RESEARCH ARTICLE

Cervical Pathology in Cytology-Negative/HPV-Positive Women: Results from Lampang Cancer Hospital, Thailand

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Abstract

Background: To evaluate the cervical pathology of cytology-negative/high-risk human papillomavirus (HR-HPV) positive-women. Materials and Methods: This study recruited 4,583 women aged 30-70 years who had undergone cervical screening by liquid-based cytology and HR-HPV test (14 HR-HPV types) at Lampang Cancer Hospital during October 2012 to July 2013. Colposcopy was carried out in all women. Results: One hundred and ninety-two (4.19%) women were found to be cytology-negative/HR-HPV-positive. However, 23 cases were excluded because of incomplete information, leaving 169 women for further analyses. Of these 169, 45 (26.6%) were infected with HPV 16/18 and 49 (29.0%) with multiple genotypes of HR-HPV. Nineteen of 169 (11.24%) women were found to have CIN 2-3. No women in the present study had AIS or invasive cervical lesions. Prevalence of CIN 2-3 among women infected with HPV 16/18 was 15.6% which was higher than the 9.68% in those with non-HPV 16/18 oncogenic types. Conclusions: Overall, 11% of cytology-negative/HR-HPV-positive women had significant cervical lesions. Risk of harboring such lesions was substantially increased among those who were HPV 16/18 positive.

Keywords: Cervical pathology - human papillomavirus - genotyping - HPV subtypes - CIN

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Introduction

Cervical cancer remains the major health problem among women living in developing countries, mainly because of the failure either to initiate or sustain effective screening strategy. In the recent population-based survey in Thailand, the highest incidence of cervical cancer was noted in Chiang Mai Province with an age-standardized incidence rate (ASR) of 28.9 per 100000, followed by Lampang Province (ASR, 22.4) (Moore et al., 2010).

The detection of cancer precursors by cervical cytology screening is generally acknowledged as an effective method for preventing cervical carcinoma. Nevertheless, cytological screening in routine practice has a notably low sensitivity. It has been suggested that testing for high-risk human papillomavirus (HPV) infection as an adjunct of cytology, or the so-called "cotest", could maximize identification of women at greatest risk of high-grade cervical intraepithelial neoplasia (CIN) or cancer (Kitchener et al., 2009; Ronco et al., 2010; Rijkaart et al., 2012; Cox et al., 2013).

In the recent consensus guidelines updated by the American Society for Colposcopy and Cervical Pathology, cotest is the preferred approach for cervical cancer screening for women aged 30-64 years. If possible, cytology-negative/HR-HPV positive women should be genotyped for HPV 16/18. With this option, women who are found to have HPV 16/18 women are recommended to undergo colposcopy whereas for those without these two high-risk types are for cotest to be repeat after 1 year (Massad et al., 2013).

Management of abnormal cervical cancer screening result mainly depends on the risk of encountering significant lesion. To our knowledge, there is no published report regarding cervical pathology among cytology-negative/HR-HPV-positive women in Thailand. Accordingly, the present study was conducted to evaluate underlying cervical pathology in cytology-negative/HPV-positive women. Our findings reflect the results from a region that has a high incidence of invasive cervical cancer.

Materials and Methods

After obtaining institutional review board approval at the Lampang Cancer Hospital, we recruited 4583 women aged 30-70 years who had undergone cervical screening by liquid-based cytology and HR-HPV test (14 HR-HPV types) at Lampang Cancer Hospital during October 2012 to July 2013. Pregnant women were excluded, as well as women with a previous history of abnormal cervical cytology of any grade or cancer of any site. Inform consent

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was obtained from each participant recruited in this study.

A gynecologic examination was conducted in which cervical samples were obtained and placed into liquid-based cytology medium (PathTezt, Biocytech Corp, Perak, Malaysia). Interpretation of cervical smear was done as per the 2001 Bethesda System. Cervical samples were also tested for 14 types of high-risk HPV DNA (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using EIA kit HPV GP HR (Diassay B.V. , Rijswijk, The Netherlands) and all positive samples were genotyped using a PCR-based assay (Inno-LiPa HPV Genotyping, Innogenetics, Ghent, Belgium).

Colposcopy was carried out in all women without cytological abnormality who found to have HR-HPV infection by KP who is a well-trained colposcopist. Colposcopic examination was performed following the application of 3-5% acetic acid solution on the upper vagina and cervix. The severity of colposcopic findings was graded based on the severity of acetowhite lesions, sharpness of the lesion margins, and vascular patterns within the acetowhite lesions. Colposcopically-directed biopsy (CDB) was taken from the area with the most severe abnormal appearance. In case without definite abnormal lesion, a CDB was randomly performed. Diagnostic conization was carried out if the initial work-up results revealed high-grade lesions or suspicion of occult invasive cervical lesion.

