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Importance of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H)

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ABSTRACT

In this study, we evaluated the biopsy counterparts of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H) cases. This was a cross-sectional, retrospective study on ASC-H cases. The follow-up results of ASC-H cases diagnosed during routine primary screening between 2011 and 2018 were evaluated, and the relationship between clinicopathological parameters, high risk Human Papilloma Virus (hrHPV) test and patients' ages at diagnosis were evaluated. Among one hundred sixty-nine ASC-H patients, high grade squamous intraepithelial lesion (HSIL) was detected in 56 (33.1%), low grade squamous intraepithelial lesion (LSIL) in 59 (35%), squamous cell carcinoma in 5 (3%) and adenocarcinoma in 1 (0.5%). HPV 16 was detected in 41 (24.4%) cases and HPV 18 in 26 (16%) cases, and 51 patients had low risk of HPV (lrHPV). The mean age of the patients with hrHPV was 31.78 ± 8.22, and the mean age of the patients with lrHPV was 34.77 ± 5.47. HPV positivity in ASC-H smears was significantly correlated with age ($p = 0.004$). General linear regression analysis showed that biopsy results were significantly correlated with HPV in high-risk patients compared to low-risk patients. hrHPV evaluation in ASC-H follow-up is significant for cervical premalignant lesion. A management strategy that requires close monitoring in women with ASCH and under 40 years old, especially in association with hrHPV, should be considered.

1. Introduction

Cervical cancer is an important global health issue: it is the third most common cancer in women worldwide and the second among women aged 15-44 years, with over 529.000 cases of cervical cancer diagnosed every year worldwide (De Sanjose S, 2012). Cervical cancer was responsible for 2.7 million age-weighted years of life lost (YLL) in 2000 (Forman, 2012). Fortunately, thanks to the widespread use of the PapSmear Test (PST) screening method, the incidence has been reduced considerably (Waxman, 2005). However, although PST is useful for cancer screening, it has high false-negative

rates and limitations such as interobserver variability. The presence of atypical cells in smears is one of the most important diagnostic problems for cervical cancer screening (Papanicolaou and Traut, 1997).

In the first Bethesda System described in 1988, "atypical squamous cells of undetermined significance" were identified (Koss, 1989). According to the 2001 Bethesda system, atypical squamous cells (ASC) were divided into two groups: Atypical Squamous Cells of Undetermined Significance (ASC-US) and Atypical Squamous Cells cannot exclude High-grade squamous intraepithelial lesion (ASC-H) (Apgar et al., 2003). ASC-H represents 5-10% of ASC diagnoses and accounts for approximately 0.2% of cytological interpretations (Apgar et al., 2003). The ASC-H category includes ASCs that exhibit characteristics equivalent to the idea of a high-grade squamous intraepithelial lesion (HSIL), but are not sufficient to diagnoss. The probability of having Cervical Intraepithelial Neoplasia-2 (CIN 2) or Cervical Intraepithelial Neoplasia-3 (CIN 3) approved by biopsy for a woman with cervical cytology result interpreted as ASCs are 5-17%, and the underlying HSIL rates in ASC-H patients range from 24% to 94% (Galliano et al., 2011; Gilani et al., 2014).

Recently, it has been shown that ASC-H is not a homogeneous category and has a broad spectrum of cyto-morphological patterns associated with different clinicopathological categories classified by biopsy and Human Papilloma Virus (HPV) test results (Solomon et al., 2002). HPV positivity rates vary between 70-86% in patients with this diagnosis (Solomon et al., 2002). Initial examination of patients diagnosed with ASC-H is recommended as colposcopy since HSIL and higher-grade lesions are more likely to be present (Massad et al., 2013). If no lesion can be shown on colposcopy, Colposcopic - histological - cytological examinations should be reviewed (Massad et al., 2013).

This study investigated the cytology and biopsy results of 169 ASC-H cases identified during routine primary screening, and analyzed abnormal biopsy results, especially in ASC-H cases with a positive test for hrHPV.

