

## RESEARCH ARTICLE

# Human Papilloma Virus Frequency and Genotype Distribution in a Turkish Population

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### Abstract

**Objectives:** To determine human papillomavirus (HPV) frequency, genotypes and the relation between cervical smear results, risk factors and types in women living in Manisa, Turkey. **Materials and Methods:** A total of 410 women were included in the study. Cervical specimens were obtained for linear array HPV genotyping and pathological testing. Conventional Pap test and Bethesda system were used for evaluation of cytology specimens. **Results:** A total of 410 women with a mean age of 34.9 years were tested. A positive result of any HPV was found in 35 patients (8.5%). Among them, 26 different serotypes of HPV were identified and the most frequent type was HPV 16 (28.5%) followed by type 45 and 53 (11.4%). Patients were infected by 65.7% high risk, 11.4% probable high risk and 22.9% low risk HPV types. Multiple HPV positive results were found in 13 patients (37.1%). Patients with single partner, history of abnormal smear or condyloma had positive HPV results and this was statistically significant ( $p < 0.05$ ). Correlation analysis showed a statistically weak relation between positive HPV and abnormal smear results ( $r = 0.120$ ). **Conclusions:** Determining HPV types of genital HPV infections is important for epidemiological studies. We have found the rate of positive HPV as 8.5% which implies the need for extended screening programs in order to diagnose oncogenic HPV at an early stage.

**Keywords:** Cervical cancer - HPV - HPV DNA - screening - Pap smear

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### Introduction

Cancer of the cervix is the second most common malignancy in women worldwide (Rock et al., 2000). More than 470,000 new cases of cervical cancer and 250,000 deaths due to it are reported annually. Approximately 75-80% of cervical cancer cases are seen in developing countries where efficient cervical screening is insufficient (Parkin et al., 2005). Early diagnosis of precancerous lesions is important because the progression to invasive neoplasia takes as long as 15-20 years giving enough time for treatment (Munger et al., 2004).

Human Papillomavirus (HPV) infection can be detected in 75-100% of cervical cancer biopsies in sexually active women and is nowadays accepted as one of the most important cancer promoting factors (Bosch et al., 1995). Some types of HPV have been well defined as the main cause of preneoplastic and neoplastic cervical lesions. HPV are divided into two groups according to their neoplastic potential. Types that cause low grade cervical lesions and genital warts, mainly HPV 6 and 11, are called "low risk" (LR) HPV types and those that cause cervical cancer are called "high risk" (HR) HPV types (Munoz et al., 2003). Type 16 is detected in 55%, type 18 is detected in 18% of all cervical cancer cases. Major types of HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

and 73) are found in 99.7% of precancerous, squamous intraepithelial lesions (Bosch et al., 1995; Trottier et al., 2006).

According to data of Globocan 2008, the incidence for cervical cancer in Turkey is 4.2/100,000 and it is the 8th cause of female deaths from cancer. According to this data 1443 women had a diagnosis of cervical cancer in 2008 and 556 had died because of it (Globocan, 2008).

There is a causal relationship between HPV and cervical cancer and HPV immunity is genotype specific, therefore it is important to determine HR-HPV prevalence in different geographic areas. We have aimed to investigate HPV frequency and genotype in women of Manisa who applied to the gynecology outpatient clinic of a university hospital and to correlate these to cervical Pap Smear results and risk factors.

### Materials and Methods

This study was approved by the local Ethical Committee of Celal Bayar University. A total of 410 women who applied to the gynecology outpatient clinic of Celal Bayar University Faculty of Medicine between January 2010-August 2011 were included in this retrospective study. Women with a diagnosis of invasive or preinvasive cervical cancer and those without sexual

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intercourse were excluded. Samples were obtained during gynecologic examination and transferred by a liquid-based media to the microbiology laboratory daily. For Pap test conventional cervical smear brushes were used. Specimens were fixed by alcohol and Bethesda system was used to classify the results (Solomon et al., 2002).

HPV Detection and Typing was done by the Linear Array (LA) HPV genotyping test (Roche Molecular Systems, Inc., Branchburg, NJ) which is a qualitative in vitro test for the determination of 37 anogenital HPV DNA genotypes [13 HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 5 probable HR types (26, 53, 66, 73, 82) and 19 LR types (6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39, and CP6108)].

This test is based on PCR amplification of target DNA using HPV primers, hybridization of the amplified product to oligonucleotide probes and their detection by colorimetric reaction. The test was performed according to the manufacturer's instructions. Briefly, DNA was amplified by PCR in a Perkin-Elmer GeneAmp PCR system 9,700 apparatus (Applied Biosystems). The denatured PCR product was then hybridized to an array strip containing immobilized oligonucleotide probes. The results were interpreted by using the reference guide and reading the matching individual types down the length of the strip.

Clinical data (age, age of menarche, number of deliveries, contraceptive method, smoking status etc) were recorded. Data were evaluated using SPSS for Windows ver 10.0 and chi-square and Spearman Correlation analysis was done.

