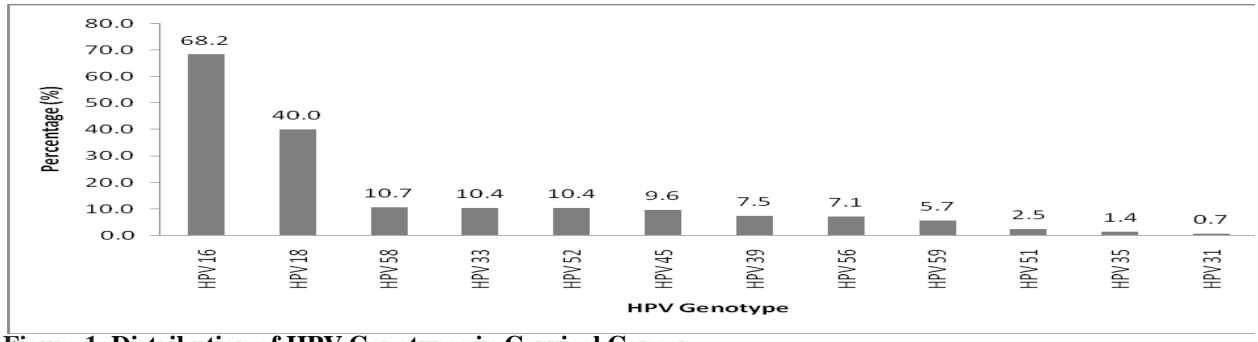

Figure 1. Distribution of HPV Genotypes in Cervical Cancer

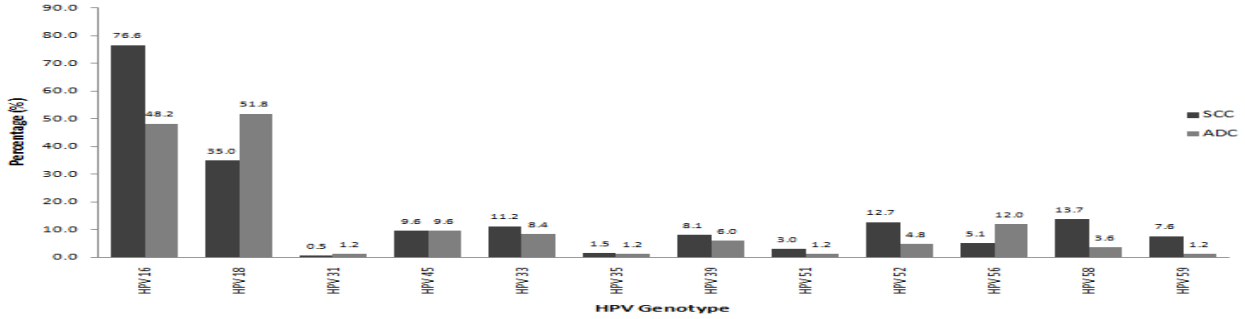

Figure 2. Distribution of HPV Genotypes in Squamous Cell Carcinoma and Adenocarcinoma

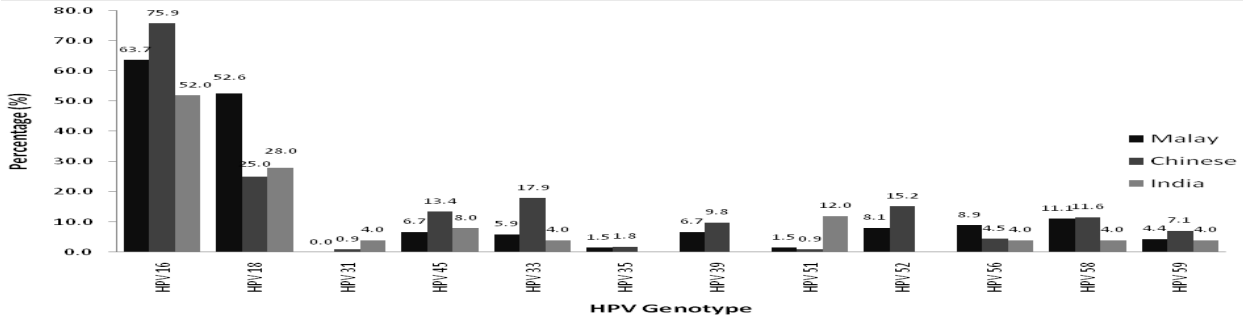

Figure 3. Distribution of HPV Genotypes in Various Ethnic Groups

(p<0.05). HPV 33 (17.9%) and 52 (15.2%) were also more commonly seen in Chinese (p<0.05).

