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Original

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HPV genotype distribution among women with normal and abnormal cervical cytology in Turkey

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ABSTRACT

Objectives. The aim of this study was to determine the human *papillomavirus* (HPV) genotype distribution and to investigate the relationship between HPV genotypes and cervical cytology in women with HPV infection.

Material and methods. In this study, 493 women who were admitted to the obstetrics clinic between 2007 and 2015 years and had HPV positivity were examined retrospectively.

Results. The median age of women included in the study was 37.3 ±10.6. The positivity of single and multiple HPV genotypes was 64.1% and 35.9%, respectively. HPV16 was the most common genotype in women with normal and abnormal cytology. The incidence of atypical squamous cells of undetermined significance (chi-square:8.32 p=0.04) and high-grade squamous intraepithelial lesion (chi-square:13.75 p<0.001) with HPV16 was significantly higher than in other HPV genotypes. In additional, abnormal cytology results in the group 1 (included HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and group 4 (included HPV40, 42, 54, 55, 61, 62, 81, 83, 84) were significantly higher than other groups (chi-square:23.15 p<0.001).

Conclusions. Group 1 genotype ratios were found to be quite high among women with abnormal cytology and women with normal cytology. For this reason, close follow-up is very important in addition to cytological findings along with genotyping, especially from an early age. We were found that multiple HPV infection was not related to the grades of cytological abnormalities. Although abnormal cytology results in group 4 were significantly higher than the other groups, it was not possible to comment on the relationship between these genotypes and cervical cancer since more than one HPV genotype was found in most of these women.

Keywords: Cervical cancer, Cytology, HPV, Genotype distribution

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Distribución del genotipo del VPH en mujeres con citología cervical normal y anormal en Turquía

RESUMEN

Objetivos. El objetivo de este estudio fue determinar la distribución del genotipo del virus del papiloma humano (VPH) e investigar la relación entre los genotipos del VPH y la citología cervical en mujeres con infección por VPH.

Material y métodos. En este estudio, 493 mujeres que ingresaron en la clínica de obstetricia entre los años 2007 y 2015, y que presentaron VPH positivo fueron examinadas retrospectivamente.

Resultados. La edad media de las mujeres incluidas en el estudio fue de 37,3 ± 10,6. La positividad de los genotipos de VPH individuales y múltiples fue de 64,1% y 35,9%, respectivamente. VPH16 fue el genotipo más común en mujeres con citología normal y anormal. La incidencia de células escamosas atípicas de importancia indeterminada (chi-cuadrado: 8,32 p: 0,04) y lesión intraepitelial escamosa de alto grado (chi-cuadrado: 13,75 p <0,001) con VPH16 fue significativamente mayor que en otros genotipos de VPH. Además, los resultados citológicos anormales en el grupo 1 (incluido VPH 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) y el grupo 4 (incluido el VPH 40, 42, 54, 55, 61, 62, 81, 83, 84) fueron significativamente más altos que en otros grupos (chi-cuadrado: 23,15 p <0,001).

Conclusiones. Se encontró que las proporciones de genotipos del grupo 1 fueron bastante altas entre las mujeres con citología anormal y las mujeres con citología normal. Por esta razón, el seguimiento cercano es muy importante además de

los hallazgos citológicos junto con el genotipado, especialmente desde una edad temprana. Se encontró que la infección múltiple por VPH no estaba relacionada con los grados de anomalías citológicas. Aunque los resultados anormales de la citología en el grupo 4 fueron significativamente superiores a los otros grupos, no fue posible valorar la relación estos genotipos y el cáncer cervical ya que se encontró más de un genotipo de VPH en la mayoría de estas mujeres.

