RESEARCH ARTICLE

Human Papillomavirus Genotype Distribution and E6/E7 Oncogene Expression in Turkish Women with Cervical Cytological Findings

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Abstract

Background: Infection with certain human papillomavirus (HPV) genotypes is the most important risk factor related with cervical cancer. The objective of the present study was to investigate the prevalence of HPV infection, the distribution of HPV genotypes and HPV E6/E7 oncogene mRNA expression in Turkish women with different cervical cytological findings in Mersin province, Southern Turkey. Materials and Methods: A total of 476 cytological samples belonging to women with normal and abnormal cervical Pap smears were enrolled in the study. For the detection and genotyping assay, a PCR/direct cycle sequencing approach was used. E6/E7 mRNA expression of HPV-16, 18, 31, 33, and 45 was determined by type-specific real-time NASBA assay (NucliSENS EasyQ®HPV v1.1). Results: Of the 476 samples, 106 (22.3%) were found to be positive for HPV DNA by PCR. The presence of HPV was significantly more common (p<0.001) in HSIL (6/8, 75%) when compared with LSIL (6/14, 42.9%), ASC-US (22/74, 29.7%) and normal cytology (72/380, 18.9%). The most prevalent genotypes were, in descending order of frequency, HPV genotype 66 (22.6%), 16 (20.8%), 6 (14.2%), 31 (11.3%), 53 (5.7%), and 83 (4.7%). HPV E6/E7 oncogene mRNA positivity (12/476, 2.5%) was lower than DNA positivity (38/476, 7.9%). Conclusions: Our data present a wide distribution of HPV genotypes in the analyzed population. HPV genotypes 66, 16, 6, 31, 53 and 83 were the predominant types and most of them were potential carcinogenic types. Because of the differences between HPV E6/E7 mRNA and DNA positivity, further studies are required to test the role of mRNA testing in the triage of women with abnormal cervical cytology or follow up of HPV DNA positive and cytology negative. These epidemiological data will be important to determine the future impact of vaccination on HPV infected women in our region.

Keywords: Human papillomavirus - consensus PCR - cycle sequencing - E6/E7 mRNA - genotype - Turkish women

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Introduction

Cervical cancer is the third most common cancer among women and a major cause of morbidity and mortality worldwide (Jastreboff and Cymet, 2002). Human papillomavirus (HPV) belongs to the *Papillomaviridae* family, small, non-enveloped, double-stranded DNA virus, established as the most important etiological agent for the development of cervical cancer (Jastreboff and Cymet, 2002; Kitchener et al., 2013).

HPV is comprised to the diverse group of viruses that represent affinity to the squamous epithelia of the skin and mucous membranes (Ciesielska et al., 2012). More than 100 different HPV genotypes have been identified and just less than a half are known to infect the mucosal epithelium of the genital tract and are classified into high-, probably high- and low-risk categories, depending on their association with malignant lesions (Muñoz et al., 2003; 2004; Coutlée et al., 2005). Infection with high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82) is known as a risk factor associated with the development of cervical cancer and its precursor lesions (Muñoz et al., 2003), especially HPV 16 and 18 are the most important, contributing to more than 70% of cases worldwide (Muñoz et al., 2004; Schiffman et al., 2005).

Early detection and treatment of cervical HPV infections through organized cervical screening programs reduces the risk of cervical cancer development. In addition to cytological screening, nucleic acid testing with HPV genotyping is the mainstay of diagnosis and follow-up (Ronco and Giorgi Rossi, 2008). Therefore, identification of high-risk HPV genotypes is very important to provide additional clinical value for those patients in order to avoid over-referral to colposcopy and over-treatment (Coutlée et al., 2005).

