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HPV burden and risk of CIN II or worse pathology in patients with atypical squamous cell of undetermined significance (ASCUS) cytology

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ABSTRACT

Objective: The aim of the study was to evaluate the Human Papillomavirus (HPV) burden and the risk of Cervical Intraepithelial Lesion grade II(CIN-II) or worse pathology in patients with Atypical Squamous Cell of Undetermined Significance (ASCUS) cytology at the time of diagnosis.

Material and Methods: The study included patients who have ASCUS cytology and underwent HPV testing between the years 2011-2021.Colposcopic biopsy were performed in patients with positive HPV.The patient's medical records and demographic information were evaluated retrospectively. The proportion of HPV positivity and the risk of CIN-II or worse pathology were evaluated by using SPSS package programme.

Results: Median age was 39 years (22-73y).749 patients had HPV test and 353(47.1 %) of them was HPV positive. 68(10.7 %) patients had HPV-16, 44(7%) had HPV-52, 39(6%) had HPV-18 and 32(5.1%) had HPV-31. Eighty-eigth (11.7%) patients had HPV-16/18 and 90(12 %) had more than three HPV types. 369(51%) patients underwent cervical biopsy and 236(64%) of them had cervicitis or normal pathology.52(14.1%) patients had CIN-I ,22(6%) had CIN-II, 57(15.4%) had CIN-III and 2(0.5%) had Carcinoma in Situ. HPV-16 and/or 18 was found to increase the risk of the presence of CIN-II+ lesions in multivariate analysis(OR: 6.2, 95%CI 3.3-11.9, P=0.00).Sensitivity and specifity of HPV-16/18 for detecting CIN-II+ lesion was 54.7% and 83% respectively and the Negative Predictive Value(NPV) was 89% and Positive Predictive Value(PPV) was 56.7%.

Results and conclusion: HPV positivity in the ASCUS population is relatively high in our institution and risk of CIN-II+ lesions sixfold increased with the presence of HPV-16/18. Moreover,HPV-16/18 testing has low PPV and high NPV in detecting CIN-II plus lesions.

Keywords: Atypical Squamous Cells of Undetermined Significance (ASCUS), Human Papillomavirus (HPV), Cervical Intraepithelial Neoplasia II plus(CIN-II+), Cervical Cancer

ÖZET

Amaç: Önemi belirsiz atipik skuamöz hücre(ASCUS) sitolojisi olan hastalarda tanı anında HPV yükü ve servikal intraepitelyal lezyon grad II(CIN II) veya üstü patoloji riskini değerlendirmektir.

Gereç ve Yöntem: Çalışmaya 2011-2021 yılları arasında ASCUS sitolojisi olan ve Human Papillomavirus(HPV) testi yapılan hastalar dahil edildi. HPV testi pozitif olan hastalara kolposkopik biyopsi yapıldı. Hastanın tıbbi kayıtları ve demografik bilgileri geriye dönük olarak değerlendirildi. HPV pozitiflik oranı ve CIN II veya daha kötü patoloji riski SPSS paket programı kullanılarak değerlendirildi.

Bulgular ve Sonuç: Ortanca yaş 39(22-73) idi. 749 hastaya HPV testi uygulandı, 353'ünde(%47,1) HPV pozitifti. 68 hastada HPV-16(%10,7), 44(%7) hastada HPV-52, 39(%6) hastada HPV-18 ve 32(%5,1) hastada HPV-31 pozitifti. Seksen sekiz hastada (%11.7) HPV-16 /18 ve 90(%12) hastada üçten fazla HPV tipi pozitifti. 369(%51) hastaya servikal biyopsi yapıldı ve bunların 236(%64)'sında servisit veya normal patoloji vardı.52(%14,1) hastada CIN-I, 22(%6)'de CIN-II, 57(%15,4)'de CIN-III ve 2(%0,05) hastada carcinoma in situ saptandı. HPV Tip-16 ve/veya 18'in multivaryant analizde CIN-2+ lezyonlarının varlığı riskini artırdığı bulunmuştur (OR:6,2, %95 GA: 3,3-11,9, P=0,00). HPV-16/18 testinin CIN-2 + lezyonunu saptamada duyarlılık ve özgüllüğü sırasıyla %54,7 ve %83 olarak bulundu ve aynı test için Negatif Öngörü Değeri (NPV) %89 ve Pozitif Öngörü Değeri(PPV) %56,7 idi.