Palabras clave: cancer cervical, citología, VPH, distribución genotipos

INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide and the leading cause of mortality in developing countries [1]. But cervical cancer is unique among common cancers in that it can be almost totally eradicated. Over 90% of women with cervical cancer are human *papillomavirus* (HPV)-positive [2]. Persistence of the HPV infection as well as viral DNA insertion in epithelial cells are the main factors leading to high-grade dysplasia with the potential of progression to carcinoma *in situ* and invasive cancer [3]. Although more than 140 HPV types have been identified and approximately 40 of them are known to infect humans and only about 15 of them cause cervical cancer and its precursor lesions [4]. Twelve viruses (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) have been found significantly associated with cervical carcinoma and classified as Group 1 "oncogenic to humans", one virus (HPV68) as Group 2A "probably oncogenic to humans", 12 viruses (HPV26, 53, 66, 67, 70, 73, 82, 30, 34, 69, 85, 97) as Group 2B "possibly oncogenic to humans", two viruses (HPV6 and 11) as Group 3 "unclassifiable as to carcinogenicity in humans" and all HPVs not included in the Group 1, 2A, 2B and 3 have been descripted as Group 4 (unclassified HPV genotypes) [5]. Several other HPV genotypes are unclassified regarding to their epidemiologic oncogenic risk although few of them have been shown to bind and to ubiquitinate p53 oncosuppressor with the same efficiency as the Group 1 oncogenic viruses [5].

According to Ministry of Health registry data, cervical cancer is the 10th most common cancer among female cancers in Turkey [4]. Overall HPV prevalence has been reported to be between 2.1% and 25% in some Turkish regional studies [6-9]. According to report of population based cervical cancer screening program using HPV testing in one million Turkish women in 2018, HPV DNA positivity was reported to be 3,5% and 19,1% of these patients was abnormal smear [10]. The aim of this study was to determine the HPV genotype distribution and to investigate the relationship between HPV genotypes and cervical cytology in women with HPV infection.

MATERIAL AND METHODS

The study was a retrospective study. The patients to be included in the study was selected among the patients who applied to the obstetrics clinic of our hospital between 2007 and 2015 years. All women had gynecological histories, cytological evaluations and HPV genotyping.

Cytological findings were classified in line with the 2004 Bethesda classification system, as follows: (1) within negative for intraepithelial lesions and malignancy (normal), (2) atypical squamous cells (a) low-grade squamous intraepithelial lesion (LSIL), (b) high-grade squamous intraepithelial lesion (HSIL), (c) atypical squamous cells of undetermined significance (ASCUS), (d) atypical squamous cells for which a high-grade lesion cannot be excluded (ASC-H), (e) invasive cervical carcinoma (ICC), (3) atypical glandular cell (a) atypical glandular cell-favor neoplasia (Agc-fn) and (b) atypical glandular cell not otherwise specified (Agc-nos).

Cervical cells were sampled with a cervical brush and they were transported in Cobas PCR Cell Collection Media (Roche). DNA purification was done using the High Pure PCR Template (Roche); HPV detection was performed using the Linear Array HPV Detection kit (Roche), and then DNA was genotyped with the Linear Array HPV Genotyping Test (Roche, Iaşi, Romania). Genotyping via this technique involves one step of PCR amplification (ABI 9700): the final volume contained 50 µl of purified DNA and 50 µl of master mix (Roche). The thermal profile included the next steps: HOLD program: 2 min/50°C; an initial denaturation of 9 min at 95°C was followed by 40 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, with a final extension for 5 min at 72°C. The amplicons have been denatured at the end of PCR amplification. HPV types were detected by hybridizations of the amplicons. Hybridization was performed in a TwinCubator (HAIN, Life Science) using Working Hybridization Buffer, Working Ambient Wash Buffer, Working Stringent Wash Buffer, Working Conjugate, Working Citrate Buffer and Working Substrate (Roche). The technique was validated through the use of positive and negative controls at each shift; the strips contain two bands for beta globin, which checks if the sample was correctly processed. HPV genotypes detected in this study were examined in five groups. These groups are as follows; Group 1 (oncogenic HPV genotypes; included HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), group 2A (probably oncogenic HPV genotype; included HPV 68), group 2B (possibly oncogenic HPV genotypes; included HPV 26, 53, 66, 67, 70, 73, 82), group 3 (unclassifiable as to carcinogenicity in humans; included HPV 6, 11) and group 4 (unclassified HPV genotypes; included HPV 40, 42, 54, 55, 61, 62, 81, 83, 84). All HPVs not included in the Group 1, 2A, 2B and 3 have been designed as Group 4 in the present study.