2. Materials and methods

The study was conducted in the Oncology Center of the Health Sciences University, Tepecik Training and Research Hospital, which provides tertiary and basic health care services for patients through many health centers. The Local Ethics Committee approved the study (University of Health Sciences, Tepecik Education and Research Hospital Ethical Committee (26/07/2018, No: 2018/9–6). The universal principles of the Helsinki Declaration were applied. In this study, we evaluated the biopsy results of 169 patients diagnosed with ASC-H between January 2010 and September 2018. Age (25-40 years), socioeconomic level (<500, 500-1000, or >1000 dollars per month), educational status (primary, secondary, high school, university), smoking status (smoker or non-smoker), parity, first sexual intercourse age (16-29) and body mass index (BMI) were recorded. Patients were divided into two groups according to the presence of HSIL and hrHPV separately. Demographic parameters were compared in HSIL and non HSIL groups. Patient ages and pathological findings were compared according to HPV risk groups.

Pregnant patients, patients in menopause, those who had a hysterectomy history as well as under hormone replacement therapy, those who had multiple HPV DNA types and those with a history of HPV vaccine were excluded. Patients who could not have satisfactory colposcopic examination or insufficient and/or absent biopsy were also excluded from the study.

Since 2014, women's participation in the HPV DNA screening program across the country has been provided by the KETEM (Cancer Early Diagnosis Screening and Training Center) affiliated with the Ministry of Health (Gultekin et al., 2018). Screening was performed by primary health care personnel at KETEM (family physicians and general practitioners trained in this field). Hybrid Capture 2 kits (Qiagen, Hilden, Germany) were sent to the KETEM screening center by the central laboratory of the Ministry of Health to collect HPV DNA samples. Two samples were taken from each woman participating in the screening, one for traditional cytology test and the other for the HPV DNA test. Cytology test results based on the 2001 Bethesda classification were reported by four pathologists in the central laboratory of the Ministry of Health. The second sample, which was obtained for HPV, was taken with a separate brush and placed in 5 mL of standard transport medium from the Hybrid Capture 2 collection kit for HPV DNA testing. Genotyping was performed using the CLART HPV kit (Genomica, Madrid, Spain) for any patient with a non-specific HPV test result (Gultekin et al., 2018). HPV genotype results were evaluated in the central laboratory of the Ministry of Health, and the results were delivered to recipients online using a fully automated operating system. HPV types 16, 18, 31, 33, 45, 51, 58, 59 and 68 were considered high-risk, 53 and 66 probable high-risk and 6, 11, 40, 54 and 70 were considered low-risk for etiopathogenesis of cervical cancer (Milutin et al., 2008).

All patients underwent colposcopic observation by experienced colposcopists with a binocular colposcope (Colposcope 1D-21100, Leisegang GmbH, 2014-03, Germany) with a green filter capable of 4.5 to 30 magnifications. Cervix was first washed with saline, then scanned at a small magnification, and vascularization pathologies were investigated with a green filter. Then, 3% acetic acid was applied and left for at least 60 seconds, and then the cervix was re-screened at small (x10) and large (x40) magnifications. After the location

of the aceto-white areas and vascular pathologies, Lugol solution was applied. After staining with cervix with lugol solution, iodine-free areas were determined, and cervical biopsy was performed with forceps from iodine-free areas and aceto-white, mosaic, punctuation, leukoplakia and atypical vascular areas. Patients with pathological colposcopic findings were monitored and treated in our center. Cervical biopsy samples were sent to the pathology unit in formaldehyde for evaluation, and biopsies were evaluated by 4 cytopathologists experienced in gynecological oncology.

One hundred sixty-nine ASC-H patients underwent follow-up tissue sampling, including colposcopic biopsy, Loop Electrosurgical Excision Procedure (LEEP), conization, and hysterectomy. LEEP tissue sampling presented satisfactory samples in HSIL cases, and the squamo-columnar junction was not deeper than the first centimeter level of the cervical canal. Conization was performed in cases with deeper problems in LEEP. Radical pelvic surgery was performed in patients with adenocarcinoma and squamous cell carcinoma. The diagnosis obtained from histological specimens were taken as the gold standard. If the cases were analyzed using histology and had more serious findings, later procedures were accepted as definitive diagnosis. Cases with normal histological samples were taken as negative results.