Anahtar Kelimeler: Önemi Belirsiz Atipik Skuamoz Hücre (ASCUS), Human Papillomavirus(HPV), Servikal İntraepitelyal Neoplazi II plus(CIN-II+) , Serviks Kanseri

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Introduction

Cervical cancer is the 4th most commonly diagnosed cancer in women and the 4th most common cause of death among women.(1)The development and proliferation of screening programs over the years have reduced the incidence and mortality rate.(2, 3, 4)

ASC-US (Atypical Squamous Cells of Undetermined Significance) are abnormal cell findings related to inflammation or premalignancy detected on microscopic examination. (5) The incidence of ASC-US in the normal population is 4.1-4.2%(6, 7). In Turkey prevalance of ASC-US in the normal population is 1.07% and incidence of ASC-US in the HPV positive population is approximately 6 %(8, 9). However, ASC-US alone is not sufficient for the detection and planning of further examinations of Cervical Intraepithelial Neoplasia II (CIN II) and more advanced pathologies (CIN II+).(10)Moreover, many countries, have published age-standardized cervical cancer screening guidelines in which cervical cytology and the HPV test are used together.

According to their carcinogenic properties, high-risk HPV types (HR-HPV); HPV-16, 18, 31, 33, 45, 52, and 58 are involved in more than 90% of cervical cancers.(11)However, the immune response of people infected with HPV usually clears the virus within 12-24 months and allows them to recover asymptomatically. However, a small portion may persist and contribute to the development of cervical dysplasia and cancer.(9, 12, 13, 14, 15)

The prevalence of HR-HPV in patients with ASC-US was reported as 30.2-32.6%, and according to HPV types, the prevalence was as followed; 8.2% for HPV 16 and 2.9% for HPV 18.(6, 16)However, the association of ASC-US and HPV may differ in various regions of the world. For this reason, the rate of co-occurrence is often reported differently (21.5%,(17) 30.3%,(16) 48.7%,(7)).

CIN I(LSIL) usually regresses on its own without causing malignancy, whereas CIN II- III (HSIL) is prone to malignancy and requires treatment.(4, 18) HPV positivity at the time of ASCUS is associated with a 0.4% risk of immediate development of CIN III, whereas the risk within five years is 4%.(19) Our goal in this study is to evaluate the viral load of HPV at the time of diagnosis and the risk of CIN II and more advanced pathologies in patients diagnosed with ASC-US.

Material and Methods

In this study, 1,947 patients with ASCUS cytology, were evaluated at the Başkent University Hospital between 2011-2021. 1198 of the 1947 patients who were diagnosed with CIN I+ in their previous biopsies before ASC-US, who did not have an HPV test within 3 months, and who did not have satisfactory data in the hospital's database were excluded from the study. A total of 749 patients who had ASCUS cytology as a result of the smear test and had an HPV testing within three months were included in the study.

Liquid-based conventional cervical cytology samples were obtained from each patient using an endocervical brush and plastic spatula following the manufacturer's instructions. The samples were used for the assay of cytology and HC2 hrHPV. The cytological evaluation was performed in Başkent University Laboratory, which is working according to the Bethesda criteria.

Hybrid Capture 2 (HC2) test (Qiagen, Gaithersburg, MD) used in our hospital for HPV testing, detects 13 hrHPV types (16,18,31,33,35,39,45,51,52,56,58,59 and 68). Swab samples taken from our patients were placed in 1ml phosphate buffer and HPV DNA was obtained by Qiacubeda (Qiagen). 4 different samples taken from the same patient were placed in the Rotor-Gene Q (Qiagen) to replicate and visualize the DNAs and to distinguish between positives and negatives. These samples were then analysed on the computer in 4 different channels. Samples above the threshold line were considered positive.

Colposcopic biopsies with or without endocervical curettage (ECC) were performed on patients within 6 weeks of their ASC-US and HPV diagnosis. Afterwards, the biopsy and ECC samples were evaluated by the pathology department of The Başkent University and diagnosed according to CIN terminology and standard criteria.

IBM SPSS Statistics 23.0 was used to perform statistical analyses. Continuous variables were documented as medians and ranges, while categorical variables were reported as frequencies and percentages. The chi-square test or Fisher exact test, where appropriate, was applied for categorical data. Univariate analyses were done by using a chi-square test to the factors affecting the presence of CIN II plus lesions. The cut-off of $p < 0.05$ was chosen as the level of significance. Multivariate analysis was performed for factors affecting the Risk of CIN II Plus lesions at

the time of diagnosis and to obtain Odds ratios (ORs) by using the Cox regression model. This study was approved by Başkent University Clinical Research and Ethics Committee.

