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The presence of advanced lesions and associating risk factors for advanced cervical carcinoma in patients with atypical squamous cells of undetermined significance

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Summary

Purpose of investigation: To characterize histopathological status, high-risk human papillomavirus (hr-HPV) infection status, and associated risk factors in patients with atypical squamous cells of undetermined significance (ASCUS). *Materials and Methods:* Cervical biopsies obtained from 130 ASCUS patients were subjected to histopathological examination and hr-HPV testing. Associations between advanced lesions and hr-HPV load or age were analyzed, and the confounding factors for high-grade cervical lesions were identified. *Results:* Cervical biopsies from ASCUS patients had a wide range of pathological states, ranging from normal to invasive cervical carcinoma. High-risk HPV infection was significantly associated with advanced cervical lesions in ASCUS patients; hr-HPV infection and the number of gestations were risk factors for developing advanced cervical disease. *Conclusions:* A significant portion of ASCUS patients harbor advanced cervical lesions. The number of gestations and hr-HPV infection can increase the risk of developing advanced cervical lesions in ASCUS patients.

Key words: Atypical squamous cells of undetermined significance(ASCUS); Cervical cancer; High-risk human papillomavirus; Viral load; Risk factors.

Introduction

Cervical cancer is the third most common cancer worldwide, with 529,800 diagnoses and 275,100 deaths in 2008 alone [1]. Approximately 85% of cervical cancers occur in developing countries [1]. The incidence and mortality rates of cervical cancer have dramatically decreased in recent years due to advances in diagnostic and testing, especially in developed nations [2]. These advanced methods include the Pap smear and the ThinPrep Pap test. The Bethesda system (TBS) was established in 1988 as a way to unify diagnostic terminology for reporting cervical cytological test results. It was subsequently revised in 1991, and again in 2001, and gives a scaled ranking of cervical abnormalities [3]. Abnormal results based on TBS include: atypical squamous cells (ASC); low grade squamous intraepithelial lesion (LGSIL or LSIL); high grade squamous intraepithelial lesion (HGSIL or HSIL); squamous cell carcinoma; atypical glandular cells not otherwise specified (AGC-NOS); atypical glandular cells, suspicious for AIS or cancer (AGC-neoplastic); and adenocarcinoma *in situ* (AIS). Within the category of ASC, there are atypical squamous cells – cannot exclude HSIL (ASC-H), and atypical squamous cells of undetermined significance (ASCUS) [4]. Often diagnosed in the cervical biopsies of patients with abnormal cytological tests are cervical intraepithelial neoplasia (CIN). These premalignant cervical lesions are graded into four groups: normal, CIN I, CIN II, and CIN II [5].

ASCUS are interpreted as undetermined significance since their phenotype is clinical enough to be attributable to reactive changes but benign enough to lack a definitive diagnosis of squamous intraepithelial lesion (SIL), either quantitatively or qualitatively. Since its introduction by the TBS, ASCUS has been problematic for several reasons. First, many scenarios can lead to an ASCUS diagnosis, either due to technical limitations such as poor specimen quality or processing, or simply the nature of the samples. For example, samples with mature intermediate-type or orangeophilic cytoplasm and those with atypical metaplasia will both be categorized as ASCUS. Second, ASCUS is a very unreliable diagnosis due to the broad spectrum of parameters and lack of uniformity [6]. Despite the lack of diagnosis standard, ASCUS accounts for a significant portion of the abnormalities observed in cervical cancer screening [7]; in the U.S., more than two million women were diagnosed with ASCUS.

There is no consensus for the optimal management of patients following an ASCUS diagnosis. Typically, however, patients are tested using follow-up procedures including

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additional cytological testing, colposcopies, and human papillomavirus (HPV) testing. The proper course of action remain contentious, with some arguing that ASCUS patients are over-treated as observed by normal follow-up pathological assessments and others arguing these patients are under-diagnosed with some ASCUS patients do harbor CIN III or even invasive carcinoma [8]. These factors, combined with the observations that patients with an ASCUS diagnosis, are at a higher risk of developing cervical cancer than patients with normal cytological diagnosis [9]. Highlight the importance of properly characterizing the histopathological status of the cervical biopsies from ASCUS patients.

