

## RESEARCH ARTICLE

# Prevalence of High Risk Human Papillomavirus Infection with Different Cervical Cytological Features among Women Undergoing Health Examination at the National Cancer Institute, Thailand

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### Abstract

High-risk (HR) human papillomavirus (HPV) testing is important in cervical cancer screening for triage colposcopy. The objective of the study was to evaluate the prevalence of HR HPV infection with different cervical cytological features among women undergoing health examination. A total of 2,897 women were retrospectively evaluated between May 2011 to December 2011. DNA was extracted from residual specimens collected during routine liquid-based cytology tests at the National Cancer Institute. Overall, HR HPV prevalence was 9.3% including 1.6% of HPV-16 and 0.4% of HPV-18. Of all 270 HPV positive samples, 211 (78.1%) were HR-HPV non 16/18; 47 (17.4%) were HPV-16 and 12 (4.4%) were HPV-18. The prevalence of HPV infection was similar in all age groups, although a higher rate was observed in women age 31-40 years. Among women with normal cytology, HR HPV positive were found in 6.7%. In abnormal cytology, HR HPV were found 46.7% in atypical squamous cells (ASC), 54.8% in low-grade squamous intraepithelial lesions (LSIL) and 80.0% in high-grade squamous intraepithelial lesions (HSIL). HPV-16 was detected in 8.6%, 6.4% and 12.0% of ASC, LSIL and HSIL, respectively. The results of this study provide baseline information on the HPV type distribution, which may be useful for clinicians to decide who should be monitored or treated more aggressively.

**Keywords:** Human papillomavirus (HPV) - prevalence - cytology - Thailand

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### Introduction

Cervical cancer is the second leading cancer among Thai women, the incidence rate was 16.7/100,00 (Khuhaprema, 2013). Cervical cancer screening based on the Papanicolaou (Pap) smear test has been credited with a significant reduction in the incidence of this disease. For a several decades, most of hospitals in Thailand using this method for cervical cancer screening. Today, certain types of human papillomavirus (HPV) have now conclusively been shown as a necessary cause of cervical cancer (Wallboom, 1999). In addition, epidemiologic studies have shown a strong association between high-risk HPV types and the development of high-grade cervical intraepithelial neoplasias (CIN) (Kjaer, 2002; Sclcut, 2001; Woodma, 2001). Alternative technologies such as liquid based cytology or human papillomavirus (HPV) screening or vaccination have been proposed as possible means to improve prevention of cervical cancer. (IARC, 2005; 2007)

According to the ASCCP (American Society for

Colposcopy and Cervical Pathology) 2001 Consensus Guidelines for the Management of Women with Cervical Cytological Abnormalities (Wright, 2002), women with ASC-US should be managed using a program of two repeated cytology tests, immediate colposcopy or HPV DNA testing for high-risk HPV types. Women with ASC-H, LSIL, HSIL and atypical glandular cells should be referred for immediate colposcopy evaluation, regardless of the result of HPV testing. Therefore, detection of HR-HPV is becoming increasingly attractive as a primary screening tool.

The aim of this study is to describes the prevalence of HPV infection and to investigate the role of HPV infection in women with normal and abnormal cervical cytology.

### Materials and Methods

During May to December 2011, a total of 2897 females, with a mean age of 39.02±12.00 years (range, 20-55 years), underwent cervical cancer screening for routine health check up at National Cancer Institute were

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recruited in the study. This study was approved by the Institution Review Board and Ethic Committee of the National Cancer Institute of Thailand.

In our gynecological outpatient clinic, cervical cancer screening were performed by the combination of HPV testing and liquid-based cytology testing. The cervical sample is taken from the patients in the usual manner, cervical smear specimen were placed in tubes with specimen transport medium. The liquid-based cytology were obtained using Thin-prep system (Hologic, Marlborough, MA). A cytotechnician reviewed all the slides and referred any abnormal findings to a cytopathologist. The cytologic results were classified according to the Bethesda system by pathology department. Abnormal cytology were defined as ASCUS or higher grade of cell abnormalities. Women underwent colposcopy (and further biopsy to visualize suspected lesions) if abnormalities were detected in cytology or HPV infection. The pathologist read the biopsies and classified them as follows: normal; cervicitis; cervical intraepithelial neoplasia (CIN) grade 1, 2, or 3, or; invasive cervical cancer (ICC).