Discussion

Our results showed a high prevalence of HPV infection in cervical cancer (92.5%) in Malaysia. Similarly, Schellekens et al. (2004) has also reported a high prevalence of HPV (95.9%) in cervical cancer in Indonesia (Schellekens et al., 2004). The prevalence of HPV infection in Malaysia is the same as the estimated worldwide prevalence (85-99%) and is comparable to other Asian countries (Walboomers et al., 1999; Bao et al., 2008; de Sanjose et al., 2010). The high prevalence of HPV that was consistently detected in cervical cancer, strengthened the previous conclusion that HPV infection is the primary causal agent for cervical cancer (Castellsague et al., 2006; Bao et al., 2008).

Our study shows that the 5 commonest HPV genotypes in cervical cancer were HPV 16, 18, 58, 52 and 33. This is similar to the findings in Indonesia, Thailand, China, Hong Kong, Taiwan, Korea and Japan (Settheetham-Ishida et al., 2005; Bao et al., 2008) (Table 3). However it is slightly different when compared to US and Europe where it was reported as HPV 16, 18, 31, 45 and HPV 16, 18, 33 and 31, respectively (Smith et al., 2007). While HPV 16 and 18 still remain the 2 commonest types across the world, the third and fourth HPV types differ across regions. Therefore, in Europe and US, HPV 45, 31 and 33 were the third, fourth and fifth commonest HPV genotypes while in Asia it was HPV 58, 52 and 33 (Bao et al., 2008). This suggests that the distribution of different HPV genotype can differs in geographic distribution, study group selected and ethnic diversity.

HPV 16 and 18 are the two commonest HPV genotypes detected in this study (62.1%) which is similar to many other studies (Smith et al., 2007; Schiffman et al., 2009; Sharifah et al., 2009; 2010). In Europe, North America and Oceania, the HPV 16 and 18 proportion is reported to be higher (74-77%) compared to Africa, Asia and South/Central America (65-70%) (Bao et al., 2008). In this study, the prevalence of combined HPV 16 and 18 genotypes was high in SCC (60.4%) and ADC (67.2%).

HPV 16 and 18 genotypes play a dominant role in cervical carcinogenesis worldwide. Bao et al. (2008) performed a meta-analysis of HPV genotyping in Asia and reported that there were eighteen HPV genotypes detected from cervical cancer, of which the 10 commonest HPV genotypes were HPV 16, 18, 58, 52, 33, 45, 31, 35, 39 and 51. Comparing these data with the current study, HPV 58 (10.7%), 52 (10.4%) and 33 (10.4%) were the 3 commonest HPV genotypes after HPV 16 and 18. Our results therefore indicate that HPV 58, 52 and 33 might

Table 3. Prevalence of Single and Multiple HPV Genotypes in ICC, (n=280)

		n	(%)	p value*
No. of HPV genotypes	Negative	21	7.5	<0.0001
	Single	103	36.8	
	Two	99	35.4	
	Three	43	15.4	
	Four	12	4.3	
	Five	2	0.7	
Total		280	100	