The main cause of cervical carcinoma and cervical precancerous lesions is HPV infection, specifically high risk strains (hr-HPV) [10]. hr-HPV strains are classified according to their tumorigenic potential and include approximately 13 strains. Several additional factors associated with incidence of cervical cancer also correlate with an increased chance of hr-HPV infection. These factors include adolescent intercourse or pregnancy, multiple parity, smoking, using oral contraceptives or intrauterine devices (IUD), multiple sex partners, abortion history, other sexually transmitted diseases, a high body mass index, and low incomes [11, 12]. Moreover, reproductive tract infection (RTI) and hr-HPV infection also induce ASCUS [13].

In this study the authors sought to characterize the histopathological status of ASCUS and identify cervical cancer-related risk factors in ASCUS patients. To accomplish this, they tested 130 ASCUS patients for hr-HPV infection and followed-up with histopathological examination. Each patient's social economical status, sexual activity, and medical history were also obtained, and the association between the cervical lesions grade and specific risk factors were analyzed. These data revealed that hr-HPV viral load correlated with the pathological grade of cervical lesions, and that hr-HPV infection and the number of gestation are the confounding factors to the risk of developing advanced cervical lesions. These findings can help identify ASCUS patients with a high risk of developing cervical cancer and facilitate the development of optimal management strategies in treating these patients.

Materials and Methods

Patient characteristics

A total of 130 patients were recruited from inpatient and outpatient clinics in the First Hospital of Jilin University between January 2011 and November 2012. All patients were diagnosed with ASCUS according to the current TBS classification in a liquid-based ThinPrep Pap test. Inclusion parameters included sexually active, non-gravid**,** and no history of radio/chemotherapy or symptoms of acute genital tract inflammation. The median patient age was 38 years (range from 20 to 66; mean 38.85 ± 8.75).

Patients were informed of the study objectives and design and signed consent forms prior to enrollment. All patients completed a questionnaire inquiring the following information: age, occupation, pertinent clinic symptoms, age of first intercourse, number of sexual partners, numbers of gestation and parity, mode of delivery, the presence of IUD, and history of genital warts in themselves and their partners. The results from this questionnaire were exclusively used for this study. Each patient received a pathological examination and an hr-HPV test. The First Hospital of Jilin University ethics committee approved the study design.

Colposcopy and cervical biopsy

Colposcopies were performed only if the patients had been free of any vaginal operation or sexual intercourse for at least 24 hours. In a dorsal lithotomy position, the vulva of each patient was examined for any suspicious lesions, a speculum was placed in the vagina, and 3% acetic acid was applied to the cervix using cotton swabs for one minute. Any white areas following acetic application or those that had abnormal vascular patterns were considered a higher priority for sampling. Three experienced pathologists conducted histopathological examinations and the histopathological classifications of the biopsies were made according to previous studies [14, 15].

hr-HPV test

For each patient, cervical tissue samples were collected using a cyto-brush with slight rotation in the endometrial canal following spatula scraping. The spatula and brush were then dropped into a collection tube. HPV DNA was isolated and detected using a Hybrid Capture 2 High-Risk HPV DNA Test Kit, a kit that assays for 13 hr-HPV species (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). The threshold for a positive HPV test was 1.0 pg/ml.

Statistical analysis

For data collection, EpiData 3.02 was used to build a database.The data were analyzed by Statistical Package for the Social Sciences (SPSS) version 17.0. A chi-square (χ^2) test was used to compare the histopathological status in each age group ($\alpha = 0.05$). A Mann-Whitney test was used to compare the association between histopathological status and hr-HPV virus load. To identify independent variables that affect the histopathological status of the cervical biopsies from ASCUS patients, unconditional logistic regression analysis ($a_{input}=0.05$, $a_{output}=0.10$) was performed. In the regression analysis, the histopathologic grade, with a threshold of CIN II, was defined as the dependent variable. All pathological, demographic, and socioeconomic factors collected were used as candidate independent variables. Since occupation, clinic symptoms, mode of delivery, and history of genital warts are multi-categorical independent variables, dummy variables were set in SPSS.

Results

Patient characteristics

Of the 130 patients diagnosed with ASCUS according to the current TBS classification, 31 were normal or had inflammatory diseases (23.90%); 29 were CIN I (22.30%), 14 were CIN II (10.80%), 55 were CIN III or cervical carcinoma *in situ* (42.30%), and one had invasive cervical carcinoma (0.80%).