Statistical analyses were performed using the Epi Info 7.2.1.0 and SPSS version 21.0 statistical software packages. Descriptive statistics such as frequency, percent, mean and standard deviation were used to summarize patients parameters. Pearson chi-square test was used to compare categorical data. Differences were considered to be statistically significant when p values were less than 0.05.

RESULTS

The study included 493 women with a median age of 37.3 ± 10.6 (min:19, max:74) years. All women are infected with any HPV type. The cytological examination was available for all women. Of the women, 316 were positive for a single HPV type (64.1%) and 177 were positive for multiple types (35.9%) (table 1).

The higher prevalence of HPVs (32.5%; 160/493) was observed among women in the age group 30-39. Single HPV infections were found in 17.7% (87/493), 21.1% (104/493), 16.6% (82/493) and 8.7% (43/493) of the women aged ≤29, 30-39, 40-49 and ≥50 years, respectively. Multiple infections were observed in 9.1% (45/493), 11.4% (56/493), 9.3% (46/493) and 6.1% (30/493) of the women aged ≤29, 30-39, 40-49 and ≥50 years, respectively. The prevalence of group 1 and other HPV groups in ≤29, 30-39, 40-49 and ≥50 age groups was 13.9 and 10.8%, 20 and 11.8%, 17.3 and 9.1%, 12.2 and 4.9%, respectively. It was found that group 1 HPV genotype positivity was higher in patients between 30 and 39 years (20%) than other age groups (table 1).

The prevalence of group 1 HPV genotypes was 68.6% (338/493). The most common genotypes were HPV16 (36.9%; 182/493), HPV45 (14.2%; 70/493), HPV18 (13.9%; 69/493), HPV31 (6.3%; 31/493), HPV51 (5.9%; 29/493), HPV33, HPV35 and HPV58 (each 5.7%; 28/493) belonging to Group1; HPV53 (7.5%; 37/493) belonging to group 2B and HPV6 (28.2%; 139/493) belonging to group 3. The frequency of all other genotypes was below 5% of all HPV infections. The distribution of HPV genotypes is shown table 2.

The cytological results of the women were as follows; normal cytology (68%; 335/493), atypical glandular cell (0.6%; 3/493) and atypical squamous cells (31.4%; 155/493). The prevalence of atypical squamous cells was significantly higher than atypical glandular cell (p<0.001). Agc-fn and Agc-nos were detected 0.2% (1/493) and 0.4% (2/493), respectively. The women that diagnosed squamous cell anomaly included ASCUS (9.5%; 47/493), ASC-H (2.2%; 11/493), LSIL (12%; 59/493), HSIL (7.3%; 36/493) and ICC (0.4%; 2/493). Of women with abnormal cytology results, the prevalence of LSIL was higher than other atypical squamous cells. However, there was not found to be statistically significant difference between atypical squamous cells (table 2).

The prevalence of group 1 HPV genotypes (with multiple HPV genotypes) found to be 63.3% (212/335) among women with normal cytology to 79.8% (126/158) in women with abnormal cytology. This distribution is shown in table 2 in women with a single HPV genotype. The most common group 1 HPV genotypes among women with normal cytology were HPV16 (32.5%; 109/335), followed by HPV45 (19.4%; 65/335), HPV18 (13.4%; 45/335), HPV31 (6.6%; 22/335), HPV51 (5.9%; 20/335), HPV 33, HPV35, HPV39, HPV52, HPV56 and HPV59 (each <5%). The most common group 1 HPV genotypes among women with abnormal cytology were HPV16 (46.2%; 73/158), HPV18 (15.2%; 24/158), HPV33 and HPV58 (each 8.9%; 14/158), HPV35 (8.2%; 13/158), HPV 52 (7.6%; 12/158), HPV51 (5.7%; 9/158), HPV39, HPV45, HPV56 and HPV59 (each <5%). The incidence of abnormal cytology was found to be significantly higher in patients with group 1 HPV genotypes compared to patients with other HPV groups (chi-square: 9.27 p= 0.002).

