RESEARCH COMMUNICATION

Clinical Relevance of Atypical Squamous Cells of Undetermined Significance by the 2001 Bethesda System: Experience from a Cervical Cancer High Incidence Region

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Abstract

The aim of this study was to evaluate the underlying lesions and factors predicting cervical intraepithelial neoplasia (CIN) 2+ in women who had "atypical squamous cells of undetermined significance" (ASC-US) on cervical cytology in the region with a high incidence of cervical cancer. This study was prospectively conducted at Chiang Mai University Hospital, Chiang Mai, Thailand. All women with ASC-US cytology undergoing colposcopic evaluation between October 2004 and August 2008 were recruited. During the study period, 208 women were enrolled. Mean age was 44.4 years. The histopathologic results at the initial evaluation were as follows: CIN 2-3, 21 (10.1%); adenocarcinoma in situ, 3 (1.4%); cancer, 5 (2.4%); CIN 1, 26 (12.5%); and no lesions, 153 (73.6%). Multivariate analysis revealed that nulliparity (adjusted odds ratio [aOR] =4.09; 95% confidence interval [CI] = 1.04-16.10) and current oral contraceptive use (aOR=2.85; 95%CI= 1.14-7.15) were independent predictors for having CIN 2+ at the initial colposcopy. At the median follow-up time of 6.7 months, CIN 2-3 lesions were additionally detected in 2 women. In conclusion, ASC-US cytology in our population has a relatively high prevalence of underlying invasive carcinoma. Nulliparity and current oral contraceptive use are independent predictors for harboring CIN 2+.

Key Words: Atypical squamous cells - histopathology - cervical cancer - cervical intraepithelial neoplasia

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Introduction

Atypical squamous cell (ASC) is a category of squamous cell abnormalities on cervical cytology. In the 1988 Bethesda System, exfoliated cervical cells equivocal for specific abnormality were designated as atypical squamous cells of undetermined significance (ASC-US) (National Cancer Institute Workshop, 1989). Possible etiologies of ASC-US widely range from reactive change, reparative process and squamous intraepithelial lesion (SIL) resulting in the notably low clinical reproducibility of interpretation. Therefore, in the Bethesda 2001 System, the interpretation of ASC-US smear was reclassified into the 3 following criteria, 1: negative for SIL or malignancy if smear favored reactive change, 2: atypical squamous cells of undetermined significance (ASC-US) if borderline abnormality was considered, and 3: atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) if smear was suspicious for some but not all of the cytomorphological criteria for high-grade SIL (HSIL) (Solomon et al., 2002).

After implementation of the 2001 Bethesda System for nomenclature of cervical cytology, several studies have consistently demonstrated a low risk of harboring significant cervical lesions among women with ASC-US (Levi et al., 2003; Srodon et al., 2006; Evans et al., 2006; Sherman et al., 2006; Boardman et al., 2006; Selvaggi, 2006; Safaeian et al., 2007; Feng et al., 2007; Feng et al., 2008; Siddiqui et al., 2008). However, in the previous studies from Chiang Mai University Hospital which is in the area with a high incidence of cervical cancer (the age-standardized incidence rate of 29.4 per 100,000), interesting findings were the high incidences of significant lesions, particularly invasive cervical cancer noted in women with ASC-H, low-grade squamous intraepithelial lesion (LSIL), HSIL and squamous cell carcinoma smears when compared to those of the previous reports (Phongnarisorn et al., 2006; Charoenkwan et al., 2006; Kantathavorn et al., 2006; Kietpeerakool et al., 2008).

Regarding such unique results, the high prevalence of underlying high-grade lesions in women with ASC-US cytology is also highly anticipated and a study confirming this hypothesis would be relevant. Therefore, this prospective study was undertaken to evaluate the

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underlying histopathology of women with ASC-US cytology by the 2001 Bethesda System criteria. This would provide more information that could lead the clinicians to manage and counsel women with ASC-US smears appropriately.

Materials and Methods

After approval of the Research Ethics Committee of the Faculty, all women with ASC-US smears who had undergone colposcopy at Chiang Mai University Hospital between October 2004 and August 2008 were prospectively evaluated. All cervical smears in this study were conventional preparations. Exclusion criteria were women with atypical squamous cells smears those did not comply to the 2001 Bethesda System, prior abnormal Pap smear, history of cervical dysplasia or cervical cancer, pregnancy, concurrent other malignancy, concurrent glandular abnormality on cytology.

