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Prevalence and types of cervical human papillomavirus among Turkish women and its relationship with demographic factors in a gynecology outpatient clinic

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Summary

Purpose of investigation: The purpose of this study was to determine the prevalence of various human papillomavirus (HPV) types and its relationship with some risk factors among Turkish women. *Materials and Methods:* A total of 673 patients were included in the study. Cervical samples were taken from the patients for routine Pap smear and HPV DNA tests. HPV DNA was studied in the cervical smear by using the PCR method. *Results:* The mean age of the patients was 40 ± 10.9 years. Of all patients, 13.5% had a positive HPV DNA test. In addition, 5.1% of the patients were HPV type 16 positive, 1.5% were HPV type 18 positive, 0.6% were HPV type 31 positive, and 0.9% were HPV type 53 positive. *Conclusion:* Findings show that awareness should be raised among Turkish women regarding HPV infection and vaccination.

Key words: Turkish women; Human Papillomavirus, Prevalence.

Introduction

Cervical cancer is one of the most common types of cancer in developing countries and accounts for 25% of all cancers among women [1]. It is second only to breast cancer on the list of cancer-related deaths around the world [2]. The rate of relationship between cervical cancer and human papillomavirus (HPV) infection was reported to be 99.9%. The prevalence of HPV types which cause cervical cancer may vary from region to region; however HPV type 16 and HPV type 18 account for 70-80% of all cases [3, 4].

In this study, the authors aimed at determining the prevalence and types of cervical HPV and its relationship with demographic factors among Turkish women.

Materials and Methods

This descriptive single-center study was approved by the ethical board of Gazi University Faculty of Medicine. A total of 673 patients who presented to Gazi University Faculty of Medicine Outpatient Clinic of Gynecology and Obstetrics with gynecological complaints between April 2007 – April 2009 were included in the study. Cervical samples were taken from the patients presenting to the outpatient clinic for routine pap smear and HPV DNA tests. HPV DNA was studied in the cervical smear and the prevalence of HPV types was determined in the patients.

A total of 673 sexually active patients of various ages were included in the study. Patients with a history of hysterectomy, cervical stenosis, cervical cancer, vulvar cancer, vaginal cancer, and chemotherapy, as well as pregnant patients were excluded from the study. Data on age, marital status, parity, age at first coitus, number of partners, smoking and educational status, as well as results of Pap smear tests were noted for each patient. Pap smears were taken by cervical brush, spread over lamina, and sent to the pathology laboratory of the present hospital for further examination. Samples were stained by hematoxylin + eosin in the pathology laboratory and evaluated by means of the Bethesda system. Smears which were taken from the endocervical canal and the whole transformation region during the gynecological examination were placed in the carrier liquid medium and sent to the molecular microbiology laboratory of the present hospital for HPV DNA tests.

Analyses

1-DNA isolation

Samples which were taken from the cervical region by sterile swab for detection and type-specific identification of HPV-DNA were placed in the carrier liquid medium and sent to the laboratory immediately. The liquid was vortexed with swab, transferred to eppendorf tubes and stored at -20 degrees Celsius until the initiation of the study. HPV was detected by means of a GenoArray Test Kit. Before type-specific identification of HPV, DNA isolation was performed. Samples which were taken by cervical swab were thawed at room temperature. For DNA isolation, the HybroBio DNA extraction kit was used and 500 µl of clinical sam-

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Table 1. — *Some demographic features of the patients.*