Group 4 HPV genotypes were detected in 54 women. In 8 of these women, single HPV genotype was detected, while 46 women had multiple HPV infection. In addition, 36 of these 46 women (78.3%) were also identified as group 1 HPV genotypes.

Distribution of ASCUS, LSIL, HSIL and ICC in the women that was detected group 1 HPV genotypes was 80.9% (38/47), 67.8% (40/59), 94.4% (34/36) and 100% (2/2), respectively. The most frequent group 1 HPV genotype in women with abnormal cytology was HPV 16 (40.1%; 73/182). Distribution of HPV 16 in women with ASCUS, LSIL and HSIL was 45.8% (22/48), 32.2% (19/59) and 72.2% (26/36), respectively. The incidence of ASCUS (chi-square: 8.32 p= 0.04) and HSIL (chi-square: 13.75 p<0,001) in women with HPV 16 was significantly higher than in other HPV genotypes.

Group 4 detected in 10.7% (36/335) of normal cytology, in 13.6% (8/59) of LSIL, in 14.9% (7/47) of ASCUS and 8.3% (3/36) of ASC-H but in none of HSIL. Among the unclassified HPVs the most frequent HPV84, 62, 54, and 42 were being found in 5.1, 5.1, 3.4 and 1.7% of women with LSIL, respectively, while the genotype 62 was the most frequent (3.9%) among women with normal cytology.

Prevalence of abnormal cytology was found to be higher in the patients with single type HPV infection (21.3%) when compared to the women with multiple HPV infection (10.8%), but this distribution was statistically insignificant (chi-square: 0.42 p= 0.5). The multiple HPV infection rates in normal cytology, ASCUS, LSIL and HSIL were 37% (124/335), 31.9% (15/47), 37.3% (22/59) and 22.2% (8/36), respectively. In additional, abnormal cytology results in the group 1 and group 4 were significantly higher than other groups (chi-square: 23.15 p <0.001).

DISCUSSION

Worldwide, cervical cancer is the second most common malignancy in women, impacting about 35 of every 100,000 women [10]. According to Turkey cancer control programme, cervical cancer is the 10th most frequently observed cancer in women [11]. Persistent infection of HPV has been definitively linked to the development of the cancer [10]. In a study featuring over 30,000 cervical cancers, the International Agency for Research on Cancer (IARC) showed that of the most frequent HPV genotypes (HPVs) that lead to cervical malignancy (16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56), HPV 16 induces more than 50% of cervical cancers, while HPV 16 and 18 together lead to over 70% of cases across the globe [10]. This study provides a comprehensive information on the HPV genotype distribution among Turkish women with normal and abnormal cytology.

Epidemiologic and biological researchers demonstrate that the statistical distribution of HPV infection and its sub-

Table 1 **HPV genotypes distribution among 493 HPV positive cases (834 different types) by age groups.**

	Total		≤29 years		30-39 years		40-49 years		≥50 years	
	n	%	n	%	n	%	n	%	n	%
Single type	316	64.1	87	17.7	104	21.1	82	16.6	43	8.7
Multiple type	177	35.9	45	9.1	56	11.4	46	9.3	30	6.1
Total	493	100	132	26.8	160	32.5	128	25.9	73	14.8
Group 1										
HPV-16	182	21.8	45	5.3	58	6.9	52	6.3	27	3.3
HPV-18	69	8.3	20	2.4	19	2.3	19	2.3	11	1.4
HPV-31	31	3.7	6	0.7	11	1.4	7	0.8	7	0.8
HPV-33	28	3.4	3	0.4	8	0.9	8	0.9	9	1.2
HPV-35	28	3.4	4	0.5	8	0.9	8	0.9	8	0.9
HPV-39	15	1.8	1	0.1	4	0.5	4	0.5	6	0.7
HPV-45	70	8.4	18	2.1	24	3	18	2.1	10	1.2
HPV-51	29	3.5	5	0.6	8	0.9	9	1.2	7	0.8
HPV-52	25	2.9	3	0.4	7	0.8	8	0.9	7	0.8
HPV-56	12	1.4	3	0.4	6	0.7	1	0.1	2	0.2
HPV-58	28	3.4	5	0.6	8	0.9	8	0.9	7	0.8
HPV-59	12	1.4	3	0.4	6	0.7	2	0.2	1	0.1
Total	529	63.4	116	13.9	167	20	144	17.3	102	12.2
Other[a]	305	36.6	90	10.8	98	11.8	76	9.1	41	4.9
Total	834	100	206	24.7	265	31.8	220	26.4	143	17.1

