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Prevalence, Types, and Associated Factors of HPV Infection Among Women With Abnormal Cervical Cytology Screening at Ramathibodi Hospital

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Background: Cervical cancer is the second leading cancer among Thai women. Human papillomavirus (HPV) is the most common cause of cervical cancer. HPV16 and HPV18 are the most prevalence types in women with cervical cancer.

Objectives: To study the prevalence and types of HPV infection and its associated risk factors among women with abnormal cervical cytology screening.

Methods: This cross-sectional study purposively recruited 376 women who had abnormal cervical cytology screening by liquid-based cytology at the outpatient clinic, Ramathibodi Hospital from August 2015 until March 2016. HPV genotyping was done by the polymerase chain reaction (PCR). Analysis of the personal characteristics was done using descriptive statistics.

Results: The prevalence of HPV infection was 41.5%. Among them, a single infection was found in 78.8% of women. The most common genotypes were HPV16 (8.5%) and HPV52 (7.7%). But HPV18 was found in only 1.9%. The characteristic which was significantly associated with HPV infection was age, nulliparity, and high-graded dysplasia ($P < .05$).

Conclusions: High prevalence rate of HPV infection was found among women with abnormal cytology. High-risk HPV prevalence and type distribution may be useful for public health authorities in assessing the cervical screening program.

Keywords: Human papillomavirus, Prevalence, Risk factors, HPV genotyping

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Introduction

In Thailand, cervical cancer is the second leading female cancer with an age-standardized rate (ASR) of 16.2 per 100 000 women. About 8622 new cases and 5015 cervical cancer deaths were recorded in 2018. Worldwide, cervical cancer ranks as the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women. In 2018, approximately 570 000 cases were diagnosed with cervical cancer and 311 000 deaths.[1]

Human papillomavirus (HPV) infection is the most common cause of cervical cancer and sexual transmitted infections. It is also associated with cervical neoplasia, anogenital warts, and other anogenital cancers. Overall, prevalence of HPV is about 99.7% of women with cervical cancer.[2] More than 100 HPV genotypes have been identified; approximately 40 types are known to infect the genital tract. These viruses can be separated into high- or low-risk type depending on their oncogenic potential; 15 HPV types were classified as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 HPV types as probable high-risk types (26, 53, and 66), and 12 HPV types as low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108).[3, 4] High-risk (HR) HPV types considered to be carcinogenic and are often associated with invasive cervical cancer (ICC). On the other hand, low-risk HPV types are mainly associated with genital warts. HPV16 and HPV18 are the most prevalence types found in 50% to 60% and 7% to 20% of cases, respectively. Women with persistent infections of HPV16 are more likely to develop cervical intraepithelial neoplasia (CIN) grade 3 (CIN3) or cervical cancer than women who have persistent infections with other high-risk HPVs. [3, 5]

The major route of genital HPV infection is sexual intercourse. Condoms offer some protection against the development of CIN2/3 and ICC and do promote the regression of CIN and penile intraepithelial lesions in concordant couples. The factors enhancing transmission are related predominantly to sexual behavior such as early age of sexual intercourse, number of sexual partners, acquisition of new partners, and the number of lifetime partners of the partner. Other factors not directly related to sexual behavior have been identified as the risk factors for HPV infection. An increased risk for HPV infection has been associated with long-term oral contraceptive use, Black or Hispanic ethnicity, and a history of chlamydia infections.[3] The significant increase in HPV infection risk was observed in women who have used contraceptives for more than 4 years. This may probably be due to the incidence of cervical ectropion, site where HPV infections preferentially induce neoplastic lesions, which serve as a potential exposure of carcinogens, and the squamo-columnar function.

HPV infection is very common in young sexually active women. Most are subclinical transient infections and low grade CIN1 may also detected by colposcopy. Most HPV infections and low-grade CIN resolve as a consequence of the development of cell-mediated immunity (CMI) accompanied usually but not inevitably by seroconversion and antibody to the major coat protein L1. Only 10% to 15% of infected women remain HPV DNA positive with a persistent viral infection and at risk for the development of high-grade intraepithelial disease and ICC.[6]

Among women who had abnormal cytology screening for cervical cancer, a high percentage of HPV infection is expected. It is still questioned that which type of HPV is common in this group of women and what are the characteristics of women who had abnormal cytology with HPV infection compare with women who did not have HPV infection.

