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Multiple genotype infection of human papilloma virus is associated with cervical cytological abnormalities

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KEYWORDS
Human Papilloma Virus; Cervical cancer; Liquid-based cytological test; High-risk HPV; Low-risk HPV.

ABSTRACT
Development of cervical cancer is associated with persistent infection of human papillomavirus (HPV). The present study is aimed to determine the association between HPV infection and cervical cytological abnormalities. HPV genotypes and liquid-based cytological analyses were performed in 351 cervical brush samples obtained from women visiting Gynecology Clinic, Khon Kaen Hospital, Khon Kaen, Thailand. HPV was detected in 23.36% (82/351) of the cases. Among the HPV positive case, 71.95% (59/82) was found with single infection and 28.05% (23/82) were found with multiple infections. Of 351 tested specimens, 7.6% (27/351) were presented with abnormal cytology, and 62% (17/27) of these 27 cases were found to be positive for HPV. The cytological anomalies presented in HPV positive cases were 52.94% (9/17) of ASCUS, 28.41% (5/17) of LSIL and 17.64% (3/17) of HSIL. Our data showed that cytological abnormalities were more frequently observed in patients with multiple HPV infection, compared to those with single infection (p-value < 0.001). However, among the patients with single HPV infection, cytological data were not different between patients with high-risk HPV (HR-HPV) and low-risk HPV(LR-HPV). In conclusion, our present study revealed the association between multiple HPV infection and cervical cytological abnormalities. This information emphasizes the importance of HPV genotype analysis for cervical cancer screening and surveillance. Those women with multiple HPV infection are strongly suggested to be treated and frequently followed up.

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Introduction

Utility of conventional Papanicolaou (Pap) screening test has a limitation for detecting high-grade squamous intraepithelial lesions (HSIL) of uterine cervix due to its low sensitivity and reproducibility[1]. Liquid-based cytological study has been used to improve test sensitivity and diagnostic accuracy. In addition, the collected samples were also applicable for molecular testing. Combination of human papillomavirus (HPV) genotypes with cytology has been recommended to enhance the diagnostic accuracy for screening of cervical cancer. Several genotypes of HPV have been identified and divided into low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV) depending on the clinical characteristics of infection. HR-HPV covers HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82; whereas, LR-HPV includes HPV 6,11, and 81[2].

Previous reports showed that persistent HPV infection plays role in the development of cervical cancer and precancerous lesions, and 99% of cervical cancer patients are associated with HR-HPV infection[3]. HPV16 and HPV18 are the most prevalent HR-HPV genotypes of cervical cancer worldwide. However, HPV16 and/or HPV18 detection alone is insufficient for predicting the development of abnormal cytology. In contrast to the Western population, the high prevalent HR-HPV genotypes among population in East Asia are HPV52 and HPV58[4,5]. The most common genotypes among Thai women were HPV-16, HPV-58, and HPV-18 and the prevalence of HR-HPV with cervical intraepithelial neoplasia grade 2-3 (CIN2-3) ranged from 64.8% to 90.1%[6]. The identification of HR-HPV is important to early detection of the disease and for efficient prophylactic vaccines[7,8]. Due to the large number of HPV genotypes, mixed infection by different HPV genotypes is commonly found in 20%-50% of HPV infected patients. The previous studies suggested that multiple-type HPV infections are associated with the development of cervical cancer and more likely to be an initiator of carcinogenesis than single-type infections[9-11]. Co-infection of HR-HPV and LR-HPV was found to be a risk to increase disease progression and morbidity. However, this information is still controversy, some other studies showed no significant differences in the risk of cervical cancer in women with single and multiple HPV infections[12]. Many studies reported that HPV-based cervical cancer screening has greater sensitivity and negative predictive values for detection of carcinoma *in situ* and adenocarcinoma, compared with cytology-based screening[13,14]. The objective of cervical cancer screening is to detect the precursor abnormalities that lead to invasive cervical cancer or to detect the early stage of cervical cancer. The Royal Thai College of Obstetricians and Gynaecologists (RTCOG) 2021 suggests to screen women between 25-65 years with cytology every two years or co-testing (HPV testing and cytology) every 5 years[15]. In our present study, we have investigated the association between HPV genotypes either single or multiple infections and the cervical cytological changes. In addition, the prevalence of HPV infection was also calculated.