The final histological diagnosis was made on the most severe histological results obtained after initial colposcopy. High-grade cervical pathology was defined as CIN 2-3, adenocarcinoma in situ (AIS) and invasive cancer.

Baseline characteristics and history related to cervical cancer risk including age at first coitus, number of sexual partner, smoking, and results of cervical pathology obtained from colposcopic procedure were abstracted.

Statistical analysis was carried out with SPSS software (IBM, Armonk, NY, USA). Descriptive statistics were used for demographic data. The association between the prevalence of high-grade cervical pathology and baseline characteristics, genotype of HR-HPV was analyzed via the χ^2 or Fisher exact test, as appropriate. $p<0.05$ was considered statistically significant.

Results

Of 4583 women who had been screened, mean age was 48.9 years with a range of 30-70 years. Negative cervical cytology result was observed in 4476 (97.66%) women. One hundred and ninety-two (4.19%) women were found to have cytology- negative/HR-HPV-positive. However, 23 cases were excluded because of incomplete information, leaving 169 women for further analyses.

Table 1 displays the association between characteristics of 4476 women having normal cytology results and risk of encountering HR-HPV infection. The risk of HR-HPV infection decreased progressively with age. For women aged 30-39 years, 47 of 667 (7.05%) had HR-HPV infection compared to that of 4.38% and 3.41% in women aged 40-49 years and 50 years and older, respectively. Postmenopausal women carried a lower risk of HR-HPV infection than premenopausal women (3.49% *vs* 4.90%, respectively). With reference to smoking behavior, women with history of smoking had a higher risk of HR-HPV infection than those who had never smoked (6.30% *vs* 5.31%, respectively).

Mean age and mean age at first coitus among 169 women who had positive HR-HPV without cytological abnormality eligible in the present study were 47.8 years and 21.6 years, respectively. Of 169 women, 45(26.63%) infected with HPV 16/18 genotypes and 49 (28.99%) infected with multiple genotypes of HR-HPV (Table 2).

Table 1. Epidemiological Variables Associated with HR-HPV Infection among 4476 Women who had Normal Cervical Smear Results

Risk factor		Total number of subjects (n=4476)	HR-HPV positive patients (n=192)	p value
Age(years)	30-39	667	47 (7.05%)	<0.001
	40-49	1554	68 (4.38%)	
	≥ 50	2255	77 (3.41%)	
Menstrual status	Premenopausae	2530	124 (4.90%)	0.025
	Postmenopause	1946	68 (3.49%)	
History of smoking	Present	651	41 (6.30%)	0.006
	Absent	3825	151 (5.31%)	
Number of Parity	0	415	22 (5.30%)	0.05
	1	865	62 (5.55%)	
	≥2	3196	122 (3.82%)	
Contraceptive method	OCPs	779	36 (4.62%)	0.934
	Others	3317	151 (4.55%)	

HR-HPV, high-risk Human Papillomavirus; OCPs, oral combined pills

Table 2. Results of HR-HPV Infectionin 169 Women with Cytology-Negative/HR-HPV Positive

Results		No.	%
Genotypes of HR-HPV infection	16/18	45	26.63
	Other	124	73.37
Number of HR-HPV infection	Single	120	71.01
	Multiple	49	28.99

HR-HPV, high-risk Human Papillomavirus

Table 3. Epidemiological Variables associated with CIN 2-3 in 169Cytology-Negative/HR-HPV Positive Women

Risk factor		No. of subjects	No. women with CIN 2-3(n=19)
Age(years)	30-39	37	3 (8.11%)
	40-49	61	8 (13.11%)
	≥ 50	71	8 (11.27%)
Menopasual status	Premenopausause	107	10 (9.35%)
	Postmenopause	62	9 (14.52%)
Parity number	0	10	0 (0%)
	1	42	4 (9.52%)
	≥2	117	15 (12.82%)
Contraceptive methods	OCPS	36	5 (13.89%)
	Others	133	14 (10.53%)
Age of first coitus (years)	15-20	73	7 (9.59%)
	21-30	90	44 (12.22%)
	> 30	6	2 (16.67%)
Number of lifetime partners	1	132	15 (11.36%)
	≥2	37	4 (10.81%)
History of smoking	Present	41	4 (9.76%)
	Absent	128	15 (11.72%)

HR-HPV, high-risk Human Papillomavirus; CIN, cervical intraepithelial neoplasia; OCPs,oral combined pills

Nineteen of 169 women (11.24%; 95%CI, 6.90-16.99) were found to have CIN 2-3. No women in the present study had AIS or invasive cervical lesions. Table 3 demonstrated baseline characteristics of 19 women who noted to have CIN 2-3.

According to the genotype of HR-HPV, 7 of 45 women with HPV 16/18 (15.56%; 95%CI, 6.49-29.46) had CIN 2-3 while it was noted in 12 of 124 women without these two high-risk types (9.68%; 95%CI, 5.10-16.29).

