

RESEARCH ARTICLE

Silent High Grade Cervical Intraepithelial Neoplasia in Atypical Smears from Liquid Based Cervical Cytology - Three Years Experience in Thammasat University Hospital

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Abstract

Purpose: To study the prevalence of CIN2+ diagnosis in women with atypical Papanicolaou (Pap) smears to suggest appropriate management option for Thai health care. **Materials and Methods:** Data from all patients with liquid based cytology with human papillomavirus (HPV) testing between May 2013 - May 2016 were collected from medical records. Women with atypical cervical Pap smears were recruited. Results for age, HPV testing, HPV 16, 18, 45 and other genotypes tested, colposcopic examination and histopathological assessment were all collected. Atypical smears were defined as atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells cannot be exclude high grade squamous intraepithelial lesion (ASC-H). **Results:** A total of 2,144 cases were recruited. Twenty six women with ASC-US on cytology had high risk (HR) HPV detection while eight cases with ASC-H had HR-HPV (40.0% VS 72.7%, $p=0.005$). Among the 26 women with ASC-US cytology and positive HR-HPV, HPV type 16 ($n=8$, 30.8%), type 18 ($n=1$, 3.8%), type 45 ($n=1$, 3.8%) and other HPV types ($n=17$, 65.4%) were found. Eight women with ASC-H and positive HR-HPV demonstrated type 16 ($n=6$, 75%) and other HPV types ($n=2$, 25%). Fifty seven women with ASC-US had normal colposcopy, CIN1 and CIN2+ at percentages of 80.7 (46/57), 14.0 (8/57) and 5.3 (3/57), respectively. In the ASC-H group, 7 out of 10 women had normal colposcopy and three (30%) had CIN2+ results. **Conclusions:** In women with ASC-US cytology, immediate colposcopy is highly recommended. HPV testing can be performed if colposcopy is not an available option because there was high prevalence (5.3%) of CIN2+ in our findings. ASCCP recommendations for ASC-H that colposcopy should be performed on all ASC-H cases regardless of HPV result are thereby supported by the findings of this investigation.

Keywords: ASC-US - ASC-H - cervical intraepithelial neoplasia - colposcopy - HPV

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Introduction

Cervical cancer is one of the most common cancer in many developing countries including Thailand (National cancer Institute., 2013). In 2015, Ministry of Public Health reported 4,500 Thai women death due to cervical cancer and 8,000 new diagnosed cases per year. Bureau of non communicable disease also reported the increasing trend of mortality rate of 0.66 to 0.94% between 2010-2014. The major problem of the high incidence and mortality rate are inadequacy of cervical screening which resulted in advanced stage disease at diagnosis.

Various screening methods, such as the Papanicolaou test (Pap test), HPV (human papillomavirus) testing and genotyping, are used in combination to achieve better detection of precancerous lesions. Because of the lowest

cost, Pap smear is generally a chosen method for early detection of premalignant cervical cancer since year 1940.

In 2012, the American Society for Colposcopy and Cervical Pathology (ASCCP) has been published the updated consensus guidelines for managing abnormal cervical cancer screening tests and precancerous precursors (Stewart et al., 2012). The acceptable management in women with atypical squamous cells of undetermined significance (ASC-US) on Pap smear is either a repeat cytology at 1 year or to perform HPV testing. The recommended management of women with atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H) is a referral for colposcopic evaluation. This is the same recommendation as previous ASCCP guideline in 2006.

Cervical biopsy results of patients diagnosed with

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ASC-US cytology revealed 0-1% cervical squamous cell carcinoma at biopsy, cervical intraepithelial neoplasia 3 (CIN3) in 3% and CIN2 in 1-5 % of the cases (Dermirtas et al., 2015). Patients diagnosed with ASC-H cytology had 4% squamous cell cancer, 39% CIN3 at biopsy, 21% CIN 2 and 26% CIN1 (Bal et al., 2012; Dermirtas et al., 2015). In Thailand, Sangkarat had reported 8% CIN2+ (CIN2/3&cancer) in women with ASC-US cytology and 39% CIN2+ in women with ASC-H (Sangkarat et al., 2014).

This investigation looked into the prevalence of CIN2+ diagnosis in women with atypical cervical Pap smear to suggest appropriate management option for Thai health care.

Materials and Methods

This retrospective study was conducted at the Department of Obstetrics and Gynaecology, Faculty of Medicine Thammasat University Hospital. The approval from Ethics Committee on clinical research of Faculty of Medicine, Thammasat University was obtained prior to the study.