Methods

Participants

The population of this descriptive cross sectional study were women aged 16 to 90 years who underwent a screening for cervical cancer by liquid-based cytology at Ramathibodi Hospital during August 2015 and March 2016. The results showed abnormal cytology according to the Bethesda system. The subjects consisted

of 376 women who had written informed consent and test for HPV infection. The number of samples was calculated by using Daniel's sample size formula as the expected prevalence was 0.25.[7]

Ethics

The study was approved by the Human Research Ethics Committee of Faculty of Medicine Ramathibodi Hospital, Mahidol University (No. MURA2017/438 on July 17, 2017). The request letter from the Dean of the Faculty of Graduate Studies was sent to the Head of the Obstetrician and Gynecologist Department for permission and cooperation for collection of the data.

Data Collection

Cervical screening records and the personal data including age, marital status, education, parity, and occupation of women who had abnormal cervical screening were collected from the logbook of gynecology record at outpatient department.

Abnormal cytology were collected for identified HPV infection. HPV typing were performed by using BD Onclarity™ HPV assay (BD Diagnostics, Burlington, NC, USA), a real-time polymerase chain reaction (PCR) technique with specific primers and fluorescent labeled detector probes for detect 14 HPV genotypes. There was 3-well, 4-channel real-time PCR test that provide genotyping information for 9 HPV high risk types which included 6 individual HPV types (HPV 16, 18, 31, 45, 51, and 52). The rest of these HR-genotypes were reported in groups which were HPV33/58, HPV56/59/66, and HPV35/39/68.

Statistical Analysis

SPSS version 23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp; 2015) was used to analyse data. Odds ratios (ORs) were used to test the association between variables. Significant level was set at 95% confidence intervals (CI). Descriptive statistics included mean, standard deviation (SD), percentage, and frequency were applied to analyze the data.

Results

In this study, most of women (44.7%) aged more than 45 years, 40% of women aged 31 to 45 years, mean (SD) age was 44.1 (13.36) years. Sixty-six percent of them were married and only 34% were single. Overall, 76% of them had bachelor's degree, 43% of them were employed and nullipara, and 88% of them had low-graded dysplasia (Table 1).

Among them, 156 women (41.5%) were positive for HR-HPV DNA. When using single probe to detect HR-HPV infection, the common HPV infection were HPV16 (8.5%), HPV52 (7.7%), HPV51 (4.5%), and HPV18 (1.9%).

Table 1. Characteristics of Women With Abnormal Cervical Cytology

Characteristic	No. (%)
Age, y	
16 - 30	57 (15.1)
31 - 45	151 (40.2)
46 - 90	168 (44.7)
Status	
Single	128 (34.0)
Married	248 (66.0)
Education	
Lower than bachelor degree	90 (23.9)
Bachelor degree	165 (43.9)
Higher than bachelor degree	121 (32.2)
Occupation	
Unemployed	90 (23.9)
Employees	165 (43.9)
Own business	121 (32.2)
Parity	
0	165 (43.9)
1	84 (22.3)
> 1	127 (33.8)
Dysplasia	
Low-graded dysplasia	331 (88.0)
High-graded dysplasia	45 (12.0)

Abbreviation: SD, standard deviation.

When using multiple probe in detection, the common types were HPV56/59/66 (11.7%) and HPV33/58 (9.9%). For single infection, the most common type was HPV16 (6.1%), HPV52 (4.8%) and HPV51 (1.9%). For mixed infection, the most common types were HPV52 (2.9%), HPV51 (2.6%) and HPV16 (2.4%). Infection with multiple HPV (≥ 3 HPV types) were detected in 1.1% (2.6% of HPV positive women) (Table 2).

The association between HR-HPV infection and personal characteristics was determined. The significant risk factors were age, parity, and severity of dysplasia. Women aged 16 to 30 years and aged 31 to 45 years were significantly associated with HPV infection ($P < .001$). Women with no parity record (OR [95% CI], 1.73 [1.08 - 2.79] had higher risk for HPV infection compared with group of women who had more than 1 children. Women with high-graded dysplasia (OR [95% CI], 2.12 [1.13 - 3.98]) had higher risk for HPV infection compared with women with low-graded dysplasia. There was no association between the risk of HPV infection and these personal factors including marital status, education, and occupation (Table 3).