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For the HPV detection and genotyping, different molecular techniques have been described in various studies. HPV DNA detection by the FDA-approved Hybrid Capture II HPV DNA test (HCII) (Digene Corporation, USA) based on signal amplification is used widely in routine analysis (Coutlée et al., 2005). Currently, consensus PCRs are the most widely used DNA amplification method for genotyping HPV for research purposes. The most commonly used consensus primers designed for broad spectrum coverage of HPV genotypes are the GP5+/6+, MY09/11, FAP59/64, PGMY09/11 and SPF1/2 that target the highly conserved region of viral L1 capsid gene (Lin et al., 2007; Shen-Gunther and Yu, 2011). Analysis and typing of PCR products were routinely done by type-specific oligonucleotide hybridizations, by real-time fluorescence PCR assays or by direct sequencing (Coutlée et al., 2005). PCR followed by reverse hybridization with immobilized probes based commercial kits for HPV typing were also available, thus allowing the simultaneous identification of a broad range of HPV genotypes (Lin et al., 2008).

The expression of HPV E6 and E7 oncogenes is responsible for the malignant transformation of HPV infected cells, resulting in the development of a cervical neoplasia (Doorbar et al., 2012). Therefore, monitoring of the activity of HPV oncogene transcripts seems to be a reasonable strategy to identify clinically relevant HPV infections with high-risk HPV genotypes. The NucliSENS EasyQ HPV v1 is a real-time nucleic acid sequence-based amplification (NASBA) and multiplex detection assay for the qualitative determination of E6/E7 mRNAs of the five most commonly identified carcinogenic HPV genotypes (HPV 16, 18, 31, 33 and 45) in cervical cancer worldwide (Jeantet et al., 2009).

HPV genotyping has essential clinical utility for the risk stratification, furthermore increased by the recent development of suitable HPV vaccines (Meijer et al., 2006). The predicted effect of the vaccines on the incidence of infection could vary depending on the regional distribution patterns of HPV genotypes (Muñoz et al., 2004). Although the importance of HPV associated cervical cancer, there is a lack of information on the incidence and distribution in Mersin province, Southern Turkey. The objective of the present study was to investigate the HPV prevalence and the distribution of HPV genotypes in Turkish women with several cervical cytological findings in our region. We also evaluated the HPV E6/E7 oncogene mRNA expression because of their importance in cervical cancer screening programs.

Materials and Methods

Study population and specimen collection

A total of 476 cervical samples were enrolled for HPV testing from women (mean age±SD, 42.15±10.70 years; range, 18-76 years) attending the Obstetric and Gynecology Clinic at Mersin University Faculty of Medicine in Mersin province, Southern Turkey, from January 2012 to March 2013 after providing written informed consent. Adult women ≥18 years of age with abnormal or normal cervical cytological findings were included in this study. Adolescents <18 years of age and those intolerant to pelvic exams or Pap smears were excluded.

Two consecutive cytological samples were taken with a cervical brush/spatula and rinsed in PreservCyt (Cytyc, MA) solution. The first specimen was sent for routine liquid-based cervical cytological examination. ThinPrep cytological slides were screened in the absence and presence of clinical signs of cervical dysplasia then the adequacy and the degree of abnormality were assessed using the criteria set out in The Bethesda System 2001 guidelines.

The second specimen was transferred to the molecular microbiology research laboratory for HPV analysis. From collected cervical smear samples for molecular analysis, PreservCyt medium (20 ml) was removed from cell pellets by centrifugation for 10 min at 2000g. The samples were aliquoted into two tubes and refrigerated at 4°C until weekly DNA or RNA extraction.

HPV detection and genotyping

HPV DNA amplification: an aliquot (200 μl) of concentrated sample was used for DNA isolation with a High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany), in accordance with the manufacturer's instructions. DNA was eluted from columns in a volume of 50 μl Elution Buffer, pH 8.0.

