

Human Papillomavirus Genotype Attribution in Premalignant-Malign Cervical Lesions: A Retrospective Tertiary Center Results

Premalign-Malign Servikal Lezyonlarda Human Papillomavirüs Subtiplerinin İlişkisi: Üçüncü Basamak Tek Merkez Analizi

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ÖZET

Amaç: Onkojenik HPV tipleri servikal kanser gelişiminde önemli rol oynarlar. Çalışmamızda HPV subtiplerinin servikal lezyonlardaki önemini ve prevalansını araştırmak istedik.

Gereç ve yöntem: Hastanemizde jinekolojik onkoloji polikliniğine başvuran ve HPV testleri ile kolposkopik biyopsi yapılan 296 kadın retrospektif olarak değerlendirildi. Kolposkopik biyopsi örnekleri histopatolojik inceleme ile HPV örnekleri ise polimeraz zincir reaksiyonu (PCR) ile değerlendirilerek DNA tipleme yapıldı.

Bulgular: Hastalarımızdaki HPV tipleri arasında en sık HPV tip 16 tespit edildi. Yüksek riskli HPV tipleri arasında en sık görülen 5 tip sırasıyla 16,31,18,51 ve 52'dir. H-SIL lezyonlarında en sık HPV tipleri 16,35,31 iken L-SIL'de 16,51,31 olarak bulundu. Bölgemizde kolposkopik servikal biyopsi yapılan 296 hastanın 129'unda servikal premalign veya malign lezyon tespit edildi. Bu 129 hastanın 110'unda etiyolojide HPV saptanmış, 19/129'unda HPV saptanmamıştır.

Sonuç: Serviks kanserinde etyolojide en önemli risk faktörü olan HPV virüsüdür. HPV ye karşı geliştirilmesi planlanan aşılardan içeriğinin en sık görülen ve yüksek risk grubu subtipler olması serviks kanserini önlemede ümit verici olacaktır.

Anahtar Kelimeler: HPV subtipleri, Servikal İntraepitelial lezyon, Serviks kanseri

ABSTRACT

Aim: Oncogenic Human papillomavirus (HPV) types play an important role in the development of cervical cancer. The cervix is an easily accessible organ that significantly improves the prognosis with early diagnosis by screening tests, and subsequent early treatment planning. In our study, we wanted to investigate the importance and prevalence of HPV subtypes in cervical neoplastic lesions.

Materials and Methods: 296 women who applied to the gynecological oncology clinic in our hospital and underwent colposcopic biopsy based on HPV tests were evaluated retrospectively. Colposcopic biopsy samples were evaluated by histopathological examination and HPV samples were evaluated by polymerase chain reaction (PCR) and DNA typing was performed.

Results: Among the HPV types in our patients, the most common HPV type 16 was detected. Among the high-risk HPV types, the 5 most common types are 16,31,18,51, and 52, respectively. While the most common HPV types were 16,35,31 in high-grade squamous intraepithelial lesions (H-SIL), and 16,51,31 in low-grade squamous intraepithelial lesions (L-SIL). In our region, a cervical premalignant or malignant lesion was detected in 129 of 296 patients who had a colposcopic cervical biopsy. In 110 of these 129 patients, HPV was detected in etiology, and HPV was not detected in 19/129 of them.

Conclusion: The HPV virus is the most important risk factor in the etiology of cervical cancer. The content of vaccines planned to be developed against HPV is the most common and high-risk group subtypes and will be promising in preventing cervical cancer.

Key words: HPV subtypes, cervical intraepithelial neoplasia, cervix carcinoma

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INTRODUCTION

Human papillomavirus (HPV) is one of the most common sexually transmitted infectious agents belonging to the papillomaviridae family (1). Cervix, vagina, and vulva in women; Penis cancers in males, and oral cavity head, neck, and anal canal cancers in both genders may develop secondary to HPV infection (2). There are more than a hundred types of HPV defined in various localizations, and more than 40 types are localized in the anogenital area (2-3). Cervical cancer is the fourth most common type of cancer among women in the world (4). Although advanced age, obesity, early sexual age, multiple partners, multiparity, low socioeconomic level, and HSV are known as risk factors, undoubtedly the most important risk factor is HPV (5). There are more than 40 types of HPV in the anogenital area, 16,18,31,33,45,51,58,59,68 of which are high risk for malignancy 53,56; defined the probability as high risk and 6,11,40,54,70 as low risk (5).