Correlation between age and histopathological status

To analyze the association between age and histopathological status, patients were stratified two groups: < CIN II containing patients who have a normal, inflammation, or CIN I diagnosis; and ≥ CIN II containing patients with CIN II, CIN III, cervical carcinoma *in situ* or invasive cervical

Table 1. — *Histopathological status of ASCUS patients.*

Age (yrs)	Case number (%)	< CIN II	≥ CIN II
20-29	19 (14.62)	11 (8.46)	8 (6.15)
30-39	50 (38.46)	23 (17.69)	27 (20.77)
40-49	47 (36.15)	17 (13.08)	30 (23.08)
50-59	12 (9.23)	8 (6.15)	4 (3.07)
≥60	2 (1.54)	1 (0.77)	1 (0.77)

$\chi^2 = 4.983$; $p = 0.289$.

Table 2. — *Correlation between hr-HPV viral load and histopathological status in ASCUS patients.*

Patient groups	N	Hr-HPV viral load	
		Mean rank	Sum of ranks
< CIN II group	27	43.56	1176.00
≥ CIN II group	99	68.94	6825.00
Total	126		

carcinoma. Each cohort was then divided into five age groups, and χ^2 analysis was used to assess the correlation between histopathological status and age group. The analysis revealed no significant correlation between histopathological status and age of ASCUS patients ($\chi^2 = 4.983$ and $p = 0.289$, Table 1).

Correlation between hr-HPV viral load and histopathological status

Next, the authors sought to analyze the correlation between hr-HPV viral load and histopathological status of the cervical lesions. They found 117 (90.00%) patients were hr-HPV positive and 13 (10.00%) were hr-HPV negative. Since four of the 13 HPV-negative patients were positive for at least one koilocyte, an indication of HPV infection, they were excluded from the subsequent study. The remaining 126 patients were ranked from low to high by their hr-HPV viral load and the ranks in the two established histopathological groups were compared by a Mann-Whitney test. The results revealed a statistically significant correlation between hr-HPV infection level and histopathological grading (U = 798.00; W = 1176.000; Z = -3.273, $p = 0.001$; Table 2).

Identification of independent variables to the risk of developing advanced cervical lesions

Finally, the authors attempted to identify independent variables that may contribute to the risk of developing advanced lesions in ASCUS patients using the demographic information collected at the time of recruitment. To assess the potential impacts of these independent variables on the histopathological status of the cervical lesions, the authors performed an unconditional logistic regression using the established histopathological group status (< CIN II and ≥ CIN II) as dependent variables. Using a forward stepwise regression process, the number of gestations and the presence of hr-HPV infection were found to have significant associations with the outcome in the regression model (Table 3). Both of these factors as independent variables also had a good overall fit by χ^2 test ($\chi^2 = 4.523$, df = 1, $p = 0.033$) and Hosmer-Lemeshow analysis ($\chi^2 = 4.314$, df = 4, $p = 0.365$). The percentage of correct prediction of the model was 53.80%, and the Nagelkerke R^2 value was 0.136. The risk of developing high-grade (≥ CIN II) cervical lesions in ASCUS patients was associated with the number of gestations, with an adjusted odd ratio of 1.424 (Table 3). The impact of being hr-HPV positive on a patient's risk in developing high-grade (≥ CIN II) cervical lesions was also significant, with an adjusted odd ratio of 15.585 (Table 3). Together, these results demonstrated that the number of gestations and hr-HPV infection are confounding factors for the development of high-grade cervical lesions in ASCUS patients.

Table 3. — *Unconditional logistic regression analysis of risk factors for developing high-grade (≥ CIN II) cervical lesions in ASCUS patients.*

Variables	*B*	*S.E.*	*Walds*	*p*	AOR	95.0% *CI*
Constant	-3.224	1.109	8.456	0.004	0.040	—
Time of pregnancy	0.353	0.172	4.243	0.039	1.424	1.017-1.993
hr-HPV infection status	2.746	1.061	6.694	0.010	15.585	1.946-124.792

Discussion

The ASCUS cytological category is problematic, mainly due to its ambiguity and lack of consensus on diagnosis and management for patients with an ASCUS diagnosis. Since ASCUS is diagnosed in a considerable portion of cervical smear cases every year [7], and there is evidence that ASCUS patients have a higher risk of developing cervical cancer [9], it is important to better understand the pathological variations and to identify risk factors associated with advanced cervical lesions in ASCUS patients.