Colposcopic examination was performed following the application of 5% acetic acid solution at the upper vagina and cervix. The severity of colposcopic finding was based on the density of acetowhite areas, sharpness of lesion margins and abnormal vascular pattern. A colposcopically-directed biopsy was taken from the area with the most abnormal appearance and endocervical evaluation using endocervical brush (ECB) was carried out in all women. Women with CIN 1 or no lesion on cervical biopsy were followed with cervical cytology at 6 months interval. A cervical conization was carried out if the biopsy results showed high-grade lesion or suspicious of occult invasive cancer.

Final histological diagnosis was made on the most severe histological results. Staging of invasive cervical cancer was clinically made according to the recommendation of the International Federation of Gynecology and Obstetrics (FIGO).

After completion of treatment, a follow-up using cervical cytology was scheduled every 4-6 months in first year and was annually thereafter if all consecutive smears were normal. Repeat colposcopy was performed in cases with abnormal cervical smears during follow-up.

The statistical analysis was carried out using SPSS computer software (SPSS Inc, Chicago). The chi-square or Fisher exact test was used to univariately analyze factors related to have underlying CIN 2-3 and invasive cancer. For those factors with a P-value of less than 0.10 in univariate analysis, a multivariate analysis using a logistic regression model was used as well to identify the independent predictors. An odds ratio with a 95% confidence interval (CI) that did not include unity was considered statistically significant.

Results

During the study period, 208 women with ASC-US on Pap smears who had undergone colposcopy and histological evaluation were enrolled. The mean age of women was 44.4 years (range; 17-79 years). Fifty-five (26.4%) women were postmenopausal and all did not receive hormonal replacement therapy. Ten (4.8%) were nulliparous. Eleven (5.3%) had positive human immunodeficiency virus (HIV) testing. One hundred-forty two (68.3%) women had satisfactory colposcopy. Hormonal contraception was currently used in 48 women (23.1%) including oral contraceptive pills (32), depot medroxyprogesterone acetate (13), and progestogen implants (3).

The histopathologic results obtained from the initial colposcopic evaluations of 208 women were as follows: CIN 2-3, 21 (10.1%); adenocarcinoma in situ (AIS), 3 (1.4%); invasive cervical cancer, 5 (2.4%); CIN 1, 26 (12.5%); and no lesions, 153 (73.6%).

Among 5 women who had invasive cervical cancer, all were squamous cell carcinoma. Two, 1, 2 patients had stage IA1, IA2 and IB1 cervical carcinomas, respectively. The univariate analysis which included the age at colposcopy, menopausal status, parity, HIV status and current oral contraceptive use was performed. Nulliparity and current oral contraceptive use were found to have a P-value of less than 0.10. Multivariate analysis using a logistic regression model, which included these 2 significant covariates, was then performed.

Both nulliparity and current oral contraceptive use remained as statistically significant predictors for having high-grade cervical lesions, with approximately 4 and 3 times the risk of harboring CIN 2+, respectively (Table1). At the median follow-up time of 6.7 months, 2 women were newly diagnosed of CIN 2-3. Accordingly, the overall prevalence of underlying CIN 2+ was 14.9 %.

Table 1. Univariate and Multivariate Analyses for Prediction of High-grade and Invasive Cancers at the Time of Initial Colposcopy

Variables		≤CIN 1	CIN2-3/AIS	Cancer	P-value	[a]OR (95%CI)	P-value
Current oral contraceptive use	Yes	23	7	2	0.023	2.85 (1.14-7.15)	0.025
	No	156	17	3			
Parity	Nulliparous	6	4	0	0.044	4.09 (1.04-16.1)	0.044
	Multiparous	173	20	5			
Menopausal status	Pre-menopausal	130	20	3	0.506	Variable removed	
	Post-menopausal	49	4	2			
HIV status	Positive	9	2	0	0.654	Variable removed	
	Negative	170	22	5			
Age	<45	84	12	2	1.000	Variable removed	
	≥45	95	12	3			

CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma *in situ*; [a]Multivariate OR, adjusted odds ratio; CI, confidence interval, Data are numbers

