Clinical research

A retrospective analysis of women diagnosed with unclassified HPV genotypes

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Abstract

Introduction: This study primarily aimed to investigate the associations between unclassified HPV genotypes and cervical lesions.
Material and methods: This was a retrospective review of 411 patients with HPV positivity. The participants were divided into two groups: Group X contained HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 while group Y contained unclassified HPV genotypes such as 42, 54, 61, 62, 71, 81, 83, 84 and 89. The X and Y groups were compared in terms of demographic characteristics and related cervical pathologies. We investigated the potential of HPV genotypes in group Y to develop cervical neoplasia.
Results: HPV 16 is the most common genotype in group X (28.5%) whereas HPV 83 is the most common genotype in group Y (4.9%). Group X and group Y were statistically similar with respect to age (p = 0.231), parity (p = 0.617), occupation (p = 0.535), marital status (p = 0.644), education level (p = 0.316), smoking (p = 0.352), gynecological findings (p = 0.201), Pap smear results (p = 0.427), and colposcopy findings (p = 0.476). When compared to group X, normal cervical biopsy was significantly more frequent (18.6% vs. 39.1%), chronic cervicitis was significantly less frequent (49.5% vs. 36.9%), CIN 1 was significantly less frequent (12.9% vs. 8.7%) and CIN 2 was significantly less frequent (5.6% vs. 2.2%) (p = 0.012). Cervical cancer was diagnosed in three patients of group X and one patient of group Y (1.6% vs. 2.2%).
Conclusions: Screening programs for cervical cancer are generally performed to detect HPV genotypes with high oncogenic potential. The importance of unclassified HPV genotypes should be investigated in large scale studies so that the success of screening programs and vaccination can be improved.

Key words: cervical cancer, colposcopy, human papillomavirus, unclassified.

Introduction

Human papillomavirus (HPV) is a sexually transmitted virus which is associated with condyloma, cervical neoplasia and carcinoma. The results of HPV transmission depend on the oncogenic potential of the virus and the immunity of the host [1].

Invasive cervical cancer is the second most common malignancy of women living all over the world. HPV infection is the most important risk factor for invasive cervical cancer [2, 3]. HPV genotypes are divided into five groups based on their oncogenic capacity (Table I). Group 1 is the highest potential risk factor for invasive cervical cancer. Groups 2A and

Table I. Distribution of HPV types based on their oncogenic capacity

Group 1 (carcinogenic to humans)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Group 2A (probably carcinogenic to humans)	68
Group 2B (possibly carcinogenic to humans)	26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97
Group 3 (unclassifiable as to carcinogenicity in humans)	6, 11
Unclassified*	42, 54, 61, 62, 71, 81, 83, 84, 89

**In our study we found 9 unclassified genotypes.*

2B include probably and possibly oncogenic HPVs. Group 3 is responsible for benign genital warts but there is not enough information about the role of unclassified HPV genotypes in the carcinogenesis of cervical tumors [4].

The prevalence of HPV genotypes varies around the world. For example, the highest HPV prevalence is about 20–30% in Africa and Latin America, and the lowest HPV prevalence is about 6–7% in Southern Europe and South East Asia [5]. The HPV genotypes vary with geographic location. For instance, the leading 5 HPV genotypes are HPV16, 53, 52, 18, and 39 for America whereas these genotypes are HPV16, 52, 58, 18, and 56 for Asia [6].

Specifying the distribution of HPV genotypes according to geographic regions is very important for determining an efficient prevention strategy that includes screening programs and vaccination. It is also important to clarify the role of unclassified HPV genotypes in the carcinogenesis of cervical tumors so that HPV vaccines and their efficiency can be improved. This study primarily aimed to investigate the associations between unclassified HPV genotypes and pre-malignant and malignant cervical lesions.

Material and methods

The present study was conducted in accordance with the approved guidelines and the principles expressed in the Declaration of Helsinki. (Ethics Committee Number: (12.09.2018-30/01)). Written informed consent was obtained from each participant.