In vaccine studies aimed at preventing high-risk HPV types, the bivalent vaccine is effective against 16-18, while the quadrivalent vaccine includes 6, 11, 16, and 18. As the last vaccine, nonavalent vaccine is effective against 6,11,16,18,31,33,45, 52 and 58. Studies show that the incidence and persistence of cervical infections decreased with the HPV vaccine (6). In our study, we wanted to investigate the pathogenicity and effect of HPV subtypes on premalignant and malignant lesions observed in the cervix.

MATERIALS AND METHODS

After obtaining the approval of the Local Ethics committee of Konya Research and Training Hospital TUEK (Date: 2020/02/07, decision no: 40/09). 296 patients who applied to the gynecological oncology clinic between 2018 and 2020, and who received colposcopic cervical biopsy were included in our study. The ethics committee approval required for the study was obtained from our hospital. It is designed retrospectively by scanning archives from the hospital system. Colposcopic biopsy and HPV subtypes results of all patients were recorded. Diagene® HC2 HPV DNA test kits were used in HPV screening tests (Qiagen GmbH, Hilden, Germany). HPV DNA; PCR was performed with LCD Array HPV 3.5 kit (Chipron GmbH, Germany). More than 100 subtypes of HPV have been defined. Therefore, high-risk common types have been specified, and some of the high-risk HPV types are classified as "other".

All patients were biopsied from suspicious areas by colposcopic evaluation. Colposcopic examinations were performed with a binocular Welch Allyn brand colposcopy device with a green filter and a magnification of 4.5 to 30. In the colposcopic examination, after washing the cervix with saline, it was scanned with a small magnification, then 3% acetic acid was applied and waited for 3 minutes. Aceto-white areas were

scanned with small and large magnifications, and abnormal vascularity was evaluated with the green filter. Lugol solution was applied in cases that could not be seen sufficiently and the clinical suspicion persisted, and the presence of iodine-free areas was screened. Biopsy with cervical biopsy forceps was taken from the areas evaluated as abnormal by colposcopic evaluation. LEEP (Loop Electrosurgical Excision Procedure) or punch (cervical biopsy device) biopsy was taken from the areas suspected during colposcopy. Biopsy materials were fixed in formaldehyde and sent to the pathology laboratory for histopathological examination. All pathology specimens were routinely evaluated under a light microscope using the H&E histochemical stain p16 and ki 67 immunohistochemical stains. Dysplasia is seen in at least the lower two third of epithelium with H&E diagnosed as HSIL. Dysplasia which is limited in the lower third of the epithelium with H&E was evaluated as LSIL. Strong and diffuse block p16 staining and continuous nuclear and cytoplasmic p 16 staining in the basal layer of dysplastic epithelium supported our dysplasia diagnosis. An increased Ki 67 proliferation index also helps us the diagnose dysplasia.

Statistical Analysis

Analysis of all data was performed using SPSS (Statistical Packages for The Social Sciences, software, edition 21, SPSS Inc. Chicago, USA).

RESULTS

The mean age of 296 female patients included in the study was $43 \pm 9,2$; The average number of colposcopic biopsy quadrants sampled from patients is 2,1. Cervical premalignant or malignant lesions (Low-grade squamous intraepithelial lesion (L-SIL), High-grade squamous intraepithelial lesion

Table 1. Frequency of HPV subtypes in all HPV-positive patients

HPV subtype	Numbers
16	80
18	13
31	25
35	5
33	2
38	11
45	7
51	13
52	12
53	3
56	7
59	5
68	9
58	3
Other	27
TOTAL	222

(HPV: Human papillomavirus)