Materials and methods

Study population

A retrospective study was performed in the women who attended the Obstetrics Clinic, Out-Patient Department of Khon Kaen Hospital, Khon Kaen, Thailand, during January 2014 to December 2020. The clinical data of women who underwent detection of HPV DNA and liquid-based cervical smear cytology test (LBC) in cervical exfoliated cells were enrolled. The data were retrieved from the Hospital Information System (HIS) and Laboratory Information System (LIS). The exclusion criteria were as follows: 1) patients with a history of pelvic chemotherapy or radiotherapy, 2) patients with a history of total hysterectomy or cervical resection, 3) patients who have received cervical physical therapy, and 4) patients with a result HPV DNA or LBC only. This study was approved by Khon Kaen Hospital Institute Review Board in Human Research (KEX63007).

Liquid-based cytological test

Cervical exfoliated cell samples (n=351) were collected during January 2014 to December 2020 by cyto-brush and were analyzed using ThinPrep Pap test (Hologic Inc.,

USA.). Cytological slides were examined by two expert cytotechnologists and the results were approved by pathologist at the Department of Anatomical Pathology, Khon Kaen Hospital. The LBC results were graded, according to the 2001 Bethesda System (TBS) classification: no intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous carcinoma, and adenocarcinoma. The cytological grades higher than NILM were classified as positive cytological change.

HPV genotyping

HPV genotype was determined in liquid-based cytology samples by multiplex real time PCR using Anyplex™ II HPV 28 Detection (Seegene®, Seoul, Korea), according to the manufacturer's instructions. The multiplex real-time PCR for HPV allows for simultaneous detection and genotyping of 19 HR-HPV genotypes including HPV-16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82 and 9 LR-HPV genotypes including HPV-6, 11, 40, 42, 43, 44, 54, 61 and 70 in a single reaction[16].

Statistical analysis

The statistical analyses were performed in SPSS software version 27 using likelihood ratio. The *p*-value less than 0.05 were considered statistically significant.

Results

Population characteristics

All 351 cervical exfoliated cells specimens were used to examine the HPV genotyping and liquid-based cytological study by ThinPrep Pap test. The mean age of subjects recruited in our study was 46 years, minimum of 18 years and maximum of 68 years. The association between age and cytological changes was not observed (Table 1). The prevalence of HPV infection was 23.36% (82/351). The subjects with HPV infection exhibited a significantly higher proportion of abnormal cytology (*p*-value < 0.001, Table 1). The cytology characteristics of HPV positive patients were 79.2% negative for intraepithelial lesion or malignancy (NILM), 10.9% with ASCUS, 6.1% with LSIL, and 3.7% with HSIL (Table 1).

The patients with single HPV infection were presented with different cytological changes: 83.0% were with NILM, 10.2% with ASCUS, and 6.8% with LSIL. The HSIL was not observed in these patients. In single HPV-genotype infected patients, the cytological characteristics between HR-HPV and LR-HPV infection were not different. Those with HR-HPV presented 12.5% ASCUS and 7.5% LSIL and LR-HPV shown 5.3% ASCUS and 5.3% LSIL. In 23 cases with mixed-infected by multiple genotypes of HPV, the cytological abnormalities were also frequently found in this group, where 13.0%, 4.3%, and 13.0% were graded as ASCUS, LSIL and HSIL, respectively. Comparing between patients with single and multiple infections, our result showed that patients with multiple infection exhibited higher grade of cytological abnormalities (*p*-value < 0.05, Table 1).