Results

Seven hundred forty-nine(749) patients were included in the study. Median age was 39 years (22-73y). 307 patients (41%) were admitted to the clinic with vaginal discharge, 101 (15%) presented abnormal bleeding, 40 (5.3%) pelvic pain, 28(4%) itching and 190 patients (25.4%) were asymptomatic. The conventional smear technique was used in 152 patients (20.3%) and the liquid-based smear technique was used in 597 patients (79.7%).

Clinicopathological and demographic characteristics are listed in Table 1. HPV test results were positive in 353(47.1%) patients and negative in 396(52.9%) patients. HPV Type-16 was detected in 68(10.7%) patients, Type-18 in 39(6%) patients, Type-52 in 44(7%) patients, Type-45 in 16(2.5%) patients, Type-31 in 32 patients (5.1%) and Type-51 in 38 patients (6%). HPV Type-16 and/or 18 was positive in 88 patients (11.7%) and 90 patients (12%) had more than 3 HPV types. Figure 1.

369(%51) patients were evaluated with a colposcopic examination and a cervical biopsy procedure. Cervicitis or normal findings were detected in 236(64%) patients, CIN I in 52(14.1%) patients, CIN II in 22(6%) patients, CIN III in 57 patients (15.4%), and carcinoma in situ (CIS) in 2(0.5%) patients. 81(%22) cases presenting CIN II or worse were analysed.

In the univariate analysis, it was observed that HPV Type-16 increased the risk of the presence of CIN II+ lesions in patients with ASC-US (OR: 5.9, 95% CI: 3.03 -11.7, P=0.00). However, HPV Types 18, 31, 45, 51, and 52 did not cause a significant increase in the risk of the presence CIN II+ lesions (p=0.3, p=0.3, p=0.055, p=0.3, and p=0.06 respectively). HPV Type 16 and/or 18 was found to increase the risk of the presence of CIN II + lesions inunivariate analysis (OR: 6.3, 95%CI 3.3-12, P=0.00) and multivariate analysis (OR: 6.2, 95%CI 3.3-11.9, P=0.00). CIN II + lesions were detected in 23(46%) of 50 HPV16 positive cases and 29(43.3%) of 67 HPV16/18 positive cases Table 2.