HPV testing were performed by Cervista HPV HR and Cervista HPV 16/18 tests (Hologic, Marlborough, MA). The former is a qualitative tests for the detection of DNA from 14 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) while latter is a genotyping test for detection of HPV 16 and 18. Both tests use the chemistry (Hologic, Inc), a signal amplification method for detection of specific nucleic acid sequences (Day SP 2009).

**Results**

The study population consist of 2897 women aged 20 to 55 years (mean±SD: 39.02±12.00 Years). 61.5% of the participants have children, 22.8% were unmarried and only 1.2% reported for smoking, these factors were not different between HPV positive or negative results (data not shown).

HPV were detected in 270 of 2897 women examined

**Table 1. HPV Prevalence in Each Age Groups**

| Age   | HPV Result       |                  |                    |            |            |
|-------|------------------|------------------|--------------------|------------|------------|
|       | HPV Negative (%) | HPV Positive (%) | HR (%) (Non 16,18) | HPV 16 (%) | HPV 18 (%) |
| All   | 2627 (90.7)      | 270 (9.3)        | 211 (7.3)          | 47 (1.6)   | 12 (0.4)   |
| 20-30 | 97 (89.8)        | 11 (10.2)        | 6 (5.6)            | 2 (1.9)    | 3 (2.8)    |
| 31-40 | 731 (88.3)       | 97 (11.7)        | 79 (9.5)           | 15 (1.8)   | 3 (0.4)    |
| 41-50 | 1083 (91.9)      | 95 (8.1)         | 79 (6.7)           | 13 (1.1)   | 3 (0.3)    |
| 51-60 | 687 (91.6)       | 63 (8.4)         | 45 (6.0)           | 16 (2.1)   | 2 (0.3)    |
| >60   | 29 (87.9)        | 4 (12.1)         | 2 (6.1)            | 1 (3.0)    | 1 (3.0)    |

**Table 4. Comparison HPV Prevalence in Different Cancer Hospitals**

| Cancer Center    | HPV Result       |                  |            |            |            |                   |
|------------------|------------------|------------------|------------|------------|------------|-------------------|
|                  | HPV Negative (%) | HPV Positive (%) | HR (%)     | HPV 16 (%) | HPV 18 (%) | HPV 16 and 18 (%) |
| Bangkok          | 2627 (90.7)      | 270 (9.3)        | 211 (7.3)  | 47 (1.6)   | 12 (0.4)   | -                 |
| Lop buri         | 1074 (88.0)      | 146 (12.1)       | 96 (7.9)   | 45 (3.7)   | 2 (0.2)    | 3 (0.3)           |
| Ubon Ratchathani | 594 (81.6)       | 134 (18.4)       | 85 (11.7)  | 30 (4.1)   | 9 (1.2)    | 10 (1.4)          |
| Udon Thani       | 926 (77.6)       | 267 (22.4)       | 208 (17.4) | 52 (4.4)   | 7 (0.6)    | -                 |

(9.3%) (Table 1). 7.3% (211/2897) of these women were infected with non 16, 18 HR HPV 47 cases (1.6%) were positive for HPV 16 and 12 cases (0.4%) were positive for HPV 18. Of all HPV positive 270 samples, 211 (78.1%) were HR-HPV non 16, 18; 47 (17.4%) were HPV-16 and 12 (4.4%) were HPV-18. The prevalence of HPV look similar in all age group with higher rate was observed in the group of 31-40 years. There were 161 (5.5%) women with abnormal cytology and 2735 (94.5%) women with normal cytology. Abnormal cervical cytology were diagnosed as follows; 53.3% atypical squamous cells (ASC), 45.2% low grade squamous cells intraepithelial lesions (LSIL) and 20% high grade squamous cells intraepithelial lesions (HSIL). Within the normal cytology samples, HPV positive were found in 6.7% (Table 2). In abnormal cytology, HPV positive were found in 53.4% of women which consist of 46.7% in ASC, 54.8% in LSIL and 80% in HSIL. Among ASC cytology sample, 34.2%, 8.6% and 3.8% were positive for non 16, 18 HR HPV 16 and 18, respectively. In LSIL sample, 48.4% and 6.4% were positive for HR and HPV 16. While, in HSIL, 56%, 12% and 12% were positive for non 16, 18 HR, HPV 16 and HPV 18, respectively.

