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Comparison of Clinical Data and Colposcopy Results of HPV-positive Women with ASC-US and LSIL

HPV Pozitif Kadınlarda ASC-US ve LSIL Sitolojilerinin Klinik Veri ve Kolposkopi Sonuçlarının Karşılaştırılması

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ÖZ

Amaç: Çalışmamızın amacı, yüksek riskli insan papilloma virüs (hrHPV) pozitif kadınlar arasında önemi belirsiz atipik skuamöz hücre (ASC-US) ve düşük dereceli skuamöz intraepitelyal lezyon (LSIL) sitolojisinin klinik verileri ile kolposkopiye yönelik biyopsi (CDB) sonuçlarını karşılaştırmaktır.

Gereç ve Yöntemler: Çalışmaya 20-67 yaşları arasında ASC-US ve LSIL sitolojisi olan toplam 359 hrHPV pozitif hasta dahil edildi. Katılımcıların yaşı, eğitim durumu, sigara içme durumu, kolposkopi sonuçları ve CDB sayısı değerlendirildi ve klinik veriler biyopsilerin histopatolojik değerlendirmesiyle istatistiksel olarak karşılaştırıldı.

Bulgular: hrHPV + ASC-US ve hrHPV + LSIL grupları arasında yaş, medeni durum, eğitim durumu ve sigara içme durumu açısından istatistiksel olarak anlamlı fark yoktu. Her iki grup CDB'lerin histopatolojik sonuçları açısından karşılaştırıldı ve gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmedi. CDB materyali sayısı ve endoservikal küretaj sıklığı hrHPV + LSIL'de hrHPV + ASC-US hastalarına göre anlamlı olarak daha yüksekti. İleri tedavi ihtiyacı açısından iki grup arasında istatistiksel olarak anlamlı fark yoktu. Hem ASC-US hem de LSIL gruplarının takibi sırasında gerçekleştirilen kontrol sitolojisi benign olarak sonuçlandı.

Sonuç: HPV pozitifliğinde ASCUS ve LSIL'e yaklaşım benzer şekilde önemlidir. Takip süremiz 1 yıllık bir süreyi içerdiğinden, daha uzun vadeli sonuçların gösterilebilmesi için daha ileri çalışmaların yapılması gerekmektedir.

Anahtar Kelimeler: ASC-US, LSIL, Colposcopy, HPV

ABSTRACT

Objective: The aim of our study is to compare the clinical data and colposcopy- directed biopsy (CDB) results of atypical squamous cell of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL) cytology among high risk human papilloma virus (hrHPV) positive women.

Materials and Methods: A total of 359 hrHPV-positive patients with ASC-US and LSIL cytology, between 20-67 years of age were included in the study. Participants' age, education status, smoking status, colposcopy results, and number of CDBs were assessed and clinical data compared with histopathologic evaluation of biopsies statistically.

Results: There were no statistically significant differences in terms of age, marital status, education status and smoking status of hrHPV + ASC-US and hrHPV + LSIL groups. Both groups were compared in terms of histopathologic results of CDBs and no statistically significant difference was observed between the groups. The number of CDB material and frequency of endocervical curettage were significantly higher in hrHPV + LSIL than hrHPV + ASC-US patients. There was no statistically significant difference between the two groups in terms of advanced treatment need. Control cytology performed during follow-up of both ASC-US and LSIL groups were resulted as benign.

Conclusion: The approach to ASCUS and LSIL is similarly important in HPV positivity. Since our follow up period includes a sort period of 1 year, further studies need to be carried out in order to demonstrate longer term outcomes.

Keywords: ASC-US, LSIL, Colposcopy, HPV

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INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide. Human papilloma virus (HPV) was detected in majority of cases. Infection is extremely common in young women in their first decade of sexual activity. Invasive cancer arises over many years, even decades, in a minority of women with precancerous lesions, with a peak risk at about 35–55 years of age (1).

High-grade squamous intraepithelial lesions (HSILs) should be evaluated by colposcopy with endocervical assessment or immediate loop electrosurgical excision. However, the approach to patients with low-grade squamous intraepithelial lesions (LSILs) or atypical squamous cells of undetermined significance (ASCUS) are not as consensus-based as in patients with HGSILs. According to American Society for Colposcopy and Cervical Pathology (ASCCP) recommendations, the management options for HPV positive patients with ASCUS and LGSILs also include details categorized as 'preferred' and/ or 'acceptable' (2) .

The ASCUS/LSIL Triage Study (ALTS), a randomized, multicenter clinical trial of the management of women with low-grade cervical cytology, compared the sensitivity and specificity of immediate colposcopy, repeat cytology, and HPV testing for the timely detection of cervical intraepithelial neoplasia grade 3 (CIN3). The results demonstrated that HPV-deoxyribonucleic acid (DNA) tests are useful for for final decision and the majority of LSILs has been confirmed to be infected with cancer-associated HPV types (3).