Data were reviewed from medical records by computerized program. Data from all patients with liquid based Pap smear who received HPV testing between May 2013 - May 2016 were collected. Inclusion criteria was patients with atypical cervical Pap smear. Exclusion criteria were women with pregnancy, pre-invasive or invasive cervical lesions or other gynecologic cancers and post-hysterectomy.

Data collected from the medical records included age, HPV testing result, HPV 16, 18, 45 and other genotypes testing, colposcopic examination and histopathological reports.

Descriptive statistics were produced for demographic data of patients. Continuous data were expressed as mean and standard deviation (SD) while categorical data were expressed as number and percentage. In comparing

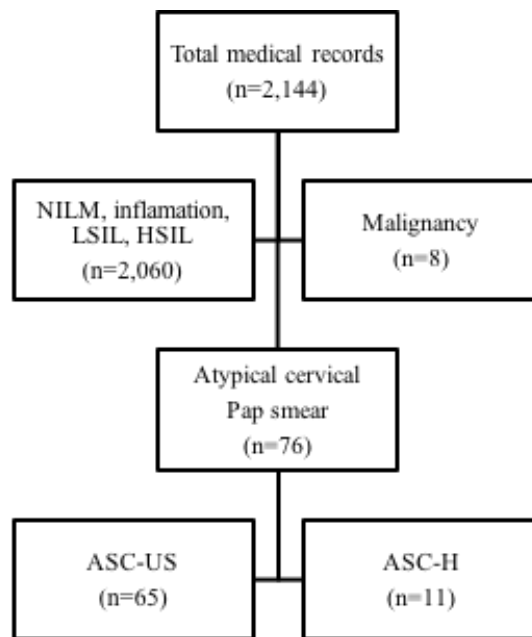


Figure 1. Medical Records Reviewed in this Study.

NILM: negative for intraepithelial lesion or malignancy, LSIL: low-grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells cannot exclude high grade squamous intraepithelial lesion

HPV genotype results between ASC-US and ASC-H patients Chi-square or Fisher’s exact test was used when appropriated. All analyses were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as a p-value of less than 0.05. All two-sided tests with p-value <0.05 were considered statistically significant.

Results

A total of 2,144 medical records were reviewed. Two thousand and sixty women who had negative

Table 1. Prevalence of HR-HPV and HPV Genotype Detection for Atypical Cervical Cytology

	HR HPV*	HPV*(n=2,103)		
		type16	type18/45	non16,18
NILM(n=1229)	110(9.0)	29(26.4)	3(2.7)	79(71.8)
Inflammation(n=768)	74(9.6)	23(31.1)	2(2.7)	49(66.2)
LSIL(n=30)	22(73.3)	2(9.1)	1(4.5)	19(86.4)
ASC-US(n=65)	26(40.0)	8(30.8)	1(3.80)	17(65.4)
ASC-H(n=11)	8(72.7)	6(75.0)		2(25.0)

*n(%), NILM: negative for intraepithelial lesion or malignancy, LSIL: low-grade squamous intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells cannot exclude high grade squamous intraepithelial lesion, HPV: human papillomavirus, HR: HPV: high risk human papillomavirus

Table 2. Cervical Histopathologic Results for Atypical Cervical Cytology

	<CIN 1			CIN 2+		
	type16	type18/45	non16,18	type16	type18/45	non16,18
NILM(n=1997)	0	0	2(0.2)	1(0.1)	1(0.1)	1(0.1)
LSIL(n=30)	0	0	7(23.3)	1(3.3)	1(3.3)	1(3.3)
ASC-US(n=57)	7(12.3)	1(1.8)	10(17.5)	1(1.8)	0	2(3.5)
ASC-H(n=10)	3(30.0)	0	1(10.0)	3(30.0)	0	0

*n(%), NILM: negative for intraepithelial lesion or malignancy, LSIL: low-grade squamous intraepithelial lesion, ASC-US: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells cannot exclude high grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia grade1, CIN2+: cervical intraepithelial neoplasia grade2,3 and cervical cancer

Table 3. Prevalence of ASC-US from the Previous Literature

	Panyanupap	Poomtavorn	Ryu	Watson	Marcos	Tangitgamol	Present
Year	2011	2011	2015	2015	2016	2016	2016
Country	Thailand	Thailand	Korea	Columbia	Brazil	Thailand	Thailand
Pap	CPP, LBP	CPP	LBP			LBP	LBP
case	1,294		40,608	993,238		4,442	2,144
ASC-US*	220 (17.0)	266	3,248(8.0)	446,957(45.0)	703	155(3.5)	64(3.0)
%Colposcopy	100	100	32	14.6	100		100
%HPV test				62.8		99.7	100
%F/U Pap				8.6			
Diagnosis							
%Normal	56.3	49.6	63.7	40.2	90		80.7
%CIN1	31.8	36.5	19.4	46.5	7.3		14
%CIN2/3	11.8	13.9	16.4	11.8	2.7		3.5
%Cancer	0	0	0.6	0.2	0		1.8