Statistical analysis

Statistical analysis of the data was performed by SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics are presented as mean ± standard deviation and median (min-max) for continuous variables and as numbers and percentages for categorical data. The distribution of normality of data for statistical test selection was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. The t-test was used to compare the age of the patients according to the HPV groups. Correlation studies between categorical variables and ratio comparisons were performed using Chi-squared test or Fisher exact test. The relationship between HPV type according to pathology result and age was examined by general linear model. Statistical significance level was accepted as $p < 0.05$.

3. Results

169 patients were included in the study. Demographic data and clinical characteristics of the patients according to HSIL pathologic results are shown in Table 1. There was no significant difference between age groups ($p = 0.425$). There was no significant relationship between biopsy results and educational status, financial status and smoking ($p = 0.320$, $p = 0.342$, $p = 0.214$, respectively). The distribution of pathologic findings and patients age according to hrHPV and lrHPV results is shown in Table 2. There was a statistically significant difference between the groups in terms of age (34.77±5.47 vs 31.78±8.22 $p = 0.004$). Cervical biopsy was performed in all the patients who participated in the study and 62 of these cases were diagnosed with HSIL and cervical carcinoma, while the remaining 107 had LSIL and other benign lesions (Table 2).

Table 1. Clinical characteristics of study population (n=169) and demographic data and evaluation of biopsy.

		<HSIL	≥HSIL	P-value
Age Mean ± SD (min-max)		33.32 ± 3.80 (30-44)	34.24 ± 4.30 (30-46)	0.425
Age of first intercourse Mean ± SD (min-max)		20.14 ± 1.48 (16-27)	21.02 ± 1.76 (16-29)	0.352
BMI Mean ± SD (min-max)		27.22 ± 2.43 (19.9-32.3)	26.18 ± 2.78 (19.4-31.7)	0.480
Gravida Mean ± SD (min-max)		2.44± 1.10 (0-5)	2.38 ± 1.19 (0-5)	0.256
Parity Mean ± SD (min-max)		2.18 ± 0.88 (0-5)	2.15 ± 0.95 (0-5)	0.282
Abortion Mean ± SD (min-max)		2.33 ± 0.85 (0-4)	2.12 ± 0.95 (0-5)	0.278
Education n, (%)	Less than high school	52(30.7)	25(14.8)	0.320
	High school	49(28.9)	33(19.6)	
	University	6(3.6)	4(2.4)	
Wage, dollars n, (%)	<500	51(21.3)	35(19.5)	0.342
	500-1000	45(25.5)	30(26.7)	
	1000<	5(3)	3(4.1)	
Smoking n, (%)	Smoking	24(6.5)	14(8.3)	0.214
	Non-smoking	73(43.8)	48(41.4)	

Data are presented as mean ± standard deviation, SD: standard deviation, min: minimum, max: maximum. A P value of <0.05 was considered as statistically significant.
Abbreviation: BMI, body mass index; ASC-H, atypical squamous cells when high-grade intraepithelial lesions (ASC-H) cannot be ruled out; HSIL, high-grade squamous intra-epithelial lesions.

Table 2. Age and biopsy results according to HPV types

		lrHPV (N=51)	hrHPV (N=118)	P-value
Age Mean ± SD, (min-max)		34.77±5.47 (29-40)	31.78±8.22 (23-40)	0.004
Cervical biopsy results	<HSIL n, (%)	42(24.9%)	65 (38.4%)	0.031
	Benign	21 (12.45%)	27 (16%)	
	LSIL	21 (12.45%)	38 (22.4%)	
	≥HSIL n, (%)	9(5.3%)	53(31.4%)	
	HSIL	9 (5.3%)	47 (27.9%)	
	Squamous CA	/	5 (3%)	
	Adeno CA	/	1 (0.5%)	