Although the majority of the low-grade lesions regress spontaneously, and follow-up CDBs reported as benign, management of ASCUS/LSIL is potential of concern, given that a small but important minority may have CIN 2 or 3, or even carcinoma on colposcopy and CDBs (4).

In this study, we aimed to compare the clinical data and CDB results of ASC-US and LSIL cytology with high-risk HPV positive women and clarify the group of patients with a high risk of developing cervical cancer.

MATERIALS AND METHODS

Between January 2014 and August 2017, 359 patients with ASC-US and LSIL cervical smear cytology, who were also positive for high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and aged between 20-67 years were admitted to the Colposcopy outpatient clinic of Ankara Etlik Zübeyde Hanım Obstetric and Gynecology Research Hospital and were included in the study. The clinical data and CDB results were compared between the groups hrHPV + ASC-US and hrHPV + LSIL. Pregnants, adolescents, and patients receiving dysplasia were excluded from the study.

Local Ethics Committee approval was obtained from the same hospital.

Cytology materials were removed by a brush, spread on the slide and fixed by spraying from a distance of 25-30 cm. Samples were evaluated by using the Bethesda classification system. The first and control smear samples at first year of follow- up were evaluated in order to evaluate progression. HPV DNAs were studied with the Hybrid Capture 2 method at the contracted external center. The HC 2 assay includes a mixture of probes for the following cervical cancer-associated HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Colposcopic examinations were performed with a binocular instrument (Leica CLS 150 XC, Heerbrugg) with 20 magnification and green filter. The examinations were performed by an expert with an assistant doctor. The standard technique was applied during colposcopy, the cervix was washed with saline, scanned at small magnification and vascular pathologies were investigated with a green filter, then 3-5% acetic acid was applied. After the application of acetic acid (60-120 seconds), cervix was re-screened in small and large magnifications, respectively. Aceto-white areas and vascular pathologies were detected by a green filter and

iodine-free areas were determined by staining with Lugol solution. Several CDBs were performed from iodine-free, aceto-white epithelium and areas with punctuation, erosion, leukoplakia, atypical vascularization by Kevorkiyan and Tischler forceps. Biopsy specimens were sent to the pathology laboratory in formaldehyde solution. Cervical biopsy specimens were evaluated by the pathologist according to the LAST classification system defined in 2012. Specimens grouped as high grade, low grade and benign. The patients with HSIL as a result of pathology were evaluated as 'High grade' (HG) and the patients with LSIL as 'Low grade' (LG). The group defined as benign included chronic cervicitis, endocervical epithelium, ectocervical epithelium, squamous metaplasia, and cervical polyps.

Statistical analyses were performed by using SPSS (version 21, IBM Corporation, NY, USA). The distribution of the data was evaluated by the Kolmogorov Smirnov test. Mean and standard deviation (SD) were used for the representation of parametric

quantitative data, median and interquartile range (IQR) for the representation of nonparametric data, number of cases (n) and percentage (%) for qualitative data. Student t-test was used for the analysis of the parametric data, Mann Whitney U was used for the analysis of the nonparametric data, and Pearson Chi-square test was used for the analysis of qualitative data and descriptive statistics (mean, median, percent distribution) were used as statistical analysis. P <0.05 was considered significant.

RESULTS

A total of 359 patients were included in this study and the patients were divided into two groups as hrHPV + ASC-US (n=189) and hrHPV + LSIL (n=170). The mean age of hrHPV + ASC-US (44.06 ± 10.13) and hrHPV + LSIL (42.20 ± 8.10)) patients were similar. The sociodemographic characteristics (educational status, marital status), smoking status and duration of the two groups were compared and no significant difference was found between groups (Table 1).

Table 1. Comparison of sociodemographic characteristics and smoking status of patients

		ASC-US (N:189)	LSIL (N:170)	*P Value*
Age (Mean±SD)		44.06(±10.13)	42.20(±8.10)	*0.06**
Marital Status (%)				*0.43*#
	Married	143(75.7)	134 (78.8)	
	Single	18 (9.5)	10 (5.9)	
	Widow	28 (14.8)	26 (15.3)	
Education Status (%)				*0.96*#
	Illiterate	5 (2.7)	3 (1.8)	
	Primary School	86 (45.5)	81 (47.6)	
	Middle School	21 (11.1)	21 (12.4)	
	High School	41 (21.7)	32 (18.8)	
	University High Bachelor	25 (13.2) 11(5.8)	24 (14.1) 9 (5.3)	
Smoking Status (%)				
	Smoker	73(38.6)	77(45.3)	
	Non-smoker	96 (50.8)	79 (46.5)	*0.43*#
	Quit Smoking	20 (10.6)	14 (8.2)	
Smoking Time (Median (IQR))				
	Package / Year	15(10)	15(10)	*0.66*&

CDB results of hrHPV + ASC-US patients were 44.8% benign, 28.7% LG and 26.5% HG, while hrHPV + LSIL patients were 46.6% benign, 28% LG and 25.4% HG. No statistically significant difference was determined between the groups (p=0,97) (Table 2).