Table 2. Distribution of HR-HPV Infection in Female With Abnormal Cervical Cytology

Category	No. (%) of HPV Infection		
	Single (n = 343)	Mixed (n = 33)	Total (N =376)
HR-HPV positive	123 (32.7)	33 (8.8)	156 (41.5)
Single probe			
HPV16	23 (6.1)	9 (2.4)	32 (8.5)
HPV18	3 (0.8)	4 (1.1)	7 (1.9)
HPV31	3 (0.8)	4 (1.1)	7 (1.9)
HPV45	2 (0.5)	4 (1.1)	6 (1.6)
HPV51	7 (1.9)	10 (2.6)	17 (4.5)
HPV52	18 (4.8)	11 (2.9)	29 (7.7)
Multiple probe			
HPV33/58	-	-	37 (9.9)
HPV56/59/66	-	-	44 (11.7)
HPV35/39/68	-	-	17 (4.5)

Abbreviations; HPV, human papillomavirus; HR, high risk.

Table 3. Association of Personal Characteristics and HPV Infection

Characteristic	No. (%)		OR (95% CI)	*P* Value*
	HPV Positive (n = 156)	HPV Negative (n = 220)		
Status				
Single	62 (48.4)	66 (51.6)	1 [Reference]	.049
Married	94 (37.9)	154 (62.1)	0.65 (0.42 - 1.00)	
Age, y				
16 - 30	31 (54.4)	26 (45.6)	2.98 (1.60 - 5.54)	< .001
31 - 45	77 (51.0)	74 (49.0)	2.60 (1.64 - 4.13)	
45 - 90	48 (28.6)	120 (71.4)	1 [Reference]	
Education				
Lower than bachelor degree	40 (37.7)	66 (62.3)	1 [Reference]	.640
Bachelor degree	87 (42.6)	117 (57.4)	1.23 (0.76 - 1.96)	
Higher than bachelor degree	29 (44.0)	37 (56.0)	1.29 (0.69 - 2.42)	
Parity				
0	79 (47.9)	86 (52.1)	1.73 (1.08 - 2.79)	.067
1	33 (39.3)	51 (60.7)	1.22 (0.69 - 2.16)	
> 1	44 (34.6)	83 (65.4)	1 [Reference]	

Table 3. Association of Personal Characteristics and HPV Infection (Continued)

Characteristic	No. (%) HPV Positive (n = 156)	No. (%) HPV Negative (n = 220)	OR (95% CI)	*P* Value*
Occupation				
Unemployed	33 (36.7)	57 (63.3)	1 [Reference]	
Employees	65 (39.4)	100 (60.6)	1.12 (0.66 - 1.91)	.199
Self employed	58 (47.9)	63 (52.1)	1.59 (0.91 - 2.78)	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

*Significant level was set at *P* value less than .05 ($P < .05$).

Discussion

This study showed the prevalence rate of HR-HPV infection among 376 women who had abnormal cervical cytology at Ramathibodi Hospital was 41.5% (156/376). The results of this study were similar to the previous studies. Sun et al[8] reported the HPV infection rate among women with abnormal cervical cytology in China was 47.28%. Laowahutanont et al[9] reported HPV infection rate was found in 53.4% of women with abnormal cervical cytology screening at National Cancer Institute. However, many studies found higher rate of HPV infection among women with abnormal cervical cytology screening. Roset et al[10] reported the rate of HPV infection among women aged 15 to 25 years who had any cytological abnormality in the PApilloma TRIal against Cancer In young Adults (PATRICIA) study in young women from various countries was 72.0%. Natphopsuk et al[11] reported the prevalence of HR-HPV infection was 85.4% among Northeast Thai women with pathology-defined squamous cell carcinoma of the cervix (SCCA). Marks et al[12] reported among women attended family planning clinics various parts of Thailand between 2002 to 2003 and were diagnosed with CIN2/3, the prevalence rate of HPV was 90.9%. As expected, the HPV positivity was varied with degree of abnormal cervical cytology. The greater in higher grade of cytological abnormalities were associated with higher rate of HPV infection. In conclusion, the overall rate of HPV infection ranged from 5% to 25%. The prevalence rate was lower among women with normal cytology findings. The rate of HPV infection increases with increasing severity of cytological abnormalities.