Discussion

In the present study, the authors evaluated cervical pathology among cytology-negative/HR-HPV positive women and the main findings are that women with cytology-negative/HR-HPV positive carry a certain risk of harboring high-grade cervical disease. This finding is even more striking among those who infected with HPV 16/18. Women with HR-HPV positive in the present study had 11.2% the risk of harboring high-grade cervical lesion, albeit negative cytology. The rate of high-grade cervical lesion among cytology-negative/HR-HPV positive women is influenced by genotype of HR-HPV. Approximately 15.6% of women with HPV 16/18 were found to have high-grade lesion compared to 9.7% of those with non-HPV 16/18 oncogenic types.

Several studies have consistently reported that cervical cancer screening using cotest is more effective than cytology alone for preventing invasive cancer, by providing a high sensitivity to detect cervical cancer precursors (Kitchener et al., 2009; Ronco et al., 2010; Rijkaart et al., 2012; Cox et al., 2013). Additionally, HR-HPV testing with separate HPV 16/18 detection from other oncogenic HPV genotypes is helpful to identify women at greatest risk of encountering cervical cancer precursor (Khan et al., 2005; Cox, et al., 2013). In the Kaiser study, 10-year cumulative incidence rates (CIRs) of CIN 3+ were 17.2% among HPV16-positive women and 13.6% among HPV 18-positive women, but only 3.0% among women infected with other HR-HPV. Based on the notably low 10-years CIR among women infected with non-HPV 16/18 oncogenic types, the authors proposed a less aggressive management of these women (Khan, et al., 2005).

The higher risk of harboring high-grade cervical lesion among women with HPV 16/18 infection was reaffirmed by the ATHENA study which is the first trial to determine the clinical usefulness of HR-HPV 16/18 genotyping among a large cohort of women undergoing routine cervical cytologic screening in the United States. In the ATHENA study, the risk of CIN 2+ in women with HPV 16/18 positive was 11.4% compared with 6.1% in those who infected with other oncogenic types. When set CIN 3+ as an endpoint, the risk was 9.8% for HPV 16/18-positive group and 2.4% for those with 12 other oncogenic types. Results of the ATHENA study support an approach incorporating additional HPV 16/18 genotyping when managing women aged 30 years or older who noted to have cytology-negative/HR-HPV-positive women (Wright et al., 2011).

In the present study, the considerably high prevalence of CIN 2-3 among women infected with HPV 16/18 (15.6%) lend support to the recommendation of immediate colposcopy for these women. Unexpectedly, the prevalence of CIN 2-3 among women infected with non-HPV 16/18 oncogenic types was approximately 10% which was relatively high as comparing with the rate of only 4.6% from the ATHENA study (Wright, et al., 2011). It should be cautiously viewed that the percentage of noncompliance with cervical cancer screening processes among Thai women can be high (Siriaree et al., 2006; Kietpeerakool et al., 2011; Rattanalappaiboon et al., 2014). These raise the question as to whether rescreening with cotest in 1-year interval is appropriate for Thai women with cytology-negative who infected with non-HPV 16/18 oncogenic types.

Impact of age on the rate of HPV positivity has been well acknowledged (ALTS Group, 2003; Leinonen et al., 2009; Chansaenroj et al., 2010; Swangvaree et al., 2010; Kim et al., 2012). For example, Leinonen et al (2009) reported that incidence of HR-HPV infection was solidly impacted by patients' age. Approximately 25% of women aged 29 years or younger had HR-HPV positive compared to 15% and 10% among women aged between 30-34 years and 35-39 years, respectively. HR-HPV infection rate was only 5% in women aged 45 years and older. In the present study, we found pattern in the relative rates of HR-HPV positivity with respect to age. Approximately 7% of women aged 30-39 years had HR-HPV infection whereas the rate of HR-HPV positivity was 4.4% and 3.4% of women aged 40-49 years and 50 years and older, respectively. In addition, postmenopausal women had a lower risk of HR-HPV infection as women in premenopausal group. Our data reaffirms the inverse association between HR-HPV positivity rate and age which currently become a basis of incorporating HPV testing in screening approach.

Smoking has been noted to increase risk of HR-HPV infection (Kim, et al., 2012; Sui et al., 2013). In our study, prevalence of HR-HPV infection among women with history of smoking was slightly higher than that of never smokers (6.3% *vs* 5.3%). However, the underlying mechanism of smoking for increasing the risk of HR-HPV infection remains inconclusive.

In conclusion, 11% of cytology-negative/HR-HPV-positive had underlying high-grade cervical lesion. Risk of harboring such lesion was substantially elevated among those with HPV 16/18 positive. However, the relatively high incidence of CIN 2-3 among women infected with non-HPV 16/18 oncogenic types in the present study needs to be confirmed in a larger study.

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