		HPV DNA (+)		HPV DNA (-)		
		n	%	n	%	OR (95% GA)
Age groups (years)	≤ 24	10	21.7	36	78.3	2.08 (0.68–6.47)
	25-34	24	13.1	159	86.9	1.13 (0.45–2.91)
	35-44	28	14.6	164	85.4	1.28 (0.52–3.24)
	45-54	21	11.4	163	88.6	0.97 (0.38–2.52)
	≥ 55	8	11.8	60	88.2	1.00
			Chi-square: 3.75			$p = 0.4413$
Marital status	Married	69	11.8	514	88.2	1.00
	Single	14	40	21	60	4.97 (2.27–10.79)
	Divorced/widowed	8	14.5	47	85.5	1.27 (0.53–2.93)
			Chi-square: 22.45			$p = 0.00001$
Age at first coitus	≤15	4	23.5	13	76.5	3.28 (0.85–11.62)
	16-19	52	21	196	79	2.83 (1.74–4.61)
	≥20	35	8.6	373	91.4	1.00
			Chi-square: 21.741			$p = 0.0001$
Smoking	Smoker	41	27	111	73	3.48 (2.14–5.66)
	Non-smoker	50	9.6	471	90.4	1.00
			Chi-square: 30.386			$p = 0.0001$
Educational status	Primary school	35	13.5	224	86.5	1.00
	High School	29	10.9	236	89.1	0.79 (0.45–1.37)
	University	23	18.3	103	81.7	1.43 (0.77–2.64)
	None	4	17.4	19	82.6	1.35 (0.36–4.54)
			Chi-square: 4.214			$p = 0.239$

ples was centrifuged. The supernatant was removed, 400 µl of solution 1 was added to the liquid, and the liquid was vortexed and kept in water-bath for 15 minutes. Later on, 400 µl of solution 2 was added to the liquid, and the liquid was kept at room temperature for two minutes and centrifuged for five minutes. The supernatant was removed following the centrifugation. Lastly, 60 µl of solution 3 was added to the liquid, and template DNA was isolated to be used for PCR reaction. The next phase was amplification.

2- Amplification

The HPV GenoArray Test Kit was used for amplification. In this method, a combination of polymerase chain reaction and flow through hybridization technology is used, and HPV infection and its 21 specific types are detected in cervical samples. For PCR amplification, firstly, PCR master mix was prepared. Secondly, 20 µl of PCR master mix and template DNA were transferred to each PCR reaction tube. Lastly, PCR reaction tubes were placed in thermal cycler, and the cycler was set to amplification mode.

3- Flow-through hybridization

Following amplification, flow through hybridization of PCR products was performed. To this end, nylon 6,6 Brodyne C membrane (HybriMem HPV-21) covered by HPV DNA probes was used. Membrane hybridization solution was added to the PCR products separately and they were mixed. Later on, blocking solution was added to the mix and the solution was incubated. Enzyme conjugate was added to the solution and the solution was left aside for three minutes. Membrane was washed with solution A. NBT/BCIP solution was added to the solution and the solution was incubated for five minutes for staining. Later on, membrane was washed with solution B for three times and rinsed with distilled water. Membranes were transferred to absorbing paper and the results were interpreted in an hour. At the end, specific HPV types were identified in the samples based on the place of the specific probes in HybriMem-21 membrane.

Statistics

The Statistical Program for Social Sciences (SPSS, version 11,5) was used for statistical analysis and data recording. Pearson chi-square test was used for correlations and Odds Ratio was used for some risk factors.

Results

A total of 673 women who presented to this gynecology outpatient clinic between April 2007 – April 2009 were screened for cervical HPV DNA. Some demographic features of the patients are demonstrated in Table 1. The mean age of the patients was 40.3 ± 10.9 (range: 19-69) years. Patients were divided into five groups according to their ages. Forty-six patients were assigned to the group aged 24 years and below (6.8%), 183 patients were assigned to the group aged between 25-34 years (27.2%), 192 patients were assigned to the group aged between 35-44 years (28.5%), 184 patients were assigned to the group aged between 45-54 years (27.3%), and 68 patients were assigned to the group aged 55 years and above (10.1%). It was found that 21.7% of patients aged 24 years and below, 13.1% of patients aged between 25-34 years, 14.6% of patients aged between 35-44 years, 11.4% of patients aged between 45-54 years, and 11.8% of patients aged 55 years and above had a positive HPV test. However, no statistically significant difference was found between positive HPV test and age ($p = 0.441$). The number of women with abnormal cervical cytology was 40 (6%) whereas the number of women with normal cervical cytology was 633 (94%). HPV DNA test gave a positive re-