Potential limitations of the present study were 2 types of probes in detecting HR-HPV types. The first one was probes of single genotyping which were HPV 16, 18, 31, 45, 51, and 52. The second type was probe which detect HR-HPV group infection which were HPV33/58, HPV56/59/66, and HPV35/39/68. This study used the BD Onclarity HPV assay which could not differentiate some of HPV genotypes. The most common HR-HPV types detected by single probe were HPV16 (16.3%) and HPV52 (14.8%) which were similar with several published studies. Roset Bahmanyar et al[10] who studied among women aged 15 to 25 years from various countries reported that the most common types were HPV16 (5.6%) and HPV18 (2.3%). Kantathavorn et al[13] who studied among women who came for cervical screening at Chulabhorn Hospital, Bangkok, Thailand, and reported that the most common types were HPV52 (10.6%) and HPV16 (9.3%). Marks et al[12] studied among women with CIN2/3 and reported the most common types were HPV52 (63.6%) and HPV16 (27.3%).

HR-HPV DNA positivity overall was highest among women aged less than or equal to 45 years (69.2%). The mean (SD) age of women included in this study was 44.1 (13.36) years. The HR-HPV was strongly associated with age. The decline in HR-HPV prevalence rate was found with increasing age of women in this study. The result was similar to the study of Kantathavorn et al[13] which reported that HPV infection was found mostly

frequent among women aged less than 30 years (24.8%), and decreased by increasing age. Sukvirach et al[7] which reported that the HPV prevalence was highest among women aged less than 35 years (9.8%) and then formed a plateau among women aged more than or equal to 35 years (4.5% - 6.0%).

Nullipara women had higher risk of HPV infection. The study of Souho et al[14] which reported that the other aspect of HPV infection that deserves more attention is its association to damages that lead to reduced fertility or sterility. It appears that HPV can be associated to i) apoptosis in sperm cells; ii) alterations of semen quality through cell count decrease, amplitude of lateral head displacement reduction, mobility reduction and increase of anti-sperm antibodies level; iii) apoptosis in embryonic cells; and iv) miscarriages or premature rupture of membrane.

Conclusions

The prevalence of HPV16 and HPV18 in this study were lower than other studies in Asian countries. The results of HR-HPV prevalence and type distribution may be useful for public health authorities in assessing the cervical screening program.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6): 394-424. doi:10.3322/caac.21492
2. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1): 12-19. doi:10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F
3. Chelimo C, Wouldes TA, Cameron LD, Elwood JM. Risk factors for and prevention of human papillomaviruses (HPV), genital warts and cervical cancer. *J Infect*. 2013;66(3):207-217. doi:10.1016/j.jinf.2012.10.02
4. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348(6): 518-527. doi:10.1056/NEJMoa021641
5. Bulkmans NW, Berkhof J, Bulk S, et al. High-risk HPV type-specific clearance rates in cervical screening. *Br J Cancer*. 2007;96(9):1419-1424. doi:10.1038/sj.bjc.6603653
6. Stanley M. Pathology and epidemiology of HPV infection in females. *Gynecol Oncol*. 2010; 117(2Suppl):S5-S10. doi:10.1016/j.ygyno.2010.01.024
7. Sukvirach S, Smith JS, Tunsakul S, et al. Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. *J Infect Dis.* 2003;187(8):1246-1256. doi:10.1086/373901
8. Sun ZR, Ji YH, Zhou WQ, Zhang SL, Jiang WG, Ruan Q. Characteristics of HPV prevalence among women in Liaoning province, China. *Int J Gynaecol Obstet*. 2010;109(2):105-109. doi:10.1016/j.ijgo.2009.11.02
9. Laowahutanont P, Karalak A, Wongsena M, et al. Prevalence of high risk human papillomavirus infection with different cervical cytological features among women undergoing health examination at the National Cancer Institute, Thailand. *Asian Pac J Cancer Prev*. 2014;15(14):5879-5882. doi:10.7314/apjcp.2014.15.14.5879
10. Roset Bahmanyar E, Paavonen J, Naud P, et al. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. *Gynecol Oncol*. 2012;127(3): 440-450. doi:10.1016/j.ygyno.2012.08.033
11. Natphopsuk S, Settheetham-Ishida W, Pientong C, et al. Human papillomavirus genotypes and cervical cancer in northeast Thailand. *Asian Pac J Cancer Prev.* 2013;14(11):6961-6964. doi:10.7314/

apjcp.2013.14.11.6961

12. Marks MA, Gupta S, Liaw KL, et al. Prevalence and correlates of HPV among women attending family-planning clinics in Thailand. *BMC Infect Dis*. 2015;15:159. doi:10.1186/s12879-015-0886-z