Detection of HPV DNA was performed by nested PCR using the general consensus primers MY09 (5'-CGT CCM ARR GGA WAC TGA TC-3')/MY11 (5'-GCM CAG GGW CAT AAY AAT GG-3') (M: A+C, R: A+G, W: A+T, Y: C+T) (Manos et al., 1989) as outer and GP5+ (5'-TTT GTT ACT GTG GTA GAT ACT AC-3')/GP6+ (5'-GAA AAA TAA CTG TAA ATC ATA TTC-3') (Snijders et al., 1990) as inner which amplify a 450 bp and 142 bp fragment of the HPV L1 conserved region, respectively. Presence of human genomic DNA was verified by amplification of a 136 bp fragment of the β-globin gene by using primers GAPDH-F (5'-GGC AGC AGC AAG CAT TCC T-3') and GAPDH-R (5'-GCC CAA CAC CCC CAG TCA-3') (Lin et al., 2007).

First and second round of PCR reactions of each sample had consisted of the same composition and were carried out in a 50 µL volume, containing 5 µL sample DNA extract as template, 1X Taq polymerase buffer, 2 mM/L of MgCl2, 0.2 mM/L of deoxynucleotide mix, 0.25 µM/L of each (sense and anti-sense) primers and 1.25 units of Taq DNA polymerase (Fermentas). PCR amplification conditions of each round were carried out after 10 min of pre-denaturation at 94°C, followed by 30 cycles of denaturation at 95°C for 45 sec, annealing at 55°C (60°C for the β-globin gene amplification) for 45 sec, and extension at 72°C for 1 min, and then by a final extension at 70°C for 7 min in a thermal cycler (Eppendorf Mastercycler, Hamburg, Germany). Positive controls were performed with purified DNA from the HPV 16-positive Caski cell line.

The MY09/MY11 and GP5+/GP6+ PCR products were electrophoretically separated on 1% agarose gel, stained with 0.5 µg/mL ethidium bromide in 1X Tris-boric aside-EDTA (TBE) buffer, and visualized on a UV

transilluminator.

HPV genotype identification: HPV genotype identification was performed by direct cycle sequencing in the presence of MY or GP amplicons. For cycle sequencing, PCR products were analyzed with using internal PCR primers (MY09 or GP5+) and the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) including dye terminator dideoxynucleotide according to manufacturer's instructions in sense sequence directions. The data of cycle sequence reactions were collected by an automated capillary sequence reader ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Obtained nucleotide sequences were subjected to the Basic Local Alignment Search Tool (BLAST®) software and compared with existing HPV reference sequences in GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The HPV genotype identification was based on the most similar and significant alignment results.

E6/E7 mRNA detection

The detection of E6/E7 mRNAs of the five most prevalent high risk HPV genotypes 16, 18, 31, 33, and 45 was performed qualitatively, with a commercial NASBA assay (NucliSENS EasyQ HPV; bioMérieux, France) test according to manufacturer's instructions. For E6/E7 mRNA detection, RNA was extracted from 200 μl sample by using a High Pure Viral RNA Kit (Roche Diagnostics GmbH, Mannheim, Germany). Human U1 small ribonucleoprotein (U1A mRNA) was used as an RNA integrity/adequacy internal control.

Ethical committee considerations

This study was approved by the Ethics Commission of the Faculty of Medicine, Mersin University. All patients signed the consent forms and were informed regarding the purpose of the proposed study. Additionally, our study was carried out in accordance with the Helsinki Declaration (The Ethic Commission Approval Report No. 2012/270).

Statistical analysis

All statistical analyses were performed with the Statistical Program for the Social Sciences (SPSS, version 13.0, Inc., Chicago, Illinois, USA). The χ^2 test was used to evaluate statistical significance between genotype, cytological lesion and age. Statistical significance was defined by a p value of less than 0.05.

Results

Of the 476 samples, 380 (79.8%) had normal cytology and 96 (20.2%) showed some cytological abnormalities. Among the samples with abnormal cytology, 74 samples (77.1%) were determined as atypical squamous cells of undetermined significance (ASC-US), 14 samples (14.6%) as low-grade squamous intraepithelial lesions (LSILs), and 8 samples (8.3%) as high-grade squamous intraepithelial lesions (HSILs).