We further investigated in the group of HPV-positive with normal cytology who underwent colposcopy. The HPV prevalence in normal, CIN I, CIN II, CIN III and cervical cancer were 57%, 90.9%, 100%, 50% and 100%, respectively (Table 3). In addition, we compared HPV prevalence among health examination women in different cancer hospitals that use the same technique. The prevalence of HPV infection in Lopburi cancer hospital,

**Table 2. HPV Prevalence and Cytology Results**

| Cytology Report | HPV Result       |                  |                    |            |            |
|-----------------|------------------|------------------|--------------------|------------|------------|
|                 | HPV Negative (%) | HPV Positive (%) | HR (%) (Non 16,18) | HPV 16 (%) | HPV 18 (%) |
| All             | 2627 (90.7)      | 270 (9.3)        | 211 (7.3)          | 47 (1.6)   | 12 (0.4)   |
| Normal          | 2551 (68.3)      | 184 (6.7)        | 146 (5.3)          | 33 (1.2)   | 5 (0.1)    |
| Abnormal        | 75 (46.6)        | 86 (53.4)        | 65 (40.4)          | 14 (8.7)   | 7 (4.0)    |
| ASC             | 56 (53.3)        | 49 (46.7)        | 36 (34.2)          | 9 (8.6)    | 4 (3.8)    |
| LSIL            | 14 (45.2)        | 17 (54.8)        | 15 (48.4)          | 2 (6.4)    | -          |
| HSIL            | 5 (20.0)         | 20 (80.0)        | 14 (56.0)          | 3 (12.0)   | 3 (12.0)   |

**Table 3. HPV Prevalence and Colposcopy Results**

| Colposcopy | HPV Result       |                  |                    |            |            |
|------------|------------------|------------------|--------------------|------------|------------|
|            | HPV Negative (%) | HPV Positive (%) | HR (%) (Non 16,18) | HPV 16 (%) | HPV 18 (%) |
| Normal     | 28 (43.0)        | 37 (57.0)        | 18 (27.7)          | 15 (23.1)  | 4 (6.2)    |
| CIN I      | 1 (9.1)          | 10 (90.9)        | 9 (81.8)           | 1 (9.1)    | -          |
| CIN II     | -                | 4 (100.0)        | 3 (75.0)           | 1 (25.0)   | -          |
| CIN III    | 2 (50.0)         | 2 (50.0)         | 1 (25.0)           | 1 (25.0)   | -          |
| CA         | -                | 1 (100.0)        | 1 (100.0)          | -          | -          |
| All        | 31               | 54               | 32                 | 18         | 4          |

Ubon Rathathani cancer hospital and Udon Thani were 12.1%, 18.4% and 22.4%, respectively (Table 4).

## Discussion

The overall HPV infection in this study was 9.3% which consist of 7.3% non 16,18 HR-HPV 1.6% HPV-16 and 0.4% HPV-18. Age-standardized HPV prevalence varied nearly 20 times between populations, from 1.4% in Spain to 25.6% in Nigeria (Clifford et al., 2005). Sukvirach et al. also reported the different of HPV prevalence in Thailand (Sukvirach et al, 2003). In low incidence area of cervical cancer in Songkhla HPV infection was found 3.8%, while in high incidence area in Lampang HPV infection was found 8.0%. Earlier studies of cervical and vulvar lesions in Thailand demonstrated HPV-16 to be most prevalent (Natphopsuk et al., 2013; Ngamkham et al., 2013; Swangvaree et al., 2013; Siriaunkgul et al., 2014).

In addition, many studies reported the prevalence of HPV infection varied according to age group (Coupe et al., 2008, Argyri et al., 2013), however others have not identified a significant relation (Burd 2003). Our results show that the prevalence of HPV infection were similar in all age groups.

As expected, HPV positivity was greater in higher grade cytology abnormalities. The prevalence of HPV infection in normal, ASC, LSIL and HSIL was 6.7%, 46.7%, 54.8% and 80%, respectively. In 2008, Bao et al. report that overall HPV prevalence in women with normal cervical cytology was estimated to be 14.4% in Asia in a meta-analysis of 79 studies. In the LSIL group, the prevalence ranged from 33.3% (India) to 74.6% (Korea and Japan). For the HSIL group, the prevalence ranged from 75.1% (China, Hong Kong) to 85.2% (Korea, Japan)

The American Society for Colposcopy and Cervical Pathology (ASCCP) suggests that women  $\geq 30$  years old, with a negative cytopathological test but a positive high-risk (HR) human papillomavirus (HPV) test should undergo HPV 16 and HPV 18 genotyping. If this test is positive, immediate cervical pathology is required. In Thailand, we conducted the last version of the clinical practice guideline for cervical cancer screening and treatment follow this recommendation. The estimated HPV16/18 positive fraction was 40.4%, 26.7% and 3.3% in women with HSIL, LSIL and normal cytology (Bao, 2008). In our study, the prevalence of HPV16/18 infection was lower than of previous report in other countries. In HSIL, LSIL and normal cytology, 24%, 6.4% and 1.3% HPV16/18 infection were found, respectively.