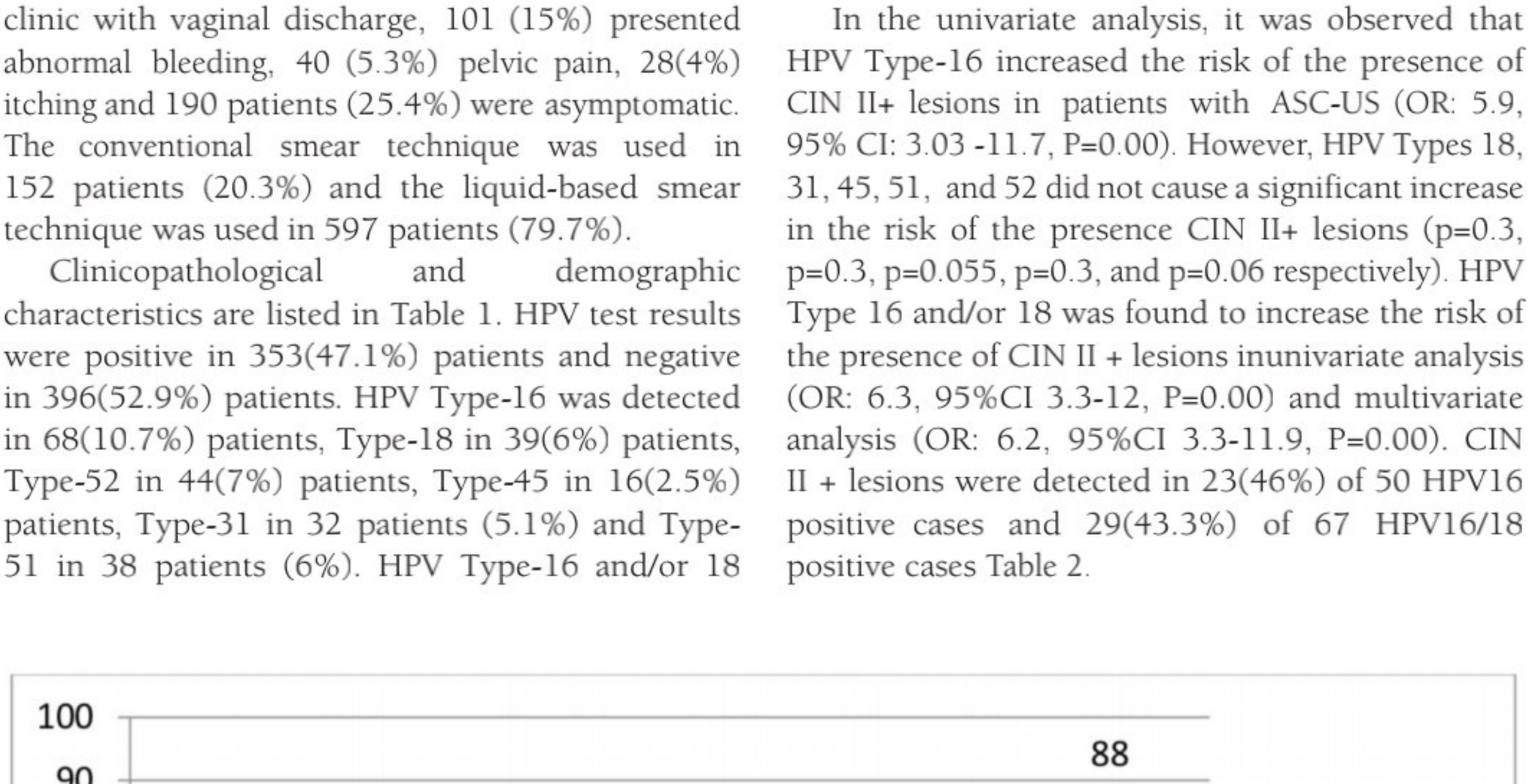

Figure 1: Distribution of HPV types
Abbreviations: HPV (Human Papilloma Virus)

Table 1 • Characteristics of patients (n=749)

Characteristics	n (%)
Median age	39 (22-73y)
Complaint	
None	190 (25.4 %)
Irreguler Bleeding	101 (15 %)
Vaginal Discharge	307 (41 %)
Itching	307 (41 %)
Pelvic Pain	28 (4%)
Unknown	73 (9.7 %)
Smoking	
Yes	172 (23 %)
No	337 (45 %)
Unknown	240 (32 %)
Oral contraceptive pills	
Yes	37 (5%)
No	415 (55 %)
Unknown	297 (40 %)
Menopausal status	
Premenopause	515 (68 %)
Postmenopause	115 (15 %)
Parity	
Nullipar	212 (29 %)
Multipar	408 (55 %)
Unknown	127 (16 %)
Smear technique	
Conventional	152 (20.3%)
Liquid base	597(79.7 %)
HPV test	
Negative	396 (52.9%)
Positive	353 (47.1 %)
Cervical Biopsy	369 (51 %)
Pathology	
Normal/ Inflamation	236 (64.0 %)
CIN I	52 (14.1%)
CIN II	22 (6.0 %)
CIN III	57 (15.4 %)
Carcinoma in-situ	2 (0.5 %)

The sensitivity of the HPV testing in detecting CIN II + lesions in patients diagnosed with ASC-US was 87%, with a specificity of 40%, Positive Predictive Value (PPV) was 92%, and Negative Predictive Value (NPV) was 29.2%. The sensitivity and specificity of HPV 16/18 genotyping was 54.7% and 83% respectively. PPV and NPV with sensitivity and specifity were given in Table 3.

Discussion

In this study, 47.1% of patients with ASC-US had positive HPV test results. When these patients were evaluated with cervical biopsy, 29.5% had CIN II+ lesions. It was found that HPV 16-18 genotypes 6.2 times increased the risk of the presence of CIN II+ lesions. In addition, it has also been observed that the HPV16/18 genotyping had low sensitivity and high specificity in detecting CIN II+ lesions in patients with ASC-US.

In the study in which more than 4 million patients were screened in Turkey, the most common HPV genotype was found to be Type-16, and the second most common genotype was Type-51.(20) Compared to that study, we have found that the most common HPV genotype was Type-16 followed by Type-52 in our study cohort.