Data are presented as mean ± standard deviation, SD: standard deviation, min: minimum, max: maximum. A P value of <0.05 was considered as statistically significant.
Abbreviation: HPV, Human Papilloma Virus; : <HSIL: normal histopathological findings or histopathological low-grade squamous intraepithelial lesion (LSIL); ≥HSIL, histopathological HSIL, squamous cell carcinoma, adenocarcinoma; hrHPV, High-risk HPV types (oncogenic or cancer-associated); lrHPV, Low-risk HPV types (nononcogenic or noncancer-associated); Squamous CA, squamous cell carcinoma; Adeno CA, Adenocarcinoma. hrHPV types are HPV DNA type 16, 18, 31, 33,35, 45, 51, 58, 59 and 68, lrHPV types are HPV DNA type 6, 11, 40, 54 and 70.

General linear regression analysis revealed that HSIL biopsy results were significantly correlated with HPV in patients with high risk (p=0.031) and that age and combined age and hrHPV presence were not affected the biopsy result (p=0.746 and p=0.349 respectively) (Table 3). In our study, no significant correlation was found between age and biopsy results in linear regression analysis (Table 3).

Table 3. General linear regression analyses of HSIL cervical biopsy results and age and HPV types.

	F-value	P-value
Age	0.168	0.746
hrHPV	5.750	0.031*
Age +hrHPV	1.126	0.349

In patients with ASC-H, only hrHPV association with age or age does not affect the results of cervical biopsy.

4. Discussion

ASC is a spectrum of both benign and precancerous lesions and have relatively rare cytological interpretation with incidence rates of 0.27-0.6% among all pap tests with this cytological diagnosis (Solomon et al., 2002). It includes squamous metaplasia with degenerative properties, atrophic changes, reserve cell hyperplasia, reactive changes and hormonal effects as benign characteristics (Solomon et al., 2002; Massad et al., 2013). ASC-H represents 5-10% of ASC diagnoses, but the presence of pre-invasive lesions is more likely (Massad et al., 2013). The criteria for the diagnosis of ASC-H are variable (Fig.1 and 2). This diagnosis is defined as the presence of cell abnormalities that resemble high-grade lesions, but there are no definite criteria for such lesions (Gultekin et al., 2018).

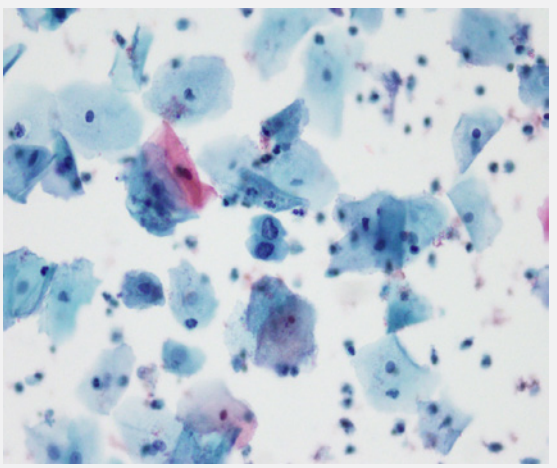

Fig. 1. Small, polygonal-shaped cells were observed on the floor consisting of polymorphonuclear leukocytes and mature squamous cells. The cytoplasms were dense and the nucleus/cytoplasm rates increased in favor of the nucleus.

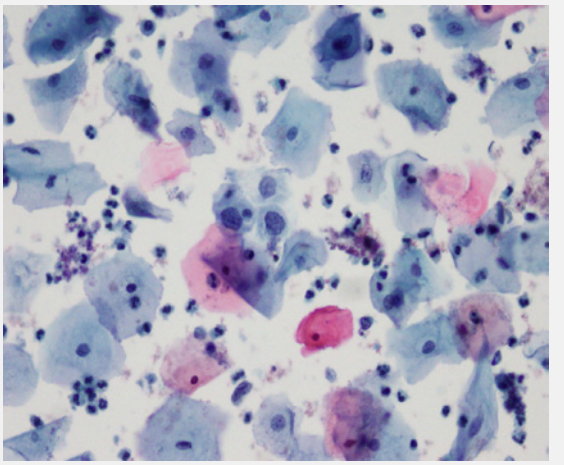

Fig. 2. The nuclei of the observed atypical squamous cells were large, the nuclear membranes were irregular, and the chromatin nets were rough.