Table 2. — *Distribution of HPV types in the patient group with abnormal cytology.*

Abnormal cytology	Negative n. (%)	Type 11 n. (%)	Type 42 n. (%)	Type 68 n. (%)	Type 16 n. (%)	Type 18 n. (%)	Type 52 n. (%)	Total n. (%)
ASCUS	19 (90.5)	1 (4.8)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	21 (100)
ASC-H	1 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	1 (33.3)	3 (100)
LSIL	5 (45.5)	0 (0)	1 (9.1)	1 (9.1)	1 (9.1)	2 (18.2)	1 (9.1)	11 (100)
HSIL	2 (40)	0 (0)	0 (0)	0 (0)	3 (60)	0 (0)	0 (0)	5 (100)
Total	27 (67.5)	1 (2.5)	1 (2.5)	1 (2.5)	6 (15)	2 (5)	2 (5)	40 (100)

sult in 13.5% of women included in the study, 32.5% of women with abnormal cervical cytology, and 12.3% of women with normal cervical cytology. As to the diagnoses of patients with abnormal cervical cytology; 52.5% had atypical squamous cell of undetermined significance (ASCUS), 7.5% had high grade squamous intraepithelial lesion which cannot be excluded (ASC-H), 27.5 % had low-grade squamous intraepithelial lesion (LSIL), and % 12.5 had high-grade squamous intraepithelial lesion (HSIL).

HPV DNA test was positive in 9.5% of patients with ASCUS, 66.7% of patients with ASC-H, 54.5% of patients with LSIL, and %60 of patients with HSIL. The most common type of HPV among women with abnormal cervical cytology was HPV type 16 (15%), followed by HPV type 18 (5%), HPV type 52 (5%), HPV type 68 (2.5%), and HPV type 11 (2.5%). Distribution of HPV types among women with abnormal cervical cytology is demonstrated in Table 2. On the other hand, the most common type of HPV among women with normal cervical cytology was HPV type 16 (4.4%), followed by HPV type 18 (1.3%), HPV type 31 (0.5%), and HPV type 53 (0.5%).

The prevalence of HPV was 11.8% among married women, 14.5% among divorced or widowed women, and 40% among single women who were sexually active. HPV DNA test was positive in 23.5% of women aged 15 years or below at first coitus and 8.6% of women aged 20 years or above at first coitus ($p = 0.0001$). Furthermore, it was positive in 27% of smokers and 9.6% of non-smokers ($p = 0.0001$). Differences between the groups were statistically significant.

Discussion

Cervical cancer is an important health issue especially in developing countries. It is the second most common type of cancer among women around the world [5]. In addition, 99.9% of patients with cervical cancer were reported to have HPV infection. Although most of the women recover without requiring treatment, women with persistent HPV infection carry the risk of having CIN II, CIN III, and cervical cancer [6]. Epidemiological and experimental studies indicate a causal relationship between cervical cancer and infection with HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66. In addition, it is estimated that HPV accounts for 5.2% of all cancers around the world [7-9].

Table 3. — *Some studies conducted in various Turkish cities regarding the prevalence of HPV.*

	N.	Method	%
Tuncer *et al.* 2006, Ankara [33]	1032	HC-II*	4
Inal *et al.* 2007, Izmir [31]	1353	HC-II	2.1
Ozcelik *et al.* 2003, Kayseri [28]	230	HC-I	6
Total	2,615		4
Ozalp *et al.* 2011, Eskisehir [6]	615	PCR**	4.2
Safi *et al.* 2002, Ankara [34]	60	PCR	3.3
Yildirim *et al.* 2013, Sivas [35]	140	PCR	6.4
Altun *et al.* 2009, Adana [17]	460	PCR	5.2
Dursun *et al.* 2009, Ankara [18]	403	PCR	20
Tunc *et al.* 2009, Ankara***	673	PCR	13.5
Total	2,351		8.7

*HC-II: Hybrid Capture II; **PCR: polymerase chain reaction;
***The present study.