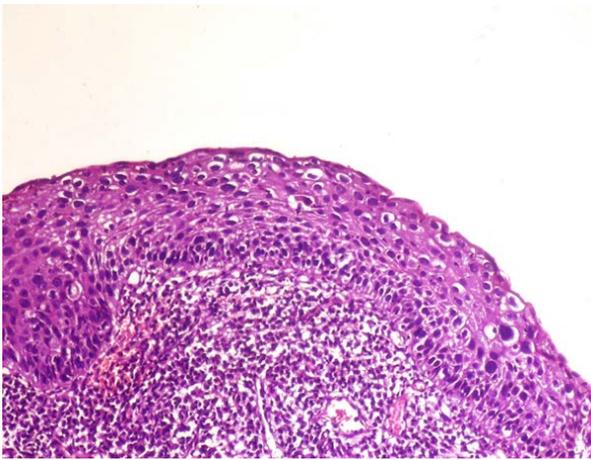


Figure 1. High grade cervical intraepithelial lesion H&E (40X)

(H-SIL), squamous cell carcinoma (SCC)) were detected histopathologically in 129 of 296 patients. In all biopsy materials diagnosed with LSIL and HSIL; pathological Ki-67 and p-16 expressions were observed (Figure 1-2-3). Of these 129 cases, 110 are HPV positive and 19 are HPV negative. 232 of 296 patients were found to be HPV positive. Only 110 of 232 HPV-positive cases were diagnosed as premalignant or malignant in biopsy materials. In 96 of 232 HPV-positive cases, HPV was 16-18 positive, and in 126 cases other HPV subtypes were positive. Among the other HPV types classified after HPV 16, the prominence of HPV 31 has drawn attention (Table 1). Of the 110 cervical premalignant lesions, 69 were reported as L-SIL, 44 as H-SIL, 1 as adenocarcinoma, and 1 as squamous cell carcinoma. HPV subtypes in H-SIL and L-SIL cases are shown in Tables 2-3.

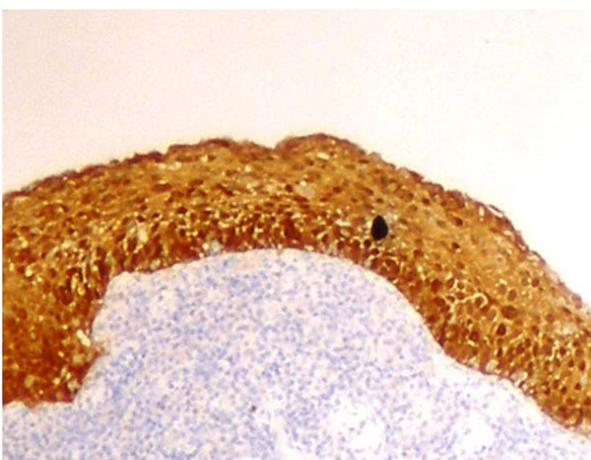
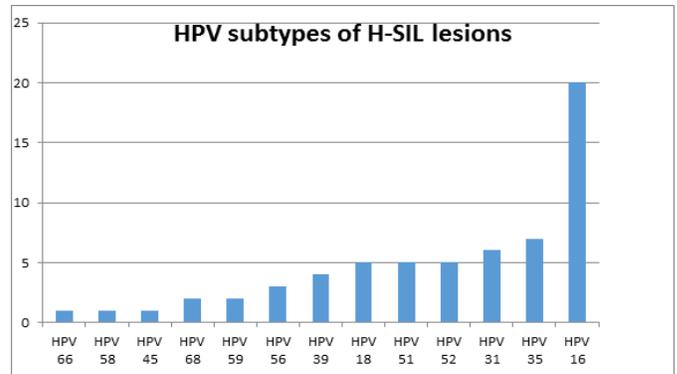


Figure 2. p16 immunohistochemistry in high grade cervical intraepithelial neoplasia. P16 cytoplasmic and nuclear staining H&E (40X)