Although this study presented here included a large number of women of a broad age range, it has some limitations. Our sample is representative of women presenting for health examination, our sample cannot be considered representative of the Thai women. The prevalence of HPV infection varied from 9.3% in Bangkok to 22.4% in Udon Thani, this might be related with hospital based patient accrual and high rate of women with abnormal cytology.

In conclusion, the prevalence of HPV-HR, 16 and 18 in our study were lower than other studies in Asian countries.

The results of this study provide baseline information on the HPV type distribution, which may useful for clinicians to decide who should be monitored or treated more aggressively. However, larger epidemiological studies in different regions in our country are needed in order to report the accurate prevalence of HPV infection.

## References

- Bao YP, Li N, Smith JS, Qiao YL (2008). ACCPAB members. Human papillomavirus type distribution in women from Asia: a meta-analysis. *Int J Gynecol Cancer*, **18**, 71-9.
- Burd E (2003). Human papillomavirus and cervical cancer. *Clin Microbiol Rev*, **16**, 1-17.
- Clifford GM, Gallus S, Herrero R, et al (2005). Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet*, **366**, 991-8.
- Coupe VMH, Berkhof J, Bulkman NW, Snijders PJ, Meijer CJ (2008). Age dependent prevalence of 14 high-risk HPV types in the Netherlands: implications for prophylactic vaccination and screening. *Br J Cancer*, **98**, 646-51.
- Day SP, Hudson A, Mast A, et al (2009). Analytical performance of the investigational use only Cervista HPV HR test as determined by a multi-center study. *J Clin Virol*, **45**, 63-72.
- Argyri E, Stefanos Pappaspyridakos, Elpida Tsimplaki, et al (2013). A cross sectional study of HPV type prevalence according to age and cytology. *BMC Infectious Diseases*, **13**, 53.
- International Agency for Research on Cancer (2005). IARC/WHO handbooks of cancer prevention. Vol 10: cervix cancer screening. IARC Press.
- International Agency for Research on Cancer (2007). IARC monographs on the evaluation of carcinogenic risks to humans. Vol 90: human papillomaviruses. IARC Press.
- Kjaer SK, van den Brule AJC, Paull G, et al (2002). Type-specific persistence of high-risk human papillomavirus (HPV) is the major indicator of high-grade cervical squamous intraepithelial lesions (SIL) in 20-29 years old women. *BMJ*, **325**, 572-3.
- Khuahaprema, T, Attasara P, Sriplung H, et al (2013). Cancer incidence in Thailand 2007-2009. National Cancer Institute. Bangkok, Thailand.
- Natphopsuk S, Settheetham-Ishida W, Pientong C, et al (2013). Human papillomavirus genotypes and cervical cancer in northeast Thailand. *Asian Pac J Cancer Prev*, **14**, 6961-4.
- Ngamkham J, Homcha-Aim P, Boonmark K, Phansri T, Swangvaree SS (2013). Preliminary study on human papillomavirus frequency and specific type-distribution in vulva cancer from Thai women. *Asian Pac J Cancer Prev*, **14**, 2355-9.
- Schlect NF, Kulafa S, Robitaille J, et al (2001). Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA*, **286**, 3106-14.
- Siriaunkgul S, Settakorn J, Sukpan K, et al (2014). HPV detection and genotyping in vulvar squamous cell carcinoma in northern Thailand. *Asian Pac J Cancer Prev*, **15**, 3773-8.
- Sukvirach S, Smith JS, Tunsakul S, et al (2003). Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. *J Infect Dis*, **187**, 1246-56.
- Swangvaree SS, Kongkaew P, Ngamkham J (2013). Frequency and type-distribution of human papillomavirus from paraffin-embedded blocks of high grade cervical intraepithelial neoplasia lesions in Thailand. *Asian Pac J Cancer Prev*, **14**, 1023-6.
- Walboomers JM, Jacobs MV, Manos MM, et al (1999). Human

papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*, **189**, 12-9.

Woodman CB, Collins S, Winther H, et al (2001). Natural history of cervical human papillomavirus in young women: a longitudinal cohort study. *Lancet*, **357**, 1831-6.

Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ (2002). 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. *JAMA*, **287**, 2120-9.