## Results

The mean age of the women was 34.9 ( $\pm 9.1$ ), mean menarche age was 13.2 ( $\pm 1.4$ ) and mean number of births was 2. Ninety-five percent of the women had a single partner. Use of contraceptive methods was as follows: 38.5% no contraceptive method, 24.1% condoms, 15% coitus interruptus, 10% intrauterine device, 7.1% tubal ligation, 5.1% oral contraceptive. Reason for application to the hospital was routine control in 33.4% of the women, the other reasons are shown in Table 1.

Positive HPV was detected in 35 out of 410 patients (8.5%). The most frequent type was type 16 (28.5%) and a total of 26 different types were distinguished. Frequencies of different HPV types are shown in Table 2. Patients positive for HPV had at least one HR type in 65.7%, probable HR type in 11.4% and one LR type in 22.9% of the women. Multiple HPV infection was seen in 13 women (37.1%). Pap test results were benign cytology in 93.2% of the patients and data of 3.4% of the patients was not available (Table 3). There was statistically no significant difference between HPV positive and negative patients in regards to age, menarche age, number of deliveries, level of education, contraceptive method, systemic disease, cancer history in family, smoking status, pelvic examination findings, ultrasonographic findings. HPV was found more frequently positive in patients with single partner, previous abnormal Pap test and women with condyloma, this difference was statistically significant

**Table 1. Reasons for Application to Gynecology Clinic**

	%
Control	33.4
Irregular bleeding	21.9
Vaginal discharge	14.4
Prolapsus uteri/leiomyoma	12.7
Infertility	9.8
Previous abnormal PAP smear	6.1
Condyloma	1.7

**Table 2. Distribution of HPV Types in the Specimens**

HPV type	n	In all women (%) (n=410)	In HPV (+) women (%) (n=35)
HR-HPV	23	5	65.7
Probable HR-HPV	4	0.9	11.4
LR-HPV	8	1.9	22.8
Multiple HPV	13	3.1	37.1
HR-HPV			
Type 16	10	2.4	28.5
Type 18	1	0.2	2.8
Type 31	2	0.5	5.7
Type 33	1	0.2	2.8
Type 35	1	0.2	2.8
Type 45	4	1	11.4
Type 52	1	0.2	2.8
Type 56	3	0.7	8.5
Type 58	1	0.2	2.8
Type 59	2	0.5	5.7
Type 68	1	0.2	2.8
Probable HR-HPV			
Type 53	4	1	11.4
Type 66	3	0.7	8.5
Type 73	2	0.5	5.7
Type 82	1	0.2	2.8
LR-HPV			
Type 6	3	0.7	8.5
Type 11	1	0.2	2.8
Type 40	1	0.2	2.8
Type 42	2	0.5	5.7
Type 54	1	0.2	2.8
Type 55	2	0.5	5.7
Type 61	1	0.2	2.8
Type 62	2	0.5	5.7
Type CP6108	2	0.5	5.7
Type 67	1	0.2	2.8
Type 84	1	0.2	2.8

**Table 3. Pap Smear Results**

	%
Benign cytology	93.2
ASCUS*	1.0
LSIL**	2.2
HSIL***	0.2

\*ASCUS: Atypic squamous cells of unknown significance, \*\*LSIL: Low grade squamous intraepithelial lesion, \*\*\*HSIL: High grade squamous intraepithelial lesion

( $p < 0.05$ ).

When patients with single type HPV infection and multiple type infection were evaluated, multiple HPV type infection was more frequent in those with single sexual partner and oral contraceptive usage, this result was statistically significant ( $p < 0.05$ ). Abnormal Pap test results were significantly more frequent in multiple type HPV infected group ( $p < 0.05$ ). Correlation analysis showed a weak association between positive HPV results and abnormal Pap test ( $r = 0.120$ ).

## Discussion

The long preinvasive duration of cervical cancer progression and the anatomically easy visualisation of the cervix lead to effective screening programs. Epidemiologic studies show that HPV infection is the major risk factor for cervical cancer. The relationship between cervical cancer and HPV infection is considered more specific than that between lung cancer and smoking (Akhan, 2007). This relation is the strongest correlation in cancer epidemiology up to date (Clifford et al., 2003; Trottier et al., 2006). HPV DNA has been isolated from nearly all (99.7%) cervical cancer cases, therefore decreasing frequency of cervical cancer depends on preventing, diagnosing and treating HPV infection (Akhan, 2007; IARC Monographs, 2007). Precursor, asymptomatic lesions can be identified by screening programs and treated efficiently.