There is a cervical cancer screening program conducted by the Ministry of Health in Turkey. The patients diagnosed with HPV positivity by the Cancer Early Diagnosis Screening and Training Center were referred to the study center between January 2014 and December 2018. This is a retrospective review of 411 patients with HPV positivity. The inclusion criteria were being between 30 and 65 years old, being sexually active, having no pregnancy during the study, having no hysterectomy or cervical resection, and not being diagnosed with cervical cancer in the past years. The exclusion criteria were being HIV positive, having vaginal bleeding during pelvic examination, having multiple HPV infections, using vaginal medications or having sexual intercourse in the last 48 h before the examination.

The participants were divided into two groups: Group X contained HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 while group Y contained unclassified HPV genotypes such as 42, 54, 61, 62, 71, 81, 83, 84 and 89. The X and Y groups were compared in terms of demographic characteristics and related cervical pathologies. The primary outcome of our study is to show the potential of unclassified HPV genotypes in the development of pre-malignant and malignant cervical lesions.

Cervical smear and HPV sampling

Cervical smears and HPV samples were collected from all participants by a doctor or nurse trained in cervical cancer screening, according to the standard sampling procedure at the Cancer Early Diagnosis Screening and Training Center.

HPV-DNA isolation and genotyping

Cervical specimens were collected and transported using the HC2 HPV DNA (Qiagen Gaithersburg, Inc. USA) Collection Device. The specimen was kept in 3 ml of sample transport medium. HPV DNA was extracted and amplified by Hybrid Capture-2 and polymerase chain reaction (PCR), using the known protocols in the literature [7–9]. The restriction fragment length polymorphism method was also used to classify the unknown HPV types. HPV genotyping was performed with the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences Corp., NJ, USA) and ABI PRISM 310 Genetic Analyzer at Iontek, Turkey.

Colposcopy

Colposcopy was performed in 224 patients (54.5%) due to previously established indications by World Health Organization (Table II) [10]. This procedure was carried out by means of a colposcope (LEICA CLS 150 XC, Germany) with a digital optical system (LEICA DFC 295, Germany). A cervical biopsy was taken from suspicious areas such as acetowhite epithelium, atypical vascularization, punctuation, mosaicism or leukoplakia.

Table II. Indications for colposcopy

Suspicious-looking cervix
Invasive carcinoma on cytology
CIN 2 or CIN 3 on cytology
Persisting (for more than 12–18 months) low-grade (CIN 1) abnormalities on cytology
CIN 1 on cytology
Persistently unsatisfactory quality on cytology
Infection with oncogenic human papillomaviruses
Acetopositivity on visual inspection with acetic acid (VIA)
Acetopositivity on visual inspection with acetic acid using magnification (VIAM)
Positive on visual inspection with Lugol's iodine (VILI)

CIN – cervical intraepithelial neoplasia.

Statistical analysis

Collected data were analyzed by SPSS version 22.0 (IBM Corp., Armonk, NY, US). Continuous variables were expressed as mean ± standard deviation (SD) or median (min.–max.), and categorical variables were denoted as numbers or percentages, if applicable. Pearson's χ^2 test and the Mann-Whitney *U* test were used for comparisons. Two-tailed *p*-values < 0.05 were considered to be statistically significant.

Results

Table III shows the distribution of HPV genotypes. HPV 16 is the most common genotype in group X (28.5%) whereas HPV 83 is the most common genotype in group Y (4.9%). Table IV shows the demographic, clinical and pathological characteristics of both groups. Group X and group Y were statistically similar with respect to age (p = 0.231), parity (p = 0.617), occupation (p = 0.535), marital status (p = 0.644), education level (p = 0.316), smoking (p = 0.352), gynecological findings (p = 0.201), Pap smear results (p = 0.427), and colposcopy findings (p = 0.476). When compared to group X, normal cervical biopsy was significantly more frequent (18.6% vs. 39.1%), chronic cervicitis was significantly less frequent (49.5% vs. 36.9%), CIN 1 was significantly less frequent (12.9% vs. 8.7%) and CIN 2 was significantly less frequent (5.6% vs. 2.2%) (p = 0.012). Cervical cancer was diagnosed in 3 patients of group X and 1 patient of group Y. One patient in group X had cervical adenocancer, 2 patients in group X had cervical squamous cell cancer and the patient in group Y had microinvasive squamous cell cancer. The distribution of cervical lesions according to HPV genotypes is displayed in Table V for group Y.