*n(%), Pap: Papanicolaou smear, ASC-US: atypical squamous cells of undetermined significance, HPV: human papillomavirus, F/U: follow up, CIN 1: cervical intraepithelial neoplasia grade1, CIN2/3: cervical intraepithelial neoplasia grade2,3, CPP: conventional cervical Pap smear, LBP: liquid based cytology

for intraepithelial lesion or malignancy (NILM), inflammation, low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) were excluded. Eight women who were diagnosed with cervical cancer also excluded. The remaining cases were 76 patients with atypical cervical Pap smear. Sixty-five and 11 women were diagnosed with ASC-US and ASC-H, respectively (Figure 1).

Women with ASC-US were younger than women with ASC-H without statistical significance (47.0 ± 1.2 VS 48.9 ± 2.4 , $p=0.920$).

Seventy six women underwent high risk HPV (HR-HPV) testing. Thirty four (44.7%) women had high risk HR-HPV detected. Twenty six women with ASC-US on cytology had HR-HPV detection while eight cases with ASC-H had HR-HPV (40.0% VS 72.7%, $p=0.005$).

Table 1 shows prevalence of HR-HPV and HPV type detection for atypical cervical Pap smear cytology. Among the 26 women with ASC-US cytology with positive HR-HPV, the following HPV genotype testing were identified. Type 16 (n=8, 30.8%), type 18 (n=1, 3.8%), type 45 (n=1, 3.8%) and other HPV types (n=17, 65.4%) were found respectively. One women was both HPV type 18 and 45 positive. 8 women with ASC-H and HR-HPV revealed type 16 (n=6, 75%) and other HPV types (n=2, 25%). No HPV type 18 and 45 were found.

From Table 2, 8 of 65 women with ASC-US denied colposcopy. The remaining 57 women had normal colposcopy with CIN1 (n=54, 94.7%) and CIN2+ (n=3, 5.3%) result. In ASC-H group, one woman refused colposcopy. In 7 out of 10 women who underwent colposcopy had normal colposcopy and three of them (30%) had CIN 2+ result.

Discussion

In Thailand, majority of cervical screening was performed by Pap smear. It is known to be quiet specific but somewhat questionable in its sensitivity (Chatzistamatiou et al., 2016). At Thammasat University Hospital, the ASCCP guideline recommendation was followed. Ninety percent of women received conventional Pap smear sponsored by the government and health

insurance scheme. Liquid based cytology was used in the rest of the screened population who paid their own screening expense.

In the 2006 ASCCP guideline for ASC-US had recommended immediate colposcopy, serial cervical cytology or colposcopy in case of positive HR-HPV testing. The latest 2012 guideline was based on using mostly liquid based analysis. They recommend HR-HPV testing and performed colposcopy in case of positive HR-HPV test. Co testing at 3 year later in case of HR-HPV negative (ASCCP). Repeat cervical cytology is another option for ASC-US cases without HR-HPV testing facility. Immediate colposcopy for women with ASC-H is still recommended.

The present study found 3.03% prevalence of ASC-US and 0.5% of ASC-H. This finding is consistent with other studies (Ryu et al., 2015; Tangjitgamol et al., 2016). This investigation found that women with ASC-H significantly had more case of positive HR-HPV test than those with ASC-US (72.7% VS 40.0%, $p=0.005$).

In ASC-US group, 26 out of 65 (40%) women had positive HR-HPV result which required immediate colposcopy per 2012 ASCCP guideline. Furthermore, three women (5.3%) were found with CIN2+ after pathological result came out. This meant that if only Pap smear was performed at 1 year on ASC-US patient, 5.3% of women would receive delayed diagnosis of high grade cervical intraepithelial neoplasia.

Seventy two percent of our subject in ASC-H group were positive HR-HPV result. They thus required immediate colposcopy per 2012 ASCCP guideline. Colposcopy resulted in 30% with CIN2+ diagnosis.

Our finding leads to a recommendation for patients with ASC-US cytology. Colposcopy should be immediately performed on this group of patients. HPV testing can be performed if colposcopy is not an available option because there was high prevalence (5.3%) of CIN2+ in our finding. Although this is not a recommendation per ASCCP guideline, however Tantitamit's, Perksanusak's and Nishimura's research groups also gave the same recommendation (Nishimura et al., 2015; Perksanusak et al., 2015; Tantitamit et al., 2015). Our recommendation is a suitable one for patients of Southeast Asia region. Our

finding also supports ASCCP recommendation for ASC-H that colposcopy should be performed on all ASC-H cases regardless of their HPV result.