The ratio of acetowhite areas that are detected after the application of acetic acid used in the colposcopic examination was compared between groups and found similar (Table 2).

Table 2. Comparison of histopathological classes and procedure- dependent variables of patients

	ASC-US (n:87)	**LSIL (n:118)**	**p value**
Class			0.97
Benign	39 (44.8)	55 (46.6)	
Low Grade	25 (28.7)	33 (28.0)	
High Grade	23 (26.5)	30 (25.4)	
	ASC-US (n:81)	**LSIL (n:86)**	**p value**
Acetowhite Area Ratio			0.29
< %25	60 (74.0)	55 (64.0)	
%25-%50	16 (19.8)	21 (24.4)	
> %50	5 (6.2)	10 (11.6)	
	ASC-US (n:189)	**LSIL (n:170)**	**p value**
ECC			<0.001
Performed	89 (47.1)	122 (71.8)	
Not performed	100 (52.9)	48 (28.2)	
	ASC-US (n:189)	**LSIL (n:170)**	**p value**
Diagnostic excisional Procedure			0.28
Unprocessed	167 (88.4)	141 (83.4)	
Conization	15 (7.9)	22 (13.0)	
LEEP	7 (3.7)	6 (3.6)	

The mean number of CDBs for the LSIL group (1.2 ± 1.14) was significantly higher than ASC-US group was (0.84 ± 1.02) (p <0,001).

ECC was performed in 89 (47.1%) patients of the ASC-US group and 122 (71.8%) patients of the LSIL group. This difference was statistically significant. (p <0,001) (Table 2). The pathologies of ECCs were not reported malignant in any patient.

When the advanced treatment status of the patients was examined, it was seen that 15 (7.9%) patients performed conization and 7 (3.7%) patients performed LEEP in the ASC-US group. In the LSIL group, 22 (13.0%) patients performed conization and 6 (3.6%)

patients performed LEEP. There was no statistically significant difference between the two groups in terms of advanced treatment (p=0,28) (Table 2).

No recurrence was observed at first year of follow- up in group of patients with high-grade biopsy results who had diagnostic excisional procedures. Four of twenty- three patients with HSIL had positive surgical margins in the hrHPV + ASC-US group. Three of these patients performed conization and one of LEEP. Two patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy after conization, and re-conization was needed for one patient with conization.

After one year of follow-up, there were no difference between ASC-US and LSIL groups in terms of cytology results, as they were all in benign subgroup of our study methodology.

DISCUSSION

ASC-US and LSIL are important cervical lesions that can lead to cervical cancer and and management strategies are of great importance for our clinical practice. Although we found the colposcopy and pathology results of patients with ASC-US and LSIL similar in our study, we found a significant difference between the approach of proffessions to ASC-US and LSIL.

Since cervical cancer is an important health problem all over the world, early diagnosis and screening of the disease have become important. Pap smear test and the widespread use of the Bethesda system worldwide have significantly reduced the mortality rate of cervical cancer. HPV and smear started to be used simultaneously after HPV DNA test was approved for cervical cancer screening by Food and Drug Administration (FDA) in 2003 (5). In our country, an HPV-based screening program has been implemented since 2014 (6). HPV has an important role in the development of precancerous lesions of cervical pathologies and HPV DNA screening test was found more sensitive than Pap Smear in detecting high grade squamous intraepithelial lesion (HSIL) (7,8,9).

Smoking is considered to be an important predisposing factor for premalignant and malignant lesions of the cervical uterus (10). In our study, median smoking duration was found to be 15 pack / year and there was no statistically significant difference between the groups (P = 0.66).