Table III. Distribution of HPV genotypes in the study group

HPV genotypes	Frequency	Percent
16	117	28.5
51	34	8.3
39	25	6.1
31	24	5.8
18	20	4.9
83	20	4.9
71	19	4.6
45	14	3.4
52	14	3.4
35	13	3.2
89	13	3.2
56	12	2.9
54	11	2.7
58	11	2.7
81	11	2.7
42	10	2.4
62	10	2.4
68	9	2.2
84	9	2.2
61	7	1.7
33	5	1.2
59	3	0.7
Total	411	100

Discussion

The role of unclassified HPV genotypes in the carcinogenesis of cervical tumors has not been fully clarified and the number of studies related to this subject is limited in the literature. Barut *et al.* found that women who had an abnormally appearing cervix during clinical examination should be evaluated by Pap smear and colposcopy [11]. This study aims to evaluate the role of HPV genotypes in the carcinogenesis of cervical tumors by Pap smear, colposcopy and colposcopy guided biopsy results.

The prevalence of HPV infections in women is strongly influenced by demographic and geographic characteristics [12]. Kulhan *et al.* previously reported about the prevalence of HPV genotypes in Turkish women. In the present study, the demographic data related to age, occupation, marital status, education level and smoking in this

Table IV. Demographic, clinical and pathological characteristics

Characteristics	Group X (*n* = 301) (%)	Group Y (*n* = 110) (%)	*P*-value
Age, mean ± SD [years]	44.7 ±9.4	43.3 ±8.5	0.231
Parity:			0.617
Nullipara	37 (12.3)	14 (12.7)	
Multipara	264 (87.7)	96 (87.3)	
Occupation:			0.535
Housewife	180 (59.8)	68 (61.8)	
Worker	121 (40.2)	42 (38.2)	
Marital status:			0.644
Single	28 (9.4)	10 (9.1)	
Married	241 (80)	85 (77.3)	
Widow	32 (10.6)	15 (13.6)	
Education level:			0.316
Illiterate	36 (11.9)	11 (10)	
Primary education	136 (45.2)	48 (43.6)	
High school	98 (32.6)	41 (37.3)	
University	31 (10.3)	10 (9.1)	
Smoking:			0.352
No	181 (60.2)	65 (59.1)	
Yes	120 (39.8)	45 (40.9)	
Gynecological findings:			0.201
Normal	233 (77.4)	86 (78.2)	
Genital warts	14 (4.7)	9 (8.1)	
Post-coital bleeding	54 (17.9)	15 (13.7)	
Pap smear results:			0.427
Normal	264 (87.7)	90 (81.8)	
ASC-US	19 (6.3)	10 (9.2)	
ASC-H	2 (0.7)	1 (0.9)	
LSIL	16 (5.3)	9 (8.1)	
HSIL	0	0	
Colposcopy findings:			0.476
Normal	213 (70.7)	63 (57.2)	
Acetowhite epithelium (ACW)	22 (7.4)	35 (31.8)	
Atypical vascularization (AV)	18 (5.9)	4 (3.7)	
ACW + AV	25 (8.4)	5 (4.6)	
Punctuation	17 (5.7)	1 (0.9)	
Mosaicism	6 (1.9)	1 (0.9)	
Leukoplakia	0	1 (0.9)	
Cervix biopsy results:			0.012
Normal	33 (18.6)	18 (39.1)	
Chronic cervicitis	88 (49.5)	17 (36.9)	
CIN1	23 (12.9)	4 (8.7)	
CIN2	10 (5.6)	1 (2.2)	
CIN3	21 (11.8)	5 (10.9)	
Cancer	3 (1.6)	1 (2.2)	
Total	178	46	

ASC-US – atypical squamous cells of undetermined significance, ASC-H – atypical squamous cell-cannot exclude high-grade squamous intraepithelial lesion, LSIL – low-grade squamous intraepithelial lesion, HSIL – high-grade squamous intraepithelial lesion, CIN – cervical intraepithelial neoplasia.