[a]Other groups different of the group 1

types varies by region, age, and some other demographic features of the population [12]. Age has been reported to have the most important effect on the prevalence of HPV in some studies [13]. The lowest prevalence of HPV was reported in women aged 14–19 years and the highest prevalence was reported in women aged 20-24 years [14, 15]. In addition, some studies reported a second peak in the prevalence of HPV in postmenopausal women [16]. However, in a multicenter study, the age-related prevalence of HPV was reported to vary according to region and population. It has been reported that the prevalence of HPV has increased around 25 years old and it decreases with age [17]. In the present study, the majority of patients were between 3rd and 4th decades (32.5%). In addition, the prevalence of HPV was found to be decreased with increasing age in accordance with the literature [16]. Our study was conducted only in a case group of patients who attended gynaecology clinic of a third stage health institution in the western region of Turkey. For this reason, we believe that our results cannot be generalized to all of Turkey. It is necessary to perform a wider scale study across the country for HPV infection and vaccination.

The results of the present study demonstrated that HPV16, HPV 6, HPV45, HPV18, HPV53 and HPV31 were the six most prevalent types in our study population. In a study by Ari et al. [18] the most common HPV types was reported to be HPV16 and HPV6. In another study from Turkey, Beyazıt et al. the most common HPV types was reported to be HPV16, HPV58, HPV6 and HPV31 [11]. In the present study was detected that HPV16, HPV 18, HPV33, HPV58 and HPV35 were the most prevalent types in women with abnormal cytology. Finan et al. [19] reported HPV types 6, 11, 16, 18 and 33 were the most common detected HPV genotypes in women with abnormal cytology and also in those with normal cytology.

The prevalence of group 1 HPV genotypes found in our study was 68.6%. The most frequent group 1 HPV genotype was HPV16 accounting for prevalence of 36.9% in all women. Moreover the prevalence of group 1 HPV genotypes and the most prevalent genotype, HPV16, were also significant among women with normal cervical cytology (63.3% and 32.5%, respectively). In a study by Al-Awadhi et al., [20] the prevalence and type specific distribution of HPV in women with normal cervical cytology was investigated. The authors reported relatively low rates (2.4%) of HPV positivity in their patient population and these low rates were attributed to unique sociodemographic and sexual behaviour characteristics of the country. In a study by Wolday et al., the prevalence of group 1 HPV

Table 2 **Distribution of HPV genotypes that single/multiple HPV genotypes found cases according to cervical cytology.**