Table 1 Association between cervical cytological grades and age and HPV infection status

	Parameter	N NILM	Cytological grading (N, %)				*p*-value*
			ASCUS	LSIL	HSIL		
Age	< 25	4	3 (75)	1 (25)	0	0	0.756
	26-35	54	46 (85.2)	5 (9.3)	1 (1.8)	2(3.7)	
	36-45	105	98 (93.3)	4 (3.8)	2 (1.9)	1 (1.0)	
	46-55	137	129 (94.2)	6 (4.4)	2 (1.4)	0	
	56-65	48	45 (93.8)	2 (4.2)	1 (2.0)	0	
	> 65	3	3 (100)	0	0	0	
HPV	Negative	269	259 (96.3)	9 (3.3)	1 (0.4)	0	< 0.001
	Positive	82	65 (79.2)	9 (10.9)	5 (6.1)	3 (3.7)	
HPV	Single infection	59	49 (83.0)	6 (10.2)	4 (6.8)	0	< 0.001
	Multiple infections	23	16 (69.5)	3 (13.0)	1 (4.3)	3 (13.0)	

Note: *Likelihood ratio, HPV=Human Papillomavirus, NILM=negative for intraepithelial lesion or malignancy, ASCUS=atypical squamous cells of undetermined significance (ASCUS), LSIL=low-grade squamous intraepithelial lesions, HSIL=high-grade squamous intraepithelial lesions.

Single and multiple infections with abnormal cytology association

Of those 82 HPV positive cases, 59 cases (16.8% of total) were presented with single infection and 23 cases (6.6% of total) had multiple infections (Figure 1). The most common single HPV genotype infection was HPV 42 and HPV 16, followed by HPV 52, 68, 39, 35, 54 and 58. The HPV16, HPV35, HPV39, HPV52 and HPV 68 accounted for 41.2% (7/17) of all cervical cytology abnormalities. Notably, HPV16 and HPV 52 were the most common type in HR-HPV with both single and multiple infections.

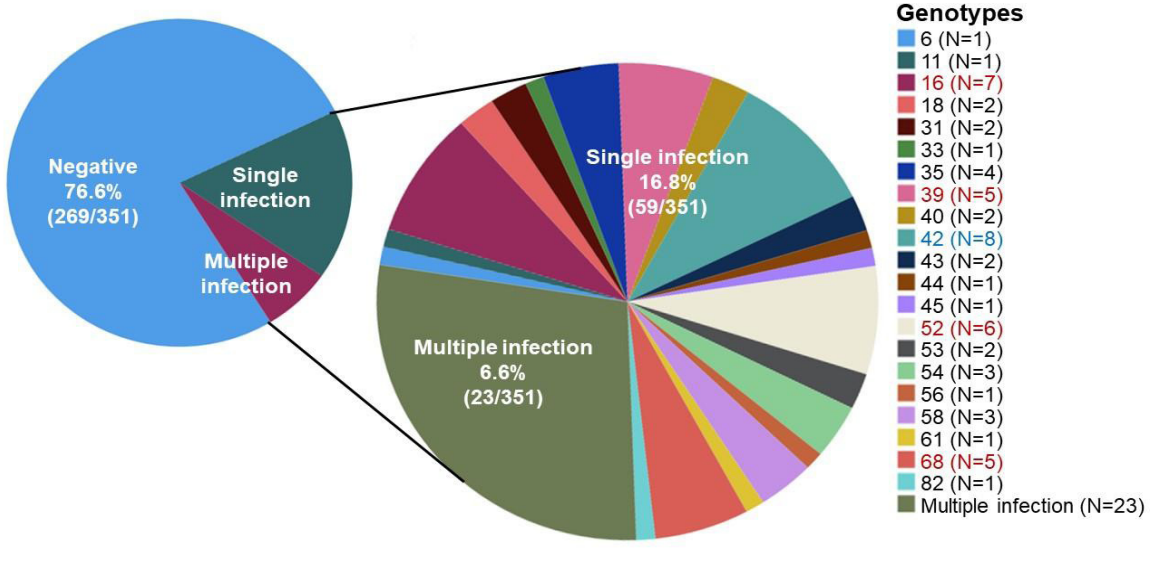

Figure 1 HPV infection status in women visited Khon Kaen Hospital, Thailand during January 2014 to December 2020.