The mean age of the women was 41.82±10.89 (range 18-76 years) and 43.43±9.87 (range 19-63 years) in women with normal and abnormal cervical cytology respectively, and there was no significant difference among these women (p=0.188).

HPV DNA was detected in 106 women (22.3%) by PCR, and the prevalence of HPV positivity was found as 18.9% (72/380) in women with normal cytology, 29.7% (22/74) with ASC-US, 42.9% (6/14) with LSIL, and 75% (6/8) with HSIL. The distribution of HPV DNA positivity according to cervical cytological findings is shown in Table 1. Increasing severity of cytological lesions was associated with higher prevalence rates of HPV DNA (p<0.001; 72/380, 18.9% in normal cytology vs. 34/96, 35.4% in abnormal cytology). The HPV positivity shows broad differences between the groups (p<0.001). The presence of HPV was significantly more common (p<0.001) in HSIL (75%) compared to LSIL (42.9%), ASC-US (29.7%) and normal cytology (18.9%).

HPV genotyping showed marked differences among cervical lesions and 20 different genotypes were detected by sequence analysis. The most common genotypes were, in descending order of frequency, HPV genotype 66 (22.6%), 16 (20.8%), 6 (14.2%), 31 (11.3%), 53 (5.7%), and 83 (4.7%). All other genotypes were detected at less frequency or only once (Table 2).

Table 1. Association between Cervical Cytological Findings and HPV DNA Positivity Rate

Cervical cytology	HPV DNA	
	Negative, n (%)	Positive, n (%)
Normal (n=380)	308 (81.1%)	72 (18.9%)
ASC-US (n=74)	52 (70.3%)	22 (29.7%)
LSIL (n=14)	8 (57.1%)	6 (42.9%)
HSIL (n=8)	2 (25%)	6 (75%)
Total (n=476)	370 (77.7%)	106 (22.3%)

*p value<0.001

Table 2. Distribution of HPV Genotypes in Women According to Each Cytological Finding

HPV genotype	Cervical cytology								Overall	
	Normal n=380		ASC-US n=74		LSIL n=14		HSIL n=8			
	n	%	n	%	n	%	n	%	n	%
HPV 66	20	27.8	2	9.1	2	33.3	0	0.0	24	22.6
HPV 16	16	23.2	3	13.6	1	16.7	2	33.3	22	20.8
HPV 6	10	13.9	2	9.1	2	33.3	1	16.7	15	14.2
HPV 31	4	5.6	6	27.3	1	16.7	1	16.7	12	11.3
HPV 53	4	5.6	1	4.5	0	0.0	1	16.7	6	5.7
HPV 83	5	6.9	0	0.0	0	0.0	0	0.0	5	4.7
HPV 62	1	1.4	2	9.1	0	0.0	0	0.0	3	2.8
HPV UC genotype	3	4.2	0	0.0	0	0.0	0	0.0	3	2.8
HPV 18	1	1.4	1	4.5	0	0.0	0	0.0	2	1.9
HPV 45	2	2.8	0	0.0	0	0.0	0	0.0	2	1.9
HPV 54	1	1.4	1	4.5	0	0.0	0	0.0	2	1.9
HPV 81	2	2.8	0	0.0	0	0.0	0	0.0	2	1.9
HPV 11	0	0.0	1	4.5	0	0.0	0	0.0	1	0.9
HPV 40	1	1.4	0	0.0	0	0.0	0	0.0	1	0.9
HPV 56	1	1.4	0	0.0	0	0.0	0	0.0	1	0.9
HPV 58	0	0.0	0	0.0	0	0.0	1	16.7	1	0.9
HPV 61	1	1.4	0	0.0	0	0.0	0	0.0	1	0.9
HPV 70	0	0.0	1	4.5	0	0.0	0	0.0	1	0.9
HPV 82	0	0.0	1	4.5	0	0.0	0	0.0	1	0.9
HPV 84	0	0.0	1	4.5	0	0.0	0	0.0	1	0.9
Total	69	100.0	22	100.0	6	100.0	6	100.0	106	100.0