Table V. Distribution of cervical lesions according to HPV genotypes in group Y

HPV type	Cervical biopsy results			
	CIN 1	CIN 2	CIN 3	Cancer
42 (*n* = 10)	–	–	–	–
54 (*n* = 11)	+	–	–	–
61 (*n* = 7)	+	–	–	–
62 (*n* = 10)	–	–	+	–
71 (*n* = 19)	+	–	++	–
81 (*n* = 11)	–	–	++	+
83 (*n* = 20)	+	–	+	–
84 (*n* = 9)	–	–	–	–
89 (*n* = 13)	–	–	–	–

(–): zero, (+): 1 case, (++): 2 case.

study were similar to those of Kulhan *et al.*, but the number of multiparous patients was lower in this study (78.5% to 87.5%) [13]. The most common colposcopy finding was acetowhite epithelium in the study of Kulhan *et al.*, but the most common finding was atypical vascularization in the study of Barut *et al.* [11]. Colposcopy findings of X and Y groups in our study were different. In the X group, the most common finding was acetowhite epithelium + atypical vascularization, but the most common finding in the Y group was acetowhite epithelium.

Similar to the present study, the most common genotype was HPV 16 in patients diagnosed with cervical cancer [13–15]. Annunziata *et al.* declared that the most common unclassified genotype was HPV 81, in Italian women [4]. However, in Turkish women included in our study, the most common unclassified genotype was HPV 83.

Annunziata *et al.* reported that none of the patients in the unclassified HPV group had a high-grade squamous intraepithelial lesion in Pap smear results, in parallel with the results of our study. However, in the literature, it was found that unclassified HPV genotypes can exert carcinogenic effects especially in immunosuppressive and HIV-positive patients [16, 17]. HIV-positive women were not included in this study, but according to the colposcopy guided biopsy results, there was one case of squamous cell carcinoma case related to HPV 81 in group Y. In this case, smoking was the only risk factor detected.

Baloch *et al.* identified 6 unclassified HPV genotypes (HPV 42, 55, 61, 71, 81, 83) in their epidemiological study in which a total of 17,898 women were evaluated. In the present study, there was no cancer case in the group of patients with unclassified HPV genotypes; however, the incidence of CIN1, CIN2 and CIN3 was higher in the HPV 81 positive group than the others [18].

It can be speculated that the prevalence of unclassified HPV genotypes and their oncogenic potentials are underestimated because researchers have focused on HPV genotypes with higher oncogenic potential [19–22]. Recently, clinicians discovered that cervical cancer can be prevented by vaccination and studies have accelerated in this direction. However, the distribution of HPV genotypes is not homogeneous and varies with respect to geographic location. Since the role of unclassified HPV genotypes in the carcinogenesis of cervical tumors has not been clarified, more studies should be performed on this subject. In this way, it is possible to improve HPV vaccines and increase their effectiveness.

This study has some limitations. First, it is a retrospective study with a small sample size. Second, the behavioral risk factors, such as condom use, multiple sexual partners and the first sexual intercourse age, have not been considered. The third disadvantage was the lack of long-term data related to the persistence of unclassified HPV genotypes.

In conclusion, the significance of cervical cancer screening programs has become prominent. These screening programs are generally performed to detect HPV genotypes with high oncogenic potential. The importance of unclassified HPV genotypes should be investigated in larger patient series so that the success of screening programs and vaccination can be improved. It should also be taken into account whether there is an additional risk factor such as smoking when deciding on the histopathological examination of women who are diagnosed with unclassified HPV genotypes.

Conflict of interest

The authors declare no conflict of interest.

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