Comparing among the patients with multiple infections, those infected with HR-HR or LR-HR were frequently presented with cytological abnormalities, comparing with LR-LR infection (Table 2, *p*-value < 0.05). However, comparing among the single-genotype infected patients, the abnormality in cytology was not different between patients with HR and LR.

Table 2 Cervical pathological grades in HPV infected patients

HPV infection	N	Cervical Pathological grading (N, %)				*p*-value*
		NILM	ASCUS	LSIL	HSIL	
Single infection						
High risk	19	17(89.0)	1(5.3)	1(5.3)	0	0.612
Low risk	40	32(80.0)	5(12.5)	3(7.5)	0	
Multiple infection						
LR and LR	2	2(100)	0	0	0	0.015
LR and HR	12	9(75)	1(8.3)	1(8.3)	1(8.3)	
HR and HR	9	5(55.6)	2(22.2)	0	2(22.2)	

Note: * Likelihood ratio, HPV=Human Papillomavirus, NILM=negative for intraepithelial lesion or malignancy, ASCUS=atypical squamous cells of undetermined significance (ASCUS), LSIL=low-grade squamous intraepithelial lesions, HSIL=high-grade squamous intraepithelial lesions, HR=high risk, LR=Low risk

Discussion

Our study presented the distribution of HPV genotypes and the association with cytological characteristics in 82 women with HPV positive in Khon Kaen, Thailand. Similar to other parts of the world, our data showed that HPV16, HPV52, HPV68, and HPV 39 were the most predominant HR-HPV genotypes among positive women. The results are in consistent with those in the previous studies performed in the same geographical area[17,18]. Among the identified HPV genotypes; HPV16, HPV52 and HPV68 were most frequently found in the samples with abnormal cytology. Also, HR-HPV genoypes with relatively high proportion in our study (i.e., HPV16, and HPV52) were consistent with the study in Asia but different from Europe, Latin America and Caribbean, Africa, and Oceania[19,20]. Multiple infections promote the development of cervical lesions and the occurrence of cervical cancer[21]. We also found that multiple HPV genotype infections increased the risk of HSIL compared with LR-HPV. The multiple infections with HR-HPV were significantly higher in women with abnormal cytology grading. It is interesting that a result of HR-HPV infection without HPV16 might be a contributor to cytological changes. Our result showed that HPV52 positivity infection seems to be associated with HSIL. This result is agreed with the previous studies in China that HPV52 infection could increase the risk of HSIL[17,22,23]. The distribution of HPV genotype and the risk of cytology abnormalities in the present study are different from those reported in the previous studies. This might be due to the variations in the study design, specimen types, and screening methods[24]. Patient's age was previously reported as one of the risks of cytological abnormalities[25]; however, our study showed the risk of HSIL was not different between the patients with different age. As a genotype analysis provided the qualitative data of HPV infection; therefore our present study could only demonstrate the association between single or multiple HPV genotype and cervical cytological changes, regardless of the infection level. Further quantitative analysis of HPV infection and its association with clinical outcomes may provide better clinical relevance. Consequently, our results suggested that the national policy for cervical cancer screening should pay attention

to the population age both of women younger and older than 46 years. Patients detected with multiple HPV infections are strongly suggested to closely followed up. Therefore, further studies either preclinical or clinical studies are required to analyze the impact of single and multiple HPV infections on development cervical diseases.

Conclusion

HPV infection is a well-established risk of cervical cancer. We have determined the occurrence of HPV in women visiting Khon Kaen Hospital for screening of cervical cancer. In the high-risk HPV, genotypes 16, 52, 68, and 39; were frequently found in the pre-cancerous lesions. Moreover, the infection with multiple genotypes of HPV was found to associated with the presentation of cytological abnormalities. This information emphasized the importance of HPV infections for cervical cancer screening method, especially in the patients infected by multiple genotypes of HPV that should be closely monitored.

Take home messages

Multiple HPV infections were more credible to a degree of cervical lesions than single infection. The multiple infection with HR-HPV was associated with cytological grading especially for HPV 16, 52, 68 and 39. The diagnosis of HR-HPV and multiple infections were important for treatment and monitoring in HPV infected women.

Conflicts of interest

The authors declare no conflict of interest.

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