Here, the present authors used pathological examination of cervical biopsies to assess the histopathological status of 130 ASCUS patients and found over 50% of the patients harbor lesions of CIN III or higher grades. Compared to previous reports [16], the present study had a higher percentage of ASCUS patients with advanced lesions. This difference may be explained by the present authors' clinical standard, which classified patients with CIN II-III with gland involvements as CIN III. The present findings supported the need for close follow-up for ASCUS patients.

HPV infection is rather common in young women. HPV infection is a main cause of cervical cancer, and 99.7% of

cervical cancer cases are HPV positive [17]. To date, there are at least 13 carcinogenic, or high-risk HPV strains [18]. HPV infection of cervical epithelial cells, specifically the expression of the E6 and E7 genes, can lead to cervical abnormalities, ranging from ASCUS to invasive carcinoma [12]. The authors found hr-HPV DNA present in 90% of ASCUS patients, and these hr-HPV-positive patients had a ~15-fold increase in the risk of developing high (≥ CIN II) grade lesions. Consistent with previous reports [19], the present authors demonstrated that in ASCUS patients, hr-HPV viral load was significantly higher in patients with advanced cervical lesions (Table 2).

In addition to HPV infection, other physiological and social factors associated with increased risk of HPV infection in women have also been reported to correlate with the development of high grade cervical lesions. For example, women between 30 and 35 year old are more likely to be diagnosed with cervical carcinoma. The present authors therefore tested for a correlation between histopathological status and age in ASCUS patients. However, they found no significant correlation between the two factors. Therefore, they recommend that ASCUS patients, regardless of their ages, should undergo further cervical evaluation.

The number of gestations has been associated with increased risk of cervical cancer. For example, women with seven or more full-term pregnancies were found to have a four-fold increase in the risk of developing cervical cancer than nulliparous women [20]. In addition, multiple parity was reported to be correlated with invasive cervical carcinomas [21], yet fewer pregnancy was correlated with a decrease in cervical cancer incidence and mortality [22]. Several mechanisms have been proposed for such correlation: first, high levels of estrogen can be immune suppressive, therefore increase the patients' susceptibility to hr-HPV infection [23, 24]. Second, elevated progesterone levels, also a hallmark of pregnancy, can upregulate E6 and E7 expression and suppress T-cell function, again increasing the patients' susceptibility to hr-HPV infection [25]. It has also been suggested that in some cases, the positive correlation between multiple gestations and parities and cervical cancer may be partly due to inadequate sterilization of medical devices used during child birth or abortion, which can cause iatrogenic HPV infection [26]. Consistent with such observation, here the present authors identified the number of gestations as a confounding factor to the risk of developing high-grade cervical.

Having sexual intercourse during adolescence increases the risk of having persistent HPV infection, and in turn developing cervical carcinoma. Women who have their first sexual intercourse before turning 17 years old have two- to three-fold higher risk of developing cervical cancer than those having their first intercourse after 20 years of age [27]. In addition, since having multiple sexual partners is often associated with unconsented intercourse with HPV-positive partner during adolescence [28, 29], it can also cause an increase in the risk of having HPV infection, and consequently cervical cancer. The present study demonstrated no significant association between high-grade cervical lesions and the age of first sexual intercourse or the number of sexual partners. The present authors reasoned the lack of association observed here may be due to sampling bias: only a very small number of patients in the study had sexual intercourse during adolescence, and most patients only had one partner as they grew up under the transitional culture influence in the 1960-1970s in China.

In conclusion, the present authors have conducted histopathological examination on cervical biopsies from ASCUS patients and analyzed the risk factors associated with the presence of advanced cervical lesion. Their findings revealed that a significant portion of ASCUS patients had high-grade cancerous lesions and suggested that HPV infection and gestation history are confounding factors to the risk of having advanced cervical lesions. These results are consistent previous studies and further support the use of hr-HPV testing in patients with ASCUS. In addition, the finding that HPV infection and gestation history are confounding factors to having advanced cervical lesions may help in developing optimal follow-up strategies for managing ASCUS patients.

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