Due to the relationship between HPV-cervical cancer, it is important to identify and type HPV. In our study we have found positive HPV rate as 8.5%. When compared to similar studies from Turkey it is seen that studies on the general population are rare. Altun et al., have found HPV DNA positive rate as 5.2% in a study with 460 women using consensus PCR with MY09/11 and GP5+/6+ primers. Yuce et al. (2012) reported HPV prevalence as 25.7 (Yuce et al., 2012) and Eroglu detected a positive rate of 32.5% in Kayseri (Eroglu et al., 2011). Studies from different countries which have all used PCR based methods report rates as 7.8% in Iran (Khodakarami et al., 2012), 10.3% in India (Sauvaget et al., 2011), 19.4% in Portugal (Pista et al., 2011), 35.9% in Italy (Piana et al., 2011) and 64.1% in France (Casalegno et al., 2011). There is a wide range between HPV prevalence rates. This may be due to differences in geographic areas, social and cultural varieties and risk factors for cervical cancer or to the different methods used and their molecular sensitivity.

According to broad prevalence studies and metaanalysis, HPV type 16 is the most frequent type in cervical atypical changes (Clifford et al., 2003a; 2003b; de Sanjose et al., 2007). We have found HPV 16 as the most frequent type with a rate of 28.5%, followed by type 45 and 53 with rates of 11.4%. Our results are comparable to Altun et al. (2011) 33.3% rate. However other prevalence studies from Ankara and Kayseri report HPV type 18 as the most frequent type (Eroglu et al., 2011; Yuce et al., 2012). We have detected HPV 18 in only 1 specimen. HPV types vary between countries, Spain reports HPV 16 and 31 as the most frequent types (de Sanjose et al., 2003), England HPV 16 and 18 (Cuschieri et al., 2004) and USA HPV 62, 84 and 53 (Dunne et al., 2007).

Cohort studies show that persistent HR-HPV infections lead to high risk for cervical neoplasia. Studies about multiple HPV infections and cervical neoplasia have conflicting results. Many studies are case control studies, and the cumulative effect of HPV exposure is not followed, therefore it is difficult to draw accurate conclusions (Trottier et al., 2006). In our study group multiple HPV infection was seen in 13 (37.1%) specimens. Other studies from Turkey show a high rate of multiple HPV infections. Yuce et al., report a rate of 23.6% (Yuce et al., 2012), Eroglu et al., report a rate of 17.5% (Eroglu et al., 2011)

for multiple type infections. However Altun et al., report a low rate of 1.1% (Altun et al., 2011) which may due to their method of DNA sequencing. DNA sequencing is considered the gold standard for detecting HPV types but is insufficient in showing multiple infections. Only the dominant genotype is shown but multiple types cannot be differentiated. Multiple probes are used in reverse hybridisation techniques to overcome this difficulty and multiple HPV typing can be done (Chinchai et al., 2011).

Risk factors for cervical cancer and cervical preinvasive lesions are early onset of sexual activity, multiple partners, unprotected sexual activity, high risk sexual partner, history of sexually transmitted disease, oral contraceptive use, multiparity, family history, low socio-economic status, smoking, immunosuppression and HPV infection (Vesco et al., 2011; Senol et al., 2012). Almost all studies Show a relation between infection with HR-HPV and especially its persistence and cervix cancer (Cuzick et al., 2003). The other risk factors have various degrees of relationship. HPV DNA is detected in sexually active women without cervical dysplasia which implies that it is a risk factor necessary but not sufficient for cervical cancer development. We have investigated the relationship between various risk factors (age, menarche age, number of deliveries, level of education, number of partners, contraceptive method, systemic disease, cancer in family and smoking) and HPV. We have found a significant higher rate of positive HPV ( $p < 0.005$ ) in women with single partner, previous abnormal Pap test and condyloma. Even though it may seem as a paradox to find having a single partner related to HPV, this may be explained by the fact that 95.1% of the study group had a single partner. Another explanation may be that women tend to conceal their sexual activity due to traditional habits.

We have seen a significantly high rate of multiple HPV infection in women with single partners and oral contraceptive users ( $p < 0.005$ ). Oral contraceptives are considered as a risk factor for cervical cancer and evidence exists showing that OC's are a cofactor to HPV in developing cervical cancer (International Collaboration, 2007). Progesterone is immunosuppressive and thus is mediator for HPV infection, therefore it is proposed as an important hormone for cervical neoplasia. HPV tends to transfect cells with progesterone receptors. Both HPV 16 and 18 contain progesterone and glucocorticoid respond elements that enhance E6 and E7 oncogene expression which are important in cell transformation (Crook et al., 1988). Experimental studies that investigate the role of estrogens in cervical carcinogenesis show that at the transformation zone where cervical neoplasia begins, estradiol is changes into 16- $\alpha$ -hydroksiestron by 16- $\alpha$ -hydroxylation and HPV transfected cells sensitive to estrogen are related to malign transformation (International Collaboration, 2007). Thus our results are consistent with literature.

In conclusion, the overall HPV positivity rate is 8.5% in our region in a low risk population. Among these positive results 65.7% are HR-HPV types. These rates are important for our community because the majority of women have only one partner who is their husband. Early detection of HPV and early diagnosis of precancerous

lesions is important, therefore country-wide screening programs are essential in the prevention of cervical cancer.

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