*p value <0.05

Table 4. Comparison of HPV Type Distribution in ICC, SCC and ADC

Reference	Country	Type of sample	Type of cancer	N cases	Prevalence	HPV prevalence							
Bao et al. 2008	Asia	Biopsies	ICC	5,954	86	16	18	58	52	33	45	31	35
	China,HK,Taiwan	Biopsies	ICC	2,698		16	18	58	52	33	31	53	45
	Korea	Biopsies	ICC	438		16	18	58	52	33	35	68	31
	Japan	Biopsies	ICC	1,185		16	18	52	33	58	31	35	51
	South east asia	Biopsies	ICC	1,141		16	18	45	52	58	59	31	33
	South central asia	Biopsies	ICC	492		16	18	45	33	35	58	31	59
Chen et al. 2009	China	Biopsies	SCC	630	97.6	16	18	31	52	58	59		
Smith et al. 2007	World	Biopsies	ICC	14,595	87	16	18	33	45	31	58	52	35
	Africa	Biopsies	ICC	1,339	94	16	18	33	45	35	31	58	52
	Asia	Biopsies	ICC	5,652	86	16	18	58	33	52	45	31	35
	Europe	Biopsies	ICC	4,373	86	16	18	33	31	45	35	58	56
	North America	Biopsies	ICC	1,354	86	16	18	31	33	45	52	35	58
	South/Central America	Biopsies	ICC	1,427	31	16	18	31	45	33	58	52	35
Franceshi et al. 2003	India	Biopsies	ICC	191	99.5	16	18	33	35	45	58	56	59
Munirajan et al. 1998	India	Fresh biopsies	SCC	43	69.8	16	18	33	58	55			
Sowjanya et al. 2005	India	Fresh biopsies	SCC	41	87.8	16	18	33	45	35			
Hamkar et al. 2002	Iran	Fixed biopsies	SCC	42	78.6	16	18	31	33				
Mortazavi et al. 2002	Iran	Fixed biopsies	ICC	69	85.5	16	18	33					
Yadav et al. 2005	Malaysia	Fresh biopsies	SCC	23	95.7	16	18	58	33				
Schelekens et al. 2004	Indonesia	Fixed biopsies	ICC	74	95.9	16	18	52	58	31	56		
Settheetham-ishida et al. 2005	Thailand	exfoliated cells	SCC	90	88.7	16	18	58	52	35			
Chicareon et al. 1998	Thailand	Fresh biopsies	ICC	377	84.7	16	18	58	52	31	45	56	
Yu et al. 2003	China	Fixed biopsies	ICC	50	96	16	18	58	52				
Clifford et al. 2003	Asia	Fixed biopsies	ADC/adenosquamous		18	16	45	59	35	52	31	58	
Clifford et al. 2003	Europe	Fixed biopsies	ADC/adenosquamous		18	16	45	31	33	59			
Clifford et al. 2003	North America/Australia	Fixed biopsies	ADC/adenosquamous		18	16	45	33	52	31			
Our study	Malaysia	Fixed biopsies	ICC	280	92.5	16	18	58	52	33	45	39	56
			SCC	197	95.9	16	18	58	52	33	45	39	59
			ADC	83	84.3	18	16	56	45	33	39	52	58

also play an important role in the development of cervical cancer in our multi-ethnic population in Malaysia. This finding is similar to the studies conducted in Indonesia, Thailand, China, Hong Kong, Taiwan, Korea and Japan (Settheetham-Ishida et al., 2005; Bao et al., 2008).

HPV variants differ in biological and chemical properties and pathogenicity, which may be an important factor in the development of cervical neoplasia (Conrad-Stoppler et al., 1999; Veress et al., 1999; Giannoudis et al., 2001). The oncogenicity of specific HPV variants appears to vary geographically and also with the ethnic origin of the population studied. Veress et al. (1999) suggested that because of increased transcriptional activity and changes in the progesterone response elements, Asian-American variants might have enhanced oncogenic activity compared to European isolates.

The percentage of HPV 16 was noted to be significantly higher in Chinese compared to Malays and Indians, while HPV 18 was significantly higher in ethnic Malays compared to Chinese and Indians. These findings could be related to differences in genetic predisposition. Magnusson et al. (2000) found that genetic heritability account for 27% of tumour development (Magnusson et al., 2000). Heritable factors which could contribute to the development of cervical cancer include susceptibility to HPV infection, ability to clear HPV infection, and time to development of disease. Environmental factors and cultural practices may also have a small contribution.

Studies have shown that with multiple HPV infections can occur (Bachtiary et al., 2002; Capra et al., 2008). Clifford et al. (2005) reported the prevalence of multiple HPV infection with a frequency ranging from 11.5% in Turin, Italy, to 42.4% in Ho Chi Minh City, Vietnam (Clifford et al., 2005; Capra et al., 2008). While Bachtiary et al. (2002) reported that multiple HR-HPV infections were seen in 46% of cervical cancer biopsies. In our study, multiple HPV infections with at least 2 HPV genotypes are commoner (55.7%) than single HPV infection (36.8%). Currently there is no report on the implication of single and multiple HPV genotypes. It is not clear whether the presence of multiple HPV genotypes can serve as a predictor of cervical cancer.

In conclusion, this study confirmed that the distribution of HPV genotypes in Malaysia is similar to other Asian countries namely, China, Hong Kong, Taiwan, Japan, Korea, Thailand, and Indonesia. HPV 16 and 18 infections accounted for 62.1% of the cases while HPV 58, 52 and 33 accounted for another 18% of cases. The current prophylactic vaccines against HPV 16 and 18 will be effective in preventing cervical cancer in a significant percentage of Malaysian women. However, to achieve wider vaccine coverage, the next generation HPV prophylactic vaccine should also include HPV 58, 52 and 33 genotypes.

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