Table 2. HPV subtypes of H-SIL lesions



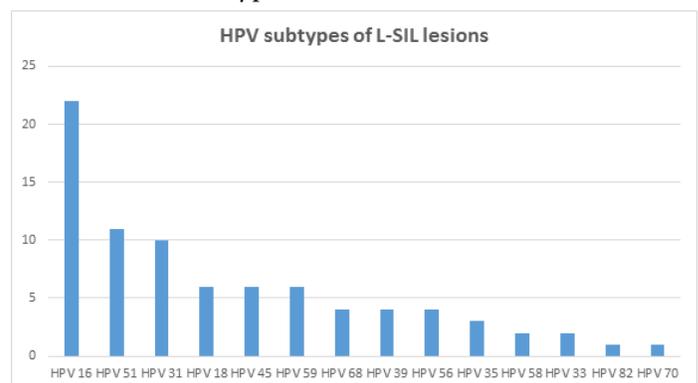
(H-SIL: High-grade squamous intraepithelial lesion, HPV: Human papillomavirus)

DISCUSSION

Cervical cancer caused by HPV is an important health problem in women. Because it is an easily accessible organ in the presence of preinvasive lesions, it is a disease suitable for early diagnosis. Cervical cancer screening methods; It is one of the few screening methods with proven efficacy in this respect, which is thought to reduce invasive cancer incidence and mortality. The Public Health Agency of Turkey conducted a community-based cervical cancer screening program was implemented (7). In the literature, there are many studies conducted in various regions on the pathogenicity and incidence of 16,18 HPV types and other HPV subtypes (8-12). In addition to these studies in the literature, our study presents single-center data in our region on the pathogenicity and frequency of 14 high-risk HPV types, including HPV 16 and 18.

The most common HPV subtypes and the most pathogenic HPV subtypes gain importance in prophylactic vaccine application. According to the results of our study, the most common subtypes in H-SIL cases were 16, 35, 31, 52, 51, and 18, respectively, while 16, 51, 31, 18, 45, and 49 were the most common subtypes in L-SIL cases. The incidence of HPV 16-

Table 3. HPV subtypes of L-SIL lesions



(L-SIL: Low-grade squamous intraepithelial lesion, HPV: Human papillomavirus)

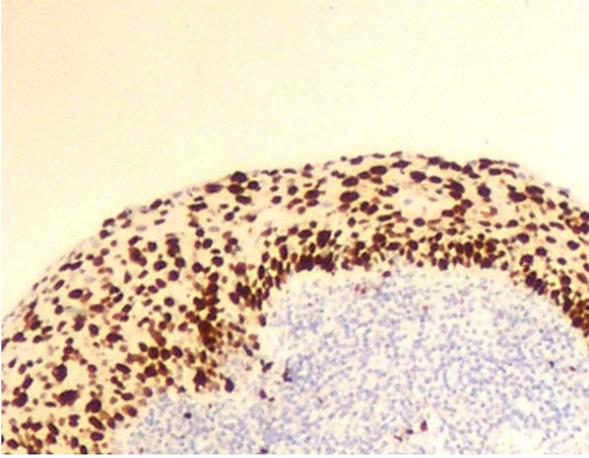


Figure 3. Ki 67 immunohistochemistry in high grade cervical intraepithelial neoplasia. Ki 67 nuclear staining H&E (40X)

18 is 96/236 (40%), and this rate was found to be 57% in a large series study conducted in our country in the literature (13). Remarkably, HPV 31 is the most common after HPV 16, and this result was found to be similar to the results of a large series study conducted in our region, middle Anatolia (13).

HPV was not detected in the etiology in 19/129 (14%) of the cervical neoplastic cases in our study. In the literature, the rate of cervical cancer without HPV in the etiology was 3.6%, and in our study, the rate of HPV-negative cervical cancer was found to be higher than in the literature (14).

In many studies conducted around the world, the most frequently observed HPV subtypes show regional differences (11-12). According to our present findings in possible prophylactic vaccine studies, the protection of the quadrivalent vaccine containing HPV 16 and 18 will be 41%, while the protection of the nonavalent vaccine containing 9 HPV subtypes will be 70%.

According to the results of our study, we believe that the protection of cervical malignant processes will be higher than the quadrivalent vaccine, since the 9-in-one vaccine, which was produced lastly in the production of the HPV vaccine, corresponds to the most frequently encountered high-risk HPV types in our study data. Also, our study will be a guide in patient management according to the subtypes of HPV-positive cases.

Etik Kurul: Ethics committee approval was received for this study from the The local ethics committee of Konya Research and Training Hospital TUEK (Date: 2020/02/07, decision no: 40/09).

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