	All cases	Normal cytology	Abnormal cytology								
			Atypical glandular cell			Atypical Squamous cell					
n (%)			Total	Agc-fn	Agc-nos	Total	ASCUS	ASCH	LSIL	HSIL	ICC
Any HPV	493 (100)	335 (68)	3 (0.6)	1 (0.2)	2 (0.4)	155 (31.4)	47 (9.5)	11 (2.2)	59 (12)	36 (7.3)	2 (0.4)
Single type	316 (64.1)	211 (42.8)	2 (0.4)		2 (0.4)	103 (20.9)	32 (6.5)	4 (0.8)	37 (7.5)	28 (5.7)	2 (0.4)
Group 1											
HPV-16	108 (21.9)	54 (10.9)	1 (0.2)		1 (0.2)	53 (10.8)	16 (3.3)	2 (0.4)	13 (2.6)	21 (4.3)	1 (0.2)
HPV-18	28 (5.7)	17 (3.5)	1 (0.2)		1 (0.2)	10 (2)	2 (0.4)	1 (0.2)	4 (0.8)	2 (0.4)	1 (0.2)
HPV-31	11 (2.2)	5 (1)				6 (1.2)	2 (0.4)		2 (0.4)	2 (0.4)	
HPV-33	0										
HPV-35	2 (0.4)	1 (0.2)				1 (0.2)			1 (0.2)		
HPV-39	2 (0.4)	1 (0.2)				1 (0.2)	1 (0.2)				
HPV-45	14 (2.8)	14 (2.8)									
HPV-51	5 (1)	2 (0.4)				3 (0.6)	2 (0.4)		1 (0.2)		
HPV-52	0										
HPV-56	4 (0.8)	1 (0.2)				3 (0.6)	1 (0.2)		2 (0.4)		
HPV-58	0										
HPV-59	5 (1)	4 (0.8)				1 (0.2)				1 (0.2)	
Total	179 (36.3)	99 (20.1)	2 (0.4)		2 (0.4)	78 (15.8)	24 (4.9)	3 (0.6)	23 (4.7)	26 (5.3)	2 (0.4)
Group 2A											
HPV-68	1 (0.2)					1 (0.2)	1 (0.2)				
Group 2B											
HPV-26	0										
HPV-53	12 (2.4)	9 (1.8)				3 (0.6)	1 (0.2)		2 (0.4)		
HPV-66	8 (1.6)	5 (1)				3 (0.6)	1 (0.2)		2 (0.4)		
HPV-67	0										
HPV-70	2 (0.4)	2 (0.4)									
HPV-73	0										
HPV-82	0										
Total	22 (4.4)	16 (3.2)				6 (1.2)	2 (0.4)		4 (0.8)		
Group 3											
HPV-6	91 (18.4)	77 (15.6)				14 (2.8)	3 (0.6)	1 (0.2)	9 (1.8)	1 (0.2)	
HPV-11	15 (3)	13 (2.6)				2 (0.4)	1 (0.2)			1 (0.2)	
Total	106 (21.4)	90 (18.2)				16 (3.2)	4 (0.8)	1 (0.2)	9 (1.8)	2 (0.4)	
Group 4											
HPV-40	0										
HPV-42	0										
HPV-54	0										
HPV-55	1 (0.2)	1 (0.2)									

Table 2 **Distribution of HPV genotypes that single/multiple HPV genotypes found cases according to cervical cytology (cont.)**

	All cases	Normal cytology	Abnormal cytology								
			Atypical glandular cell			Atypical Squamous cell					
n (%)			Total	Agc-fn	Agc-nos	Total	ASCUS	ASCH	LSIL	HSIL	ICC
HPV-61	2 (0.4)	1 (0.2)				1 (0.2)	1 (0.2)				
HPV-62	3 (0.6)	3 (0.6)									
HPV-81	1 (0.2)	1 (0.2)									
HPV-83	0										
HPV-84	1 (0.2)					1 (0.2)			1 (0.2)		
Total	8 (1.6)	6 (1.2)				2 (0.4)	1 (0.2)		1 (0.2)		
Multiple type											
1	65 (13.2)	45 (9.1)				20 (4.1)	6 (1.2)	4 (0.8)	4 (0.8)	6 (1.2)	
2A	0										
2B	2 (0.4)	1 (0.2)				1 (0.2)			1 (0.2)		
3	1 (0.2)					1 (0.2)			1 (0.2)		
4	0										
1+2A	9 (1.8)	8 (1.6)				1 (0.2)	1 (0.2)				
1+2B	16 (3.3)	8 (1.6)	1 (0.2)	1 (0.2)		7 (1.4)	1 (0.2)		4 (0.8)	2 (0.4)	
1+3	31 (6.3)	27 (5.5)				4 (0.8)	1 (0.2)		3 (0.6)		
1+4	17 (3.5)	9 (1.8)				8 (1.6)	4 (0.8)	1 (0.2)	3 (0.6)		
2A+2B	3 (0.6)	2 (0.4)				1 (0.2)			1 (0.2)		
2A+3	0										
2A+4	0										
2B+3	2 (0.4)	1 (0.2)				1 (0.2)			1 (0.2)		
2B+4	4 (0.8)	2 (0.4)				2 (0.4)		1 (0.2)	1 (0.2)		
3+4	3 (0.6)	3 (0.6)									
1+3+4	11 (2.2)	9 (1.8)				2 (0.4)		1 (0.2)	1 (0.2)		
1+2B+4	6 (1.2)	3 (0.6)				3 (0.6)	1 (0.2)		2 (0.4)		
1+2B+3	2 (0.4)	2 (0.4)									
1+2B+3+4	2 (0.4)	2 (0.4)									
2B+3+4	3 (0.6)	2 (0.4)				1 (0.2)	1 (0.2)				
Total	177 (35.9)	124 (25.2)	1 (0.2)	1 (0.2)		52 (10.6)	15 (3)	7 (1.4)	22 (4.5)	8 (1.6)	