*UC: Unclassified

The distribution of HPV genotype varied among cytological lesions. The most common genotype, HPV 66 (n=24, 22.6%) was found with the following distribution: 27.8% in normal cytology samples, 9.1% in ASC-US, 33.3% in LSILs, and 0.0% in HSILs. The second most frequent HPV 16 (n=22, 20.8%): 23.2% in normal cytology, 13.6% in ASC-US, 16.7% in LSILs, and 33.3% in HSILs. The third genotype HPV 6 (n=15, 14.2%): 13.9% in normal cytology, 9.1% in ASC-US, 33.3% in LSILs, and 16.7% in HSILs (Table 2). This group was followed by HPV genotypes 31 (11.3%), 53 (5.7%), 83 (4.7%), 62 (2.8%) and unclassified genotypes (2.8%). HPV genotypes18, 45, 54 and 81 were detected at same frequencies, 1.9% and other genotypes, HPV 11, 40, 56, 58, 61, 70, 82 and 84 were detected only once (0.9%) (Table 2). Only the frequency of genotype 31 increased significantly from normal cytology to ASC-US and genotype 6 from normal cytology to LSIL and HSIL (Table 2).

The distribution of age groups was found homogeneous in normal and abnormal cervical cytology (p=0.111). The distribution of HPV DNA positivity according to age groups didn't show any significant statistical difference (p=0.902) (Table 3).

E6/E7 mRNA expression

Cervical smear samples were included from 38 women who were found positive for HPV genotype 16, 18, 31 and 45 by PCR/sequencing. The NucliSens-EasyQ-mRNA (Biomerieux) positivity (12/476, 2.5%) of HPV E6/E7 oncogenes was lower than DNA positivity (38/476, 7.9%) within women. Presence of viral E6/E7 mRNAs was detected in 31.5% (12/38) of the samples. The positivity rate was 30.4% (7/23) in the normal cervical cytology group and 33.3% (5/15) in abnormal cervical cytology group. HPV genotype 16 mRNA was observed as the most common (5/12, 41.7%), followed by genotype 31 (4/12, 33.3%), 18 (2/12, 16.7%) and 45 (1/12, 8.3%) (Table 4).

Co-infections were found in 2 samples by NucliSens-EasyQ-mRNA, one of them was found in normal cytology with HPV 16/31/53, and the other one was in HSIL with HPV 16/31.

Table 3. The Distribution of HPV DNA Positivity According to Age Groups

Age	Total		Cervical cytology				HPV DNA positivity	
			Normal		Abnormal			
	n	%	n	%	n	%	n	%
<35	124	26.1	106	27.90	18	18.8	25	23.6
35-45	172	36.1	139	36.60	33	34.4	40	37.7
45-55	127	26.7	93	24.50	34	35.4	28	26.4
>55	53	11.1	42	11.10	11	11.5	13	12.3
Total	476	100.0	380	100	96	100	106	100

Table 4. Prevalence of HPV DNA and mRNA Expression of the E6/E7 Oncogenic Gene in Cervical Samples According to Cytological Diagnosis

	Normal	ASC-US	LSIL	HSIL	Total
HPV genotype detected by PCR/sequencing n (%)					
	(n=23)	(n=10)	(n=2)	(n=3)	(n=38)
16	16 (69.6%)	3 (30.0%)	1 (50.0%)	2 (66.7%)	22
18	1 (4.3%)	1 (10.0%)	-	-	2
31	4 (17.4%)	6 (60.0%)	1 (50.0%)	1 (33.3%)	12
33	-	-	-	- -	
45	2 (8.7%)	-	-	- 2	
HPV E6/E7 mRNA detected by NucliSens-EasyQ-mRNA n (%)					
	(n=7)	(n=2)	(n=1)	(n=2)	(n=12)
16	3 (42.9%)	-	1 (100%)	1 (50.0%)	5
18	1 (14.2%)	1 (50.0%)	-	-	2
31	2 (28.6%)	1 (50.0%)	-	1 (50.0%)	4
33	-	-	-	-	-
45	1 (14.2%)	-	-	-	1