Table 2. Underlying Cervical Lesions in ASC-US Cytology from Various Reports

Reference	Year	Country	No.	CIN1	CIN 2-3	Cancer
Levi et al	2003	USA	86	24.5	7.0	0
Srodon et al	2005	USA	631	11.1	5.9	0
Evans et al	2006	USA	64	14	12.5	0
Sherman et al	2006	USA	497	NA	8.0	0
Boardman et al	2006	USA	215	NA	15.0	0
Selvaggi	2006	USA	127	36.2	11.8	0
Safaeian et al	2007	USA	3,326	NA	15.3	0.06
Feng et al	2007	USA	846	33.9	9.5	0
Feng et al	2008	USA	136	30.9	3.7	0.74
Siddiqui et al	2008	USA	200	29.5	25.5	0
This study	2008	Thailand	208	12.5	12.5	2.4

Data are Number and %. CIN, cervical intraepithelial neoplasia; NA, not available

Discussion

Generally, management options for women with abnormal cervical smears depends on the risk of harboring significant cervical lesions i.e. CIN 2-3, AIS and invasive cervical carcinoma. As aforementioned, the prevalence of underlying significant cervical lesions particularly invasive cancer in women with abnormal cervical cytology in the areas with a high incidence of cervical cancer is relatively high when compare to that in low incidence population (Law et al., 2001; Chichareon et al., 2002; Phongnarisorn et al., 2006; Charoenkwan et al., 2006; Kantathavorn et al., 2006; Kietpeerakool et al., 2008). For ASC-US smears, several studies from North America, where the incidence of cervical cancer is considerably low, have consistently demonstrated that the risk of having invasive lesion is less than 1% (Table 2) (Levi et al., 2003; Srodon et al., 2006; Evans et al., 2006; Sherman et al., 2006; Boardman et al., 2006; Selvaggi, 2006; Safaeian et al., 2007; Feng et al., 2007; Feng et al., 2008; Siddiqui et al., 2008). An interesting finding in this study was that the prevalence of invasive cervical cancer among women with ASC-US smears was notably high as it accounted for 2.4%. This finding reaffirmed the impact of background incidence of cervical cancer on the underlying histopathology of women with abnormal cervical smears and should be taken into account in decision making on patient management.

Infection with high-risk human papillomavirus (HPV) is now considered as a causal factor for cervical cancer. However, less than 1% of women with such infection eventually developed cervical cancer (Tsuda et al., 2003). This raises the possibility of cofactors for cervical carcinogenesis. Oral contraceptives have been noted as a strong cofactor of HPV infection for cervical cancer. In the IARC multicenter case-control study, use of oral contraceptives was the significant cofactor for cervical cancer development, particularly in those who used for more than 5 years (Moreno et al., 2002). Because HPV testing is unavailable in the majority of developing countries, information regarding HPV status is unknown. Therefore, oral contraceptive user should be considered as a population at a high risk of cervical cancer and appropriate screening and management programs are mandatory. In this study, current oral contraceptive use was a significant independent predictor for having CIN 2+.Women who currently used oral contraceptives were approximately 3 times the risk of having CIN 2+. Based on this finding, current oral contraceptive use should be considered in management of women with ASC-US smears.

Interestingly, nulliparity was also noted as independent predictor for having CIN 2+. Nulliparous women had approximately 4 times increased in the probability of having CIN 2+. However, because of the relatively small number of nulliparous women in this study, there is a possibility of statistical significance given by chance. So, further study is warranted to verify this result.

During follow-up, CIN 2-3 lesions were additionally detected in 2 women. Generally, the possible explanations for missing CIN 2-3 at the time of initial colposcopy were the operator/technique-related aspects associated with colposcopy and biopsy, a small focal lesion of CIN 2-3 which may be lost during tissue processing for histological examination and errors by the final evaluator (Bonvicino et al., 2007). Therefore, close follow-up after initial colposcopy is mandatory even in cases whose initial results are not suggestive for high-grade disease in order to detect a possible missing significant lesion.

One strength of this study is that it is prospective by nature, which allowed the investigators to use a uniform management protocol. Additionally, all final diagnoses are histologically confirmed. The limitation of this study includes a lack of central slide review. Despite this limitation, results from this study could be implemented in the areas where the system for slide tracking and review are not widely established.

In conclusion, this study emphasizes important data regarding the following issues: (1) reporting ASC-US cytology in the investigator's area is clinically significance because of a notably higher prevalence of underlying invasive lesion than those previous reports and (2) nulliparity and current oral contraceptive use are independent predictors for harboring CIN 2+ at the initial colposcopic evaluation. These findings should be cautiously considered during patient counseling and treatment planning.

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