Acknowledgements

Colposcopy should be immediately performed in women patients with ASC-US or ASC-H cytology. Repeat cervical cytology should be done in ASC-US cases with negative HR-HPV testing.

References

Bal MS, Goyal R, Suri AK, et al (2012). Detection of abnormal cervical cytology in Papanicolaou smears. *J Cytol*, **29**, 45-7.

Chatzistamatiou K, Moysiadis T, Moschaki V, et al (2016). Comparison of cytology, HPV DNA testing and HPV 16/18 genotyping alone or combined targeting to the more balanced methodology for cervical cancer screening. *Gynecol Oncol*, **142**, 120-7.

Demirtas GS, Akman L, Demirtas O, et al (2015). Clinical significance of ASCUS and ASC-H cytological abnormality: a six-year experience at a single center. *Eur J Gynaecol Oncol*, **36**, 150-4.

Khunamornpong S, Settakorn J, Sukpan K, et al (2014). Performance of HPV DNA testing with hybrid capture 2 in triaging women with minor cervical cytology abnormalities (ASC-US/LSIL) in Northern Thailand. *Asian Pac J Cancer Prev*, **15**, 10961-6.

Marcos Lopes AC, Campener AB, Henrique LQ (2016). Prevalence of high-grade intraepithelial neoplasia in patients with cytology presenting atypical squamous cells of undetermined significance. *Acta Cyto*, **60**, 139-44.

National Cancer Institute, Ministry of health (2013). The prevalence of cancer patients in Thailand. Bangkok: National Cancer Institute.

Nishimura M, Miyatake T, Nakashima A, et al (2015). Clinical significance of atypical squamous cells of undetermined significance among patients undergoing cervical conization. *Asian Pac J Cancer Prev*, **16**, 8145-7.

Panyanupap A, Thaweekul Y, Poomtavorn Y, et al (2011). Prevalence of high-grade cervical intraepithelial neoplasia (CIN) in patients with atypical squamous cells of undetermined significance (ASC-US) Pap smear: hospital based, dynamic population area. *J Med Assoc Thai*, **94**, 159-63.

Perksanusak T, Sananpanichkul P, Chirdchirm W, et al (2015). Colposcopy requirement of Papanicolaou smear after atypical squamous cells of undetermined significance (ASC-US) by follow-up protocol in an urban gynaecology clinic, a retrospective study in Thailand. *Asian Pac J Cancer Prev*, **16**, 4977-80.

Poomtavorn Y, Suwannarurk K, Thaweekul Y, et al (2011). Risk factors for high-grade cervical intraepithelial neoplasia in patients with atypical squamous cells of undetermined significance (ASC-US) Papanicolaou smears. *Asian Pac J Cancer Prev*, **12**, 235-8.

Ryu K, Lee S, Min K, et al (2015). Reflex human papillomavirus test results as an option for the management of Korean women with atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion. *Gynecol Oncol*, **20**, 635-9.

Sangkarat S, Laiwejpithaya S, Rattanachaiyanont M, et al (2014). performance of Siriraj liquid-based cytology: a single center report concerning over 100,000 samples. *Asian Pac J Cancer Prev*, **5**, 2051-5.

Stewart M, Mark H, Warner K, et al (2013). 2012 updated consensus guidelines for the management of abnormal cervical screening test and cancer. *J Low GenitTract Dis*, **17**, 1-27

Tangjitgamol S, Kantathavorn N, Kittisiam T, et al (2016). Prevalence and associated factors of abnormal cervical cytology and high risk HPV DNA among Bangkok metropolitan women. *Asian Pac J Cancer Prev*, **17**, 3147-53.

Tantitamit T, Termrungruanglert W, Oranratanaphan S, et al (2015). Cost-effectiveness analysis of different management strategies for detection CIN2+ of women with atypical squamous cells of undetermined significance (ASC-US) Pap smear in Thailand. *Asian Pac J Cancer Prev*, **16**, 6857-62.

Tokmak A, Guzel A, Ozgu E, et al (2014). Clinical significance of atypical squamous cells of undetermined significance in detecting preinvasive cervical lesions in post-menopausal Turkish women. *Asian Pac J Cancer Prev*, **15**, 6639-41.

Watson M, Nenard V, Lin L, et al (2015). Provider management of equivocal cervical cancer screening results among underserved women, 2009-2011 : follow -up of atypical squamous cells of undetermined significance. *Cancer Causes Control*, **26**, 759-64.