Agc-fn: atypical glandular cell-favor neoplasia; Agc-nos: atypical glandular cell not otherwise specified; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells for which a high-grade lesion can not be excluded; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ICC: invasive cervical carcinoma. Group 1: Including the oncogenic viruses. Group 2A: Including the probably oncogenic viruses. Group 2B: Including the possibly oncogenic viruses. Group 3: Including the unclassifiable as to carcinogenicity in humans. Group 4: Including the unclassified HPV genotypes.

genotypes and HPV16 was found 71% and 20.3% among women with normal cytology, respectively [21]. Previous studies from Turkey have revealed prevalence of group 1 HPV genotypes ranging from 1.5 to 27% in women presenting with normal cytology [22-26]. Compared to these studies in our country, our high rate can be explained by the changing social policies of the health ministry which includes more regular gynecological controls with the usage of more sensitive HPV methods and by the reduction of social and religious influences on the young population (resulting in liberal sexual life). Recently, in two studies from Turkey the rate of HPV positivity among women with normal cytology were detected by 49% and 73%,

respectively [11, 27]. These patients which have group 1 HPV genotypes with normal cytology must be regularly followed up in accordance with international guidelines. Because these patients have a high risk of serious HPV induced lesions in the future. Delayed follow-up may have jeopardized these patients because of risk for serious epithelial changes [11]. According to the American Society of Colposcopy and Cervical Pathology (ASCCP) guidelines, management of cytology-negative/HPV-positive women may follow two pathways. These are a repeat co-test a year later or immediate HPV genotype-specific testing for HPV-16 or HPV-16/18. If patients are HPV-16/18 positive, they must be directly referred to colposcopy. If the result is negative for HPV16/18, the ASCCP advises that the co-test be repeated 12 months later [27].

The prevalence of group 1 HPV genotypes positivity was detected 79.8% among women with abnormal cytology in our study. Additionally, the prevalence of group 1 HPV genotypes in women with LSIL, HSIL and ASCUS in currently study was found to be 67.8%, 94.4% and 80%, respectively. This rates were reported in the several studies as range between 46.5 and 89% [4, 5, 11], 86.7% and 100% [4, 5, 11, 21], 72.5% and 94% [4, 11], respectively. The prevalence of group 1 HPV genotypes in women with LSIL, HSIL and ASCUS in our study was consistent with those in findings of these studies [4, 5, 11, 21]. As expected, the prevalence of group 1 HPV genotypes, especially HPV16, was higher in women with HSIL (72.2%) than women with LSIL (32.2%) and women with normal cytology. In conclusion, although in women with slight cell alterations (such as ASCUS and LSIL) follow-up testing is commonly recommended, in women with HSIL colposcopy with endocervical sampling has been considered the best management [11].