Discussion

Collected samples for this study were obtained from a group of women who attended to Obstetrics and Gynecology Clinics because of a routine gynecologic control or previous abnormal Pap tests results. Several studies have been carried out concerning genotype distribution in different regions of Turkey and HPV prevalence rate changes from 4.9% to 57.5% depending on the examination methods and study groups (Ozturk et al., 2004; Yuce et al., 2012; Abike et al., 2013; Akcali et al., 2013; Dursun et al., 2013; Sahiner et al., 2014; Yildirim et al., 2013), but no data available about the distribution in our restricted region, Mersin in Southern Turkey.

The overall HPV prevalence in women with normal and abnormal cytology was 22.3% in our study. This rate is high, compared to the study of Yildirim et al. (2013) from Central region and the Akcali et al. (2013) from Aegean region of Turkey which reported HPV prevalence rates of 6.7% and 8.5%, respectively, but also quite close when compared to the multicenter hospital based study of Dursun et al. (2013) which reported overall HPV prevalence of 25% in women with normal and abnormal cytology in Turkey. This high prevalence might be due to the fact that the women in our study had attended to genecology service for cervical complain or previous abnormal Pap test results.

Our study shows as a wide range distribution of HPV genotypes in the Southern Turkey. HPV genotype 66 (22.6%) was the most frequent genotype in our area, followed by HPV 16 (20.8%), 6 (14.2%) and 31 (11.3%), with a low prevalence of HPV 53 (5.7%) and 83 (4.7%). HPV genotype 62, unclassified HPV genotypes, 18, 45, 54, 81, 11, 40, 56, 58, 61, 70, 82 and 84 (varying from 2.8% to 0.9%) were detected at less frequency or only once. These data are consistent with other reports belonging to our country except for HPV genotype 66. When distribution data of HPV genotypes was examined, most frequently observed genotypes were HPV 16 (19.5-45.5%) (Ergunay et al., 2008; Abike et al., 2013; Akcali et al., 2013; Sahiner et al., 2014) and HPV 6 (8.5 and 40%) (Abike et al., 2013; Akcali et al., 2013), followed by HPV 53 (11.4 and 22.7%) (Ergunay et al., 2008; Akcali et al., 2013), HPV 18 (7.2 and 23.2%) (Abike et al., 2013; Sahiner et al., 2014), HPV 68 (7.2 and 18.2%) (Ergunay et al., 2008; Sahiner et al., 2014), 58 (11.6 and 13.6%) (Ergunay et al., 2008; Sahiner et al., 2014), and HPV 31 (5.7-9.1%) (Ergunay et al., 2008; Akcali et al., 2013; Sahiner et al., 2014).

The prevalence and genotype distribution of HPV

varies around the world. A worldwide overall HPV prevalence of 11-12% was found in women with normal cytology. It was estimated that the prevalence of HPV infection is 20% among women in Africa, 17.1% in America, 12.3% in Europe and 8.4% in Asia. The five most prevalent genotypes worldwide are HPV 16 (3.2%), 18 (1.4%), 52 (0.9%), 31 (0.8%) and 58 (0.7%) (Bosch et al., 2013). Compared with some Asian countries, HPV prevalence was found consistent reported here with the result of 24.5% in China (Chen et al., 2012) and 14.1% in Thailand (Natphopsuk et al., 2013) among women with normal cervical cytology. In international multicenter case control study, the 15 most common genotypes were found as in descending order of frequency, 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 39, 51, 73, 68 and 66. Higher than average proportions of HPV genotype 16 were found in Northern Africa, of genotype 18 in South Asia, of genotype 45 in sub-Saharan Africa and of genotype 31 in Central/ South America (Muñoz et al., 2004). These differences might be related to various geographic distributions of HPV genotypes, age, specimen type (histological lesion), sensitivity of HPV testing protocols and interaction between different HPV genotypes such as multiple infections (Li et al., 2011).