13. Kantathavorn N, Mahidol C, Sritana N, et al. Genotypic distribution of human papillomavirus (HPV) and cervical cytology findings in 5906 Thai women undergoing cervical cancer screening programs. *Infect Agent Cancer.* 2015;10:7. doi:10.1186/s13027-015-0001-5

14. Souho T, Benlemlih M, Bennani B. Human papillomavirus infection and fertility alteration: a systematic review. *PLoS One.* 2015;10(5): e0126936. doi:10.1371/journal.pone.0126936

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ความชุก ชนิด และปัจจัยที่มีความสัมพันธ์กับการติดเชื้อ HPV ในสตรีที่มีผลการตรวจคัดกรองมะเร็งปากมดลูกผิดปกติที่โรงพยาบาลรามาธิบดี

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บทนำ: มะเร็งปากมดลูกมีสาเหตุหลักจากการติดเชื้อไวรัสฮิวแมนแพปพิวโลมาหรือเอชพีวี (Human papillomavirus, HPV) สายพันธุ์เชื้อไวรัสเอชพีวีที่พบส่วนมากในสตรีที่เป็นมะเร็งปากมดลูกคือ เอชพีวี 16 และเอชพีวี 18

วัตถุประสงค์: เพื่อศึกษาความชุก ชนิด และปัจจัยที่เกี่ยวข้องต่อการติดเชื้อไวรัสเอชพีวีในสตรีที่พบความผิดปกติในการตรวจคัดกรองมะเร็งปากมดลูก

วิธีการศึกษา: การวิจัยภาคตัดขวาง ในกลุ่มตัวอย่างสตรีที่ถูกคัดเลือกอย่างเฉพาะเจาะจงและมีผลการตรวจคัดกรองมะเร็งปากมดลูกผิดปกติโดยวิธี Liquid-based ที่คลินิกผู้ป่วยนอกสตรี โรงพยาบาลรามาธิบดี จำนวน 376 คน ตั้งแต่เดือนสิงหาคม พ.ศ. 2558 ถึงเดือนมีนาคม พ.ศ. 2559 การตรวจหาการติดเชื้อและชนิดของไวรัสเอชพีวีโดยวิธี PCR และวิเคราะห์ข้อมูลโดยใช้สถิติเชิงพรรณนา

ผลการศึกษา: ความชุกของการติดเชื้อไวรัสเอชพีวี ในสตรีที่มีผลการตรวจคัดกรองปากมดลูกผิดปกติ คิดเป็นร้อยละ 41.5 โดยร้อยละ 78.8 ติดเชื้อสายพันธุ์เดี่ยว สายพันธุ์ที่พบมากที่สุดคือ เอชพีวี 16 (ร้อยละ 8.5) และเอชพีวี 52 (ร้อยละ 4.5) แต่พบเอชพีวี 18 เพียงร้อยละ 1.9 ปัจจัยที่มีความสัมพันธ์ต่อการติดเชื้อไวรัสเอชพีวีอย่างมีนัยสำคัญ ได้แก่ อายุ การไม่มีบุตร และความผิดปกติของเซลล์ปากมดลูกระดับรุนแรง ($P < .05$)

สรุป: ความชุกของการติดเชื้อไวรัสเอชพีวีในสตรีที่มีผลคัดกรองมะเร็งปากมดลูกผิดปกติอยู่ในระดับสูง โดยความชุกและการกระจายชนิดของเชื้อที่สัมพันธ์กับมะเร็งปากมดลูกจะเป็นประโยชน์ต่อหน่วยงานสาธารณสุขที่เกี่ยวกับการตรวจคัดกรองมะเร็งปากมดลูก

คำสำคัญ: ไวรัสฮิวแมนแพปพิวโลมา ความชุก ปัจจัยเสี่ยง การจำแนกสายพันธุ์

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