Co-infection with multiple HPV genotypes is commonly encountered in HPV studies [28]. The prevalence of infection with multiple HPV genotypes which might have been affected by the characteristics of the study population, and geographic distribution [28] have been reported between 4.4% and 78.3% [29-31]. A high incidence of co-infection by multiple genotypes (35.9%) was observed in currently study. The carcinogenic effects of multiple infections are still unclear. In the some studies were reported that multiple HPV infections were associated with the grades of cervical abnormalities, and the cervical cancer risk of patients who suffered from multiple HPV infections was higher than those with single HPV infection [28, 32, 33]. Contrary to these literatures the prevalence of abnormal cytology was found to be higher in the patients with single type HPV infection (21.3%) in our study. Additionally in this study, the multiple HPV infection rates in normal cytology, ASCUS, LSIL and HSIL were 37%, 31.9%, 37.3% and 22.2%, respectively. Hence, the incidence of multiple HPV infections indicated that multiple HPV infection was not related to the grades of cytological abnormalities. Carrillo-García et al. showed that multiple HPV infections are not associated to the severity of cervical lesions since they were as common in ICC or HSIL as in LSIL or normal cytology [34]. Additional studies must be needed in order to determine the association between multiple infections and pathogenesis.

Interestingly, we have identified 12 unclassified genotypes in 10.7% of normal cytology, in 13.6% of LSIL, in 14.9% of ASCUS and 8.3% of ASC-H but in none of HSIL. Among the unclassified HPVs the most frequent HPV84, 62, 54, and 42 were being found in 5.1, 5.1, 3.4 and 1.7% of women with LSIL, respectively, while the genotype 62 was the most frequent (3.9%) among women with normal cytology.

Annunziata et al. found that among the unclassified HPVs the genotypes were the most frequent 81, 42, 62 and 91 and the prevalence of these were 2.7, 2.2, 2.2 and 1.8% of women with LSIL, respectively [5]. It has been reported that some of the unclassified HPV genotypes can important in cases which weaken the immune system such as HIV infection. The compromised immune system could be not able to limit the "weak oncogenic" activity of some unclassified viruses [5]. Garbuglia et al. found that HPV62 and HPV81 were associated, as single infections, with 9.1 and 4.5% of HSIL, respectively, among HIV positive women [36]. In our study, more than one HPV genotype was identified in most (85.2%) of women infected with unclassified HPV genotypes. In addition, at least one group 1 HPV genotype was detected in 64.8% of these women. Although abnormal cytology results in unclassified HPV genotypes were significantly higher than the other groups, we cannot comment on the relationship between unclassified HPV genotypes and abnormal cytology. Further studies about unclassified HPV genotypes will be of importance in establishing, especially helpful in formulating a strategy for further second-generation vaccine development as well as follow-up on the effectiveness of the currently used vaccines.

HPV diagnosis, which is regarded as a major factor in cervical cancer aetiology, is of great importance today. Cervical cancer differs from other cancer types as it is a "preventable" cancer type. For that reason, scanning, early diagnosis and treatment are important for HPV-related infections. The recently licensed nonavalent HPV vaccine targets the seven group 1 HPV genotypes (HPV16, 18, 31, 33, 45, 52 and 58) and the low risk HPV genotypes (HPV6 and 11) [26]. Considering the frequency of these nine HPVs among the analyzed women, the use of nonavalent vaccine would be able to prevent more than 80% of HPV infections. In particular, vaccination would prevent 74.6% and 97.2% of LSIL and HSIL, respectively.

Close follow-up is quite important even in women with normal cytology, due to the presence of high group 1 HPV ratios. We were found that multiple HPV infection was not related to the grades of cytological abnormalities. However, additional studies must be needed in order to determine the association between multiple infections and pathogenesis. In addition, we found that the most common genotypes among the unclassified HPVs were HPV84, 62, 54, and 42. Nevertheless, we could not comment on the relationship between these genotypes and cervical cancer because most of these women have co-infection with multiple HPV genotypes. The future studies should also focus on these rare uncommon HPV genotypes, especially on the different distributions of these rare HPV genotypes in particular regions. Thus, a strategy for the development of second generation vaccines in a given region can be formulated.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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