Differences in HPV positivity have been reported for most cytological and histological categories in several regions of the world and overall HPV prevalence increases in women with the severity of the lesion with rate of 52% in ASC-US, 76% in LSIL and 85% in HSIL (Guan et al., 2012). HPV prevalence in our current study was significantly higher in women with abnormal cervical cytology compared to women with normal cytology (35.4% vs. 18.9%) and rate of positivity was 29.7% in ASC-US, 42.9% in LSIL, and 75% in HSIL. These prevalence rates were not completely consistent with limited reports so far from Turkey. HPV distribution reported as range 34.5% to 72.6% in abnormal cytology and 34.8% to 37%, 27% to 84.15%, and 20% to 95% in ASC-US, LSIL and HSIL, respectively (Batmaz et al., 2009; Abike et al., 2013; Dursun et al., 2013; Sahiner et al., 2014). These discrepancies may be associated with used detection methods, sample quality and regional differences in distinct areas of our country.

According to our study results, HPV genotyping showed no marked differences among cervical cytology findings. But, increasing severity of cytological lesions was associated with higher prevalence rates of HPV genotype 6 and 31 infection. The most prevalent HPV genotypes were: HPV 66 (27.8%) in normal cervical cytology, HPV 31 (27.3%) in ASC-US, HPV 6 and 66 (33.3% each one) in LSIL and HPV 16 (33.3%) in HSIL. Although HPV 16 is a carcinogenic genotype, it was more frequently detected in women with normal cervical cytology. HPV 6 and 53 was found with the same frequency in women with normal and abnormal cervical cytology. HPV 83 was detected only in normal cervical cytological samples. HPV 62, unclassified HPV types, 18, 45, 54, 81, 11, 40, 56, 58, 61, 70, 82 and 84 (varying from 2.8% to 0.9%) were uncommon in our study as mentioned above (Table 2). In the current study, potential carcinogenic HPV genotypes were also found in important rate. Concerning genotypes were HPV 66, 16, 31, 53, 18, 45, 56, 58 70 and 82 and their frequencies were 22.6%, 20.8%, 11.3%, 5.7%, 1.9-0.9%, respectively.

HPV infection is crucial etiological factor for inducing cervical cancer especially in developing countries due to lack of extensive screening programs. HPV prevalence among women with cervical cancer range between 43%-93.3% and most common seen genotypes were HPV 16 (30-68%), HPV 18 (8-40%), HPV 58 (10.7 and 17.8%), HPV 33 (4.7% and 10.4%) and HPV 45 (1.8% and 5%) in some Asian countries (Turki et al., 2013; Natphopsuk et al., 2013; Hamzi Abdul Raub et al., 2014). The overall HPV, HPV 16 and HPV 18 prevalence of the worldwide were reported as 89.9%, 56.6% and 16%, respectively. Other most common identified genotypes were HPV 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56, in order of decreasing prevalence (Li et al., 2011).

The spontaneous regression of HPV infection commonly takes place within about 2 years, but a small part of the population infected with high-risk HPV genotypes is at risk to develop invasive cervical cancer after a long lasting latency period of primary infection (Evander et al., 1995; Schiffman et al., 2005). Base on a study of Wang et al. (2013), the integration of HPV DNA into the human gene has been proposed as a potential marker of cervical neoplastic progression and they suggested that HPV 16 and 58 were found the most frequently integrated genotypes. Although cytology screening has significantly reduced the incidence and mortality rate of cervical cancer, its usefulness is limited to samples collected from the site of the lesion, resulting in its low sensitivity and unsuitability for use in medical screening programs (Gibb, 2011).

Identification and genotyping of HPV infection in genital samples has a great importance for the prevention and monitoring of cervical cancer development. PCR and following direct sequence analysis that we used are a substantial method for the detection of HPV in cytological samples but not convenient in routine diagnosis. The data presented here suggest a wide distribution of HPV genotypes in the analyzed population. Otherwise, most commonly used hybridization-based assays are able to detect only a limited number of specified genotypes (Coutlée et al., 2005).