The cytoplasm of cells in ASC-H are characteristically wider than in HSIL (Nieh et al., 2005). Because of the above-mentioned malignant and benign characteristics, the follow-up histology results of ASC-H cases are also highly variable and show significant differences in use between laboratories and in different clinical settings (Galliano et al., 2011). While some laboratories may see ASC-H as benign reactive processes, which can increase the ASC-H reporting rate, other laboratories may interpret some HSIL cases as ASC-H, resulting in a lower reporting rate (Koss, 1989).

62 (36.7%) of 169 patients were diagnosed as HSIL+. Cervical carcinoma was detected in 6 patients. These findings confirmed the opinion of referring all patients with ASC-H cytology to colposcopy and cervical biopsy. Histological follow-up studies of ASC-H cytology have shown that 70% of women show CIN 2 or above CIN 2 biopsy results and some studies have shown this to be a relatively high-risk category (Galliano et al., 2011; Gilani et al., 2014). In the study of Louro et al. 171 (79%) of 217 patients with ASC-H were found to have SIL or advanced lesions with biopsy results (Louro et al., 2003). In this study group, ASC-H ratio was reported as 0.84%. Saad et al. reported that in the comparison of premenopausal and postmenopausal PST results of biopsy results in patients with ASC-H, 22% of premenopausal patients and only 6% of postmenopausal patients were associated with HSIL (Saad et al., 2006). Young patients with ASC-H have been associated with a higher rate of HSIL than older women. Similarly, Selvaggi et al. Reported a high pre-prevalence of up to 68% in ASC-H patients in the young population aged 19-34 (Selvaggi et al., 2003).

In our study, we did not find a significant relationship between age and histopathology results. HrHPV positivity alone had an association with the degree of cervical biopsy results of patients with

ASC-H. In patients with ASC-H, hrHPV association only with age or age alone does not affect the results of cervical biopsy. Contrary to this finding, HrHPV positivity with younger age or age alone has no effect on the histopathology results of patients with ASC-H histology. On the contrary, Gilani et al. and Patton et al. found that advanced age was a determining risk factor for CIN in ASC-H patients (Patton et al., 2008; Gilani et al., 2014). The ALTS (ASCUS / LSIL Triage Study) study group reported that age was an important parameter in these patients and that the hrHPV rate was 85% in patients 35 years old and younger with ASC-H, while it decreased to 40% over the age of 35. The ALTS group identified a 40.5% risk of CIN 2+ lesion diagnosis in a patient with ASC-H and pointed out the need for studies to increase the efficiency of HPV DNA testing in the group over 35 years of age (Solomon et al., 2002; Sherman et al., 2001). Sung et al. suggested that the hr-HPV DNA test and p16INK4a test may be preferred for their higher specificity than HC2 and that these tests should be re-checked after 6-12 months if found negative (Sung et al., 2010). The rate of hr-HPV positivity in women with ASC-H cytology varies across the population (51-90%), and hr-HPV (+) was detected in 118 (66.2%) of 169 patients undergoing HPV testing in our study. It has been shown that the prevalence of ≥ CIN 2 is in a wide range (13-66%, mean value 34%) in women with ASC-H cytology (Patton et al., 2008; Gilani et al., 2014). In our study, this rate was found as 36.7%.