Table 2 • Risk of CIN II or worse pathology at the time of ASCUS according to HPV types

	Univariant Analyses			Multivariant cox-regression analyses		
HPV Type	**OR**	**95 % CI (lower-higher)**	**P value**	**OR**	**95 % CI (lower-higher)**	**P value**
16	5.9	3.03-11.7	0.00	1.3	0.8-1.2	0.12
18	1.4	0.4-3.6	0.3			
31	1.7	0.5-6.2	0.3			
51	1.6	0.6-4.4	0.3			
52	0.4	0.01-1.12	0.06	1.8	0.7-2.14	0.3
45	0.2	0.06-1.01	0.055	1.4	0.9-1.7	0.4
16-45	5.9	3.03-11.7	0.00	1.2	0.2-5.4	0.7
16-52	4.9	2.5-8.9	0.00	2.1	0.7-6.3	0.1
16-18	6.3	3.3-12	0.00	6.2	3.3-11.9	0.00

HPV(Human Papilloma Virus), OR(Odds Ratio), CI(Confidence Interval)

Table 3 • The sensitivity and spesificity of HPV testing and HPV 16/18 genotyping testing

	Sensitivity	**Specificity**	**PPV**	**NPV**
HPV	87 %	40 %	92 %	29.2 %
HPV 16/18	54.7 %	83 %	56.7 %	89 %

HPV(Human Papilloma Virus), PPV(Positive Predictive Value), NPV(Negative Predictive Value)

In another study, where 697 patients had an ASC-US or LSIL cervical cytology, high-risk HPV was found in 62.8% of them, again, with Type-16 being the most common HPV genotype.(21) In the ATHENA study, in which 40,901 patients were screened, HPV+ was seen in 29.7% of 2,617 patients with abnormal cytology. While HPV16 positivity was seen in 0.7% of patients with normal cytology, this rate increased 9-fold to 6.3% in patients with abnormal cytology.(22) Since our study was hospital-based, HPV genotyping was found to be higher than in other studies. We ascertained the HPV16/18 association as 13.9% and found that the incidence of CIN II+ lesions in HPV 16/18 positive patients was lower than in patients with only the HPV Type-16 genotype. In the ATHENA study, HPV positivity was 32.6%-31.5%, Type-16 positivity was 8.2% and Type-18 positivity was 2.9% in 1578 patients diagnosed with ASC-US. HPV 16/18 was observed in 44% of patients with CIN II and 61% of patients with CIN III+.(22)

In another study, where 1089 HPV+ patients were analysed, abnormal cervical cytology was observed in approximately 40% of patients and HSIL was observed in 14.65% of these patients. When all HPV-positive patients were examined, the percentage of HSIL was 5.79. (11)

In another study conducted in Turkey, which included patients with ASCUS cytology, the prevalence of HPV-16 positivity was found to be 36%, HPV-18 positivity was 5%, HPV-31 positivity was 8%, HPV-45 positivity was 4%, and HPV-52 positivity was 4%. When these patients were examined through colposcopic biopsy procedures, it was found that among those with a co-occurrence of ASCUS and HPV, CIN2+ pathologies were detected at a rate of 24.3%, and among patients with a co-occurrence of HPV 16 and/or 18, CIN2+ pathologies were detected at a rate of 41.7%.(23) These data substantiate our findings.

In our study, HPV positivity and the risk of CIN II+ lesions were found to be higher than in other studies because most of the patients presented with complaints.

In another hospital-based study, when 50,943 patients were screened, HPV negativity was observed in 26,291 patients, and CIN II+ lesions were detected in 1.38% of these patients. Whereas in HPV-positive patients, CIN II+ was detected in 19.95% of patients, so approximately 15 times higher.(24)

Comparable to current study a study from japan analysed the incidence of CINII+ lesions in ASCUS population according to HPV status and they showed that the incidence in HPV positive patients was 35.4%. (25)

In a study conducted on colposcopy, cytology, HPV, and ASC-US, 889 out of 2661 patients were evaluated on HPV, while the remaining patients were evaluated on colposcopy and repeated cytology. The patients evaluated with HPV were mostly women between the ages of 20 and 30; 82.1% were multiparous, 22.7% have never used contraceptives and 77.7% had never smoked before.(26) 41% of the patients who were examined under the HPV section had high-risk HPV,(26) the average age of patients who consulted was 30. In our study, most of the women were non-smokers, 55% were multiparous, 68% were premenopausal, oral contraceptive use was 5%, and the most common complaint was about vaginal discharge.

The fact that our study had some limitations, which were: its retrospective design, being a monocentric study, a relatively small number of patients who mostly applied due to pre-existing complaints, and lack of colposcopic findings.

It has also been shown in that HPV negative patients with Ascus and patients who do not have any intraepithelial lesion or malignancy in their cytology have similar risk of developing CIN III or CIN II lesions which is very low. Therefore, HPV positivity has a great importance in terms of lesions.(25)

In conclusion, we have found that the HPV viral load can reach up to 50% in patients presenting within the ASCUS population. Moreover, HPV genotyping should be performed to assess the risk of CIN II+ lesions in these patients.

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