Recently available new generation assays are based on simultaneous genotyping and detection of the persistent mRNA expression of the viral oncogenes E6 and E7 of high-risk HPV genotypes to increase the positive predictive value for detection of cervical carcinoma (Jeantet et al., 2009). Monitoring the activity of corresponding mRNAs in the context of clinically relevant symptoms and/or abnormal cytology is considered to be a suitable strategy for proven HPV-associated carcinomas and further evaluation for primary screening, triage and follow-up after treatment (Villa and Denny, 2006; Jeantet et al., 2009; Giorgi Rossi et al., 2013). On the other hand, several study implied that the determination of HPV E6/E7 mRNA has a diagnostic and prognostic value in order to decrease the follow-up intensity in women with HPV DNA positive and negative colposcopy or histology (Benevolo et al., 2011; Giorgi Rossi et al., 2013).

In this study, we preliminarily evaluated the E6/E7

mRNA expression from the five most carcinogenic HPV genotypes for the detection of oncogenic activity by the commercial NASBA E6/E7 mRNA test. HPV E6/E7 mRNA testing for high-risk genotypes seems to correlate better with the severity of the lesion compared with HPV DNA testing, and might have a role as the potential predictive marker for the identification of women at risk of developing cervical carcinoma (Table 4). The study designed to evaluate the efficacy of molecular testing, was suggested that mRNA testing had a higher clinical efficacy than DNA testing in a population attending to colposcopy units in combination with cytological results (Spathis et al., 2012). Although the higher specificity of mRNA tests could potentially help to reduce colposcopy attempts, widespread application of these tests is likely be limited by the test complexity, targeted only 5 high risk HPV genotypes and needs expensive laboratory instruments. Because of the low rate mRNA expression of high risk HPV genotypes, the investigation of mRNA expression would be the most reasonable routine test after the determination of these genotypes by sequencing in low income countries like Turkey. Hence, more studies are required to fully assess the performance of the system for diagnostic laboratories in Turkey.

It was suggested that HPV sequencing studies in various populations are required to understand the epidemiological distribution of HPV genotypes, in order to predict the protectiveness of the current vaccine and to develop new vaccine strategies targeting uncommon genotypes (Speich et al., 2004; Pannier-Stockman et al., 2008). Although a vaccine including genotypes 16 and 18 could potentially prevent, respectively, 40-70% and 15% of cervical cancers in worldwide (Muñoz et al., 2004), HPV vaccine genotypes 6, 11 (low-risk types) and 16, 18 (high-risk types) were detected in 14.2%, 0.9% and 20.8%, 1.9% of women respectively, according to our study results. Most frequent genotypes (66 and 31) in our population are not covered by the current vaccine. Since October 2007, HPV vaccine has also been licensed in Turkey, but not involved in national vaccination programs. It is estimated that the results of the present study will provide a major help to determine the regional needs of our population for the cervical cancer vaccination or the benefits of detection tests.

In conclusion, determining the HPV genotypes of genital HPV infections is important for epidemiological studies. This study demonstrates an important prevalence and wide distribution of the HPV genotypes in women with evidence of normal and abnormal cervical cytology. We have found the rate of HPV positivity as 22.3% in all women which implies the need for extended screening programs in order to diagnose oncogenic HPV at an early stage. Our data show that HPV genotype 66, 16, 6, 31, 53 and 83 are the most prevalent HPV genotypes in the different cervical cytological findings in this region in Southern Turkey, which constitutes instructive information for the development of new screening strategies and second generation HPV vaccines. Because of the differences between HPV E6/E7 mRNA and DNA positivity in our study, further studies are required to test the role of mRNA testing in the triage of women with abnormal cervical cytology or in the follow up of HPV DNA positive and cytology negative cases. These epidemiological data will be important to determine the future impact on HPV infected women in our region.

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