Follow-up results of patients diagnosed with ASC-H in pap smear test vary between studies. Mokhtar et al. reported that 59.4% of 123 cases diagnosed as cytologically ASC-H were reported as HSIL in subsequent biopsies (Mokhtar et al., 2008). Cytryn et al. showed that the prevalence of CIN 2/3 was 19.3% in 57 patients with ASC-H cytology (Cytryn et al., 2009). Kietpeerakool et al. reported the underlying prevalence of CIN 2 or higher lesions as 69.4% in 69 ASC-H patients. In this study, HSIL was relatively low compared to previous studies, which ranged from 24% to 94% (Kietpeerakool et al., 2008). Sun et al. reported that 52.3% of patients under 40 years of age with ASC-H in Pap smear had preinvasive lesions such as HSIL and higher (Sun et al., 2011). We think that the underlying reason for the high incidence of HSIL in our study may be related to the fact that the subjects were under 40 years of age. However, considering that we could not find any difference between the results of biopsy in patients under 30 years and 30-40 years of age, we conclude that smear interpretations with ASCH should be used more carefully in patients under the age of 40.

HPV test results are another helpful method for assessing the risk level of the ASC-H category. Gilani et al. found a high predictive value (87.2%) of the HPV test for the diagnosis of CIN in patients with ASC-H and suggested that these patients should be followed closely and carefully (Gilani et al., 2014). The American Society for Colposcopy and Cervical Pathology (ASCCP) recommends that women with ASC-H pap smears be evaluated by colposcopy regardless of performing HPV test (Wright et al., 2007). According to the ASCCP assessment, HPV (-) ASC-H patients have a higher risk of cervical cancer than expected, and HPV testing in ASC-H patients is already high (+) in studies, and HPV testing is not mandatory. Colposcopic examination instead of follow-up is appropriate for the benefit of the patient (Wright et al., 2007). However, there are also some reports advocating that these patients should be evaluated by HPV test even if they were examined by colposcopy (Louro et al., 2003; Milutin et al., 2008; Gultekin et al., 2018)

Although our approach was to apply colposcopy to all ASCH patients in accordance with ASCCP recommendations, colposcopic evaluation was recommended in patients with persistent cytological abnormalities in repeated tests or if the HPV test was positive in a study that opposed this opinion (Wright et al., 2007).

In the context of possible outcomes, this study has a few more values. Considering the inclusion of many registered young patients and the similar appearance of ASCH with secondary changes such as metaplasia, inflammation and atrophy, the exclusion of this group of patients was an important addition to the literature on this subject. First, a homogeneous condition was studied, although it had a retrospective design. Patients were selected between 23-40 years of age, and perimenopausal and/or postmenopausal patients were excluded from the study; therefore, our study was composed of younger patients. We analyzed all samples with the same instrument for the entire study period and for the entire study group. In addition, we excluded several obstetric and medical conditions that had the potential to influence the parameters examined, which also increase the quality of our study. Factors such as hormone replacement therapy are less likely to affect the outcome of the study. These secondary changes are more common in older women, which may explain why ASC-H has a higher incidence of HSIL in younger patients. Although ASC-H is in clinical use due to its association with underlying HSIL, it is still difficult to predict ASC-H results. This study showed that ASC-H was closely related to HSIL or higher lesions, especially in young (under 40) women with positive smear testing for HPV DNA.

Our study had some limitations. First, the study may have been affected by lifestyle and the number of partners. Second, this study was conducted in a single institution. We believe that the results of this study may help the early and effective treatment of ASC-H. The

performance of the markers used in this study in multi-center studies will strengthen our results.

Since HPV test was studied together with cytology as a co-test in some cases, the limited number of patients was among the missing elements of our study. In conclusion, patients with ASC-H cytology carrying hr-HPV had a risk of 2.647-fold in terms of ≥ CIN 2 lesions compared to patients without hr-HPV. Considering our study and the current literature, colposcopic evaluation is an indispensable step since ASC-H patients have a high risk of ≥ CIN 2 lesions. Performing HPV test in ASC-H patients may lead us to minimize errors and discrepancies in cytology and colposcopy. With national cervical cancer screening in our country, it is thought that conducting large series of studies including the value of HPV test on ASC-H patients in the following years will contribute to science not only in our country but also in the world. In conclusion, patient age, positive HPV DNA test and recurrent abnormal cytology may help predict the underlying pathology of HSIL or higher lesions in women with ASC-H.

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