Up to date, many studies on HPV DNA have been published. In the HART (HPV Addition to Routine Testing) study conducted by Cuzick J. et al. 11085 women patients between 30 and 60 years of age who had ASC-US/ILS or had positive HPV DNA but with negative cytology were scanned. They were immediately followed by colposcopy or by HPV DNA, cytology, colposcopy for 12 months. In conclusion, adding HPV DNA to screening program was found more sensitive in detecting HSIL, decreasing the referral rates to colposcopy and the follow-up compliance was same as a colposcopy (72% in single HPV DNA, 73% in single colposcopy); since adding cytology to HPV DNA has less cost than follow up of HPV DNA alone, and treatment based on only HPV DNA may lead to excessive treatment and increase the cost (11). In our country, Gultekin M. et al. performed a cervical cancer scan by using HPV test and cytology in one million women and detected a cytological abnormality in 19.1% of 37.515 patients with HPV positivity. Cancer was detected in 85 of 3499 patients who were referred to colposcopy, and 1.985 patients had a normal cytology, 708 had CIN1, 285 had CIN2, and 436 had CIN3. They demonstrated that only the pap-smear program could miss 45.9% of CIN3 and cancer cases, thus the Co-test was found much more effective than conventional pap-smear (12).

Since HPV DNA positivity has been shown to affect colposcopy results and follow-up, in our study we investigated patients with high-risk HPV positivity in both ASC-US and LSIL groups. When both groups were compared in terms of colposcopy results, no statistically significant difference was observed between the groups (p = 0.97). These results were found to be similar to the results of the ALTS group study conducted by Cox JT et al. in 2003. They prospectively examined 897 LSIL and 1193 ASC-US patients with high oncogenic HPV infection by direct colposcopy and biopsy for two years. In the two-year follow-up of these patients, CIN2 or 3 was found to be by 10%, CIN1 by 12.5%, normal colposcopy by 12.8%, negative biopsy by 10.6%, and no difference was found between the groups (13). In addition, Siebers et al. investigated the risk of CIN 3 in the presence and absence of coilocytosis in patients with hrHPV + ASC-US and hrHPV + LSIL. They found the risk of CIN3 to be similar for the ASC-US and LSIL groups in the presence and absence of coilocytosis (14).

Fallani et al. compared colposcopic biopsy results of cytologically reported cases as ASCUS and LSIL. Out of 584 women, 358 were diagnosed with ASCUS and 226 with LSIL.Of the patients reported as ASCUS, 36.3% had CIN1, 15.7% had CIN2-3 and in situ ca, and only 1 case had invasive ca. CIN1 was detected in 67.7% of the patients reported as LSIL, CIN 2-3 and in situ ca were detected in 20.8%, and invasive ca was detected in only 2 cases. According to these results, colposcopic

examination was recommended for all patients with cytological diagnosis of ASCUS or LSIL (15). However, in our study, 87 of 189 patients diagnosed with ASCUS and 118 of 170 patients diagnosed with LSIL required colposcopic examination. This result indicated that the cytological diagnosis of ASCUS could be considered less important than LSIL in case of HPV positivity. In addition, when the ASCUS (n=81) and LSIL (n= 86) groups were compared in terms of acetic acid application in colposcopic examination, no statistically significant difference was observed between the groups (p= 0.29).

Wright et al. examined ASCUS and LSIL cases similarly in their study in 2019 and underlined that HPV positivity is more important. In case of high-risk HPV positivity, colposcopy was recommended to patients whose cytology was found to be ASCUS or LSIL, while colposcopy was avoided in case of low-risk HPV positivity and annual follow-up was recommended (16). When the diagnostic excisional procedures were examined, 15 (7.9%) of the patients in the ASCUS group were treated with conization and 7 (3.7%) with LEEP; In the LSIL group, 22 (13.0%) of the patients underwent conization and 6 (3.6%) of them underwent LEEP. When evaluated in terms of advanced treatment, no statistically significant difference was found between the two groups (p=0.28). When evaluated in terms of the number of biopsies performed, the mean number of biopsies was 0.84 (1.02) in the ASCUS group, while it was 1.2 (1.14) in the LSIL group, and there was a statistically significant relationship between the groups (p0.001). In the examination of patients who underwent endocervical curettage, 89 (47.1%) patients were diagnosed with ASCUS and 122 (71.8%) patients were diagnosed with LSIL, which was statistically significant (p0.001). In addition, since no abnormality was detected in the colposcopic examination, no biopsy or similar intervention was required in 91(48.1%) patients with a diagnosis of ASCUS and 46 (27.1%) with a diagnosis of LSIL, all of whom were HPV positive.

When HPV positive patients were examined in terms of endocervical curettage requirement, the number of patients with ASCUS was lower than those with LSIL. However, the fact that all ECC results were reported as benign raises the question of whether ECC is necessary or not.

In conclusion, it was observed that the approach was not the same in our study examining patients diagnosed with ASCUS and LSIL with high-risk HPV positivity. However, although there is no significant relationship between pathology results, it can be concluded that ASCUS cases with HPV positivity are as important as LSIL and approaches should be similar. Although all of the results were in the group we defined as benign in the cytological examination at the annual follow-up after treatment, more studies are needed for a longer-term evaluation.

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