The prevalence of HPV varies around the world. In a study which was conducted in China among 16,803 women with normal cervical cytology, the prevalence of HPV was found to be 14.4% [10]. In another study conducted in Russia in 2007, the prevalence of HPV was reported to be 27.2% [11]. In two other studies which were conducted in different Greek regions, the prevalence of HPV was found to be 2.5% and 23.6%, respectively [12, 13]. In a study which was conducted in Kenya, 44.3% of the women were reported to have a positive HPV DNA test [14]. The prevalence of HPV was found to be 3% in a study conducted in Spain [15]. In addition, HPV infection was detected in 16,6% of the patients in an Argentinian study [16].

Results of the studies which were conducted in various Turkish cities for determination of HPV prevalence are demonstrated in Table 3. In their study which was conducted in Eskisehir, Turkey, Ozalp *et al.* screened 615 women and reported that 4.2% of the women were HPV DNA positive [6]. In another study conducted in Cukurova, Turkey, the prevalence of HPV DNA was reported to be 5.2% [17]. Polat *et al.* found in 2009 that the prevalence of HPV DNA was 20% among women [18]. In the present study, on the other hand, the authors found that 13.5% of their patients had a positive HPV DNA test. This was the second highest rate reported in the studies conducted in Turkey. Differences in the results may be explained by differences in study populations and in sensitivity of HPV DNA tests that are used.

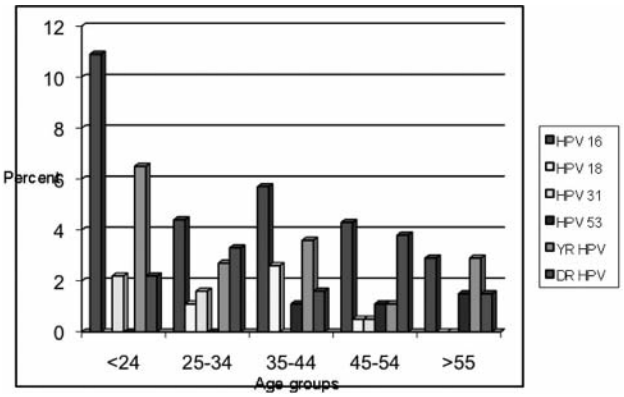

Figure 1. — Distribution of HPV types among age groups.

In a meta-analysis of 78 studies, de Sanjosé *et al.* reported in 2007 that the prevalence of cervical HPV DNA was 10.4% among women with normal cervical cytology around the world [19]. This prevalence was found to be higher in less developed countries (15.5%) compared to developed countries (10%). In the same study, the most common type of HPV was found to be HPV type 16 (2.5%), followed by HPV type 18 (0.9%), HPV type 31 (0.7%), HPV type 58 (0.6%), and HPV type 52 (0.6%) among women with normal cervical cytology [19]. In the present study, women with normal cervical cytology constituted 94.1% of the study population. In agreement with the aforementioned studies, the present authors found that the prevalence of HPV DNA was 12.3% among Turkish women. In addition, in line with the literature, the most common types of HPV DNA were HPV type 16 (4.4%), HPV type 18 (1.3%), HPV type 31 (0.5%), and HPV type 53 (0.5%).

Bao *et al.* included 5954 women with invasive cervical cancer, 1,653 women with HSIL, 958 women with LSIL and 16,803 women with normal cervical cytology in their study and reported that high risk (HR) HPV DNA was positive in 5.9%, 81%, 72.9%, and 14% of these cases, respectively [10]. In their study, Park *et al.* reported that the prevalence of HR HPV DNA was 17.6% among women with normal cervical cytology, 73.5% among women with LSIL, 92.2% among women with HSIL, and 95.2% among women with invasive cervical cancer [20]. In their study of 84 patients with LSIL, HSIL or cervical cancer, Serra *et al.* found that the prevalence of HR HPV DNA was 83.3%, 58.4%, and 100%, respectively [21]. In agreement with the aforementioned studies, the authors found in the present study that the HR HPV DNA was positive in 54.6% of women with LSIL and 60% of women with HSIL.

It is reported in the literature that there is an inverse relationship between positive HPV DNA test and age, and the prevalence of HPV is higher among women under the age of 25 and decreases with age [14, 15, 22]. In a study which was conducted in the USA for determination of the prevalence of HPV among various age groups, the prevalence of HPV was found to be 24.5% among women aged between 14-19 years, 44.8% among women aged between 20-24 years, 27.4% among women aged between 25-29 years, 27.5% among women aged between 30-39 years, 25.2% among women aged between 40-49 years, and 19.6% among women aged between 50-59 years [23]. According to the results of the present study, the prevalence of HPV is the highest among women aged between 20-24 years, which represent the most active years of sexual life and remarkably decreases after the age of 40.

In a study which was conducted in Turkey, Dursun *et al.* reported that the prevalence of HPV infection was 34% among women under the age 30 [18]. In the present study, the prevalence of HPV among women under the age of 24 was 21.7%. No statistically significant relationship was found between positive HPV DNA test and age. However, the mean age of HPV DNA (+) patients was lower than that of HPV DNA (-) patients. Distribution of HPV types among age groups is demonstrated in Figure 1.

In their study, de Sanjosé *et al.* reported that the prevalence of HPV infection was the highest among women under the age of 24 compared to the other age groups. In all the continents except for Asia, the prevalence of HPV was shown to peak for a second time in women over the age of 44. In this respect, three hypotheses were put forward as follows: 1) decline of the immune system as a result of the hormonal changes caused by menopause; 2) changing sexual behaviors of the woman and her partner due to advanced age; 3) increased extra-marital sexual activities of the husband [19]. In the present study, the authors could not find such a peak in their patients over the age of 44. The prevalence of HPV DNA was 11.4% in this age group, and there was an insignificant increase in the prevalence of HPV DNA among women over the age of 55 (11.8%).

Several studies reported a relationship between smoking and development of cervical cancer [24, 25]. Although the exact mechanism of such a relationship is not known, it is considered that smoking interacts with HPV and leads to premalignant changes. It is further considered that carcinogenic metabolites of smoking cause viral persistency via immune-modulation or genetic damages caused by genotoxins [26]. In the present study, HPV DNA was found to be positive in 27% of smokers and 9,6% of non-smokers. The relationship between smoking and positive HPV DNA test was statistically significant ($p = 0.0001$).

In a study which was conducted in Spain, the prevalence of HPV DNA was found to be 6.7 times higher among divorced women compared to married women [15]. Similarly in another study, divorced women were reported to be exposed to HPV infection in higher rates compared to married women [27]. In the present study, 86.6% of the patients were married, whereas 5.2% were single and 8.2% were either widowed or divorced. In addition, HPV DNA was positive in 11.8% of the married women, 40% of the single women,

and 14.5% of the widowed or divorced women. The relationship between marital status and positive HPV DNA test was statistically significant ($p = 0.00001$). It is considered that higher rates of positive HPV among single and sexually active women, as well as divorced women, may be associated with the number of partners.

The most common type of HPV is HPV type 16 around the world, followed by HPV type 18 in western countries, and HPV type 58 in Asia [28, 29]. In a study which was conducted in Turkey, Dursun *et al.* reported that the most common types of HPV were HPV types 16, 6, and 18 among the Turkish population [18]. Rota *et al.* also reported that the most common types of HPV were 16 and 18 [30]. In addition, Inal *et al.* found that HPV types 16, 11, 6, 18 , and 31 were the most common types of HPV among their study population [31]. Similarly in the present study, the most common types of HPV were type 16, 18, 6, 11, 31, and 53. These results show that vaccination against HPV the present country.

In their study, Bao *et al.* reported that the prevalence of HPV was 81% among women with HSIL. Furthermore, they listed the most common types of HPV as follows: 16, 58, 52, 18, 33, 51, 31, 56, 35, and 45. Inal *et al.* found that the prevalence of HPV was 2% in their study. In addition, HPV DNA was positive in all women with abnormal cervical cytology [31]. Onan *et al.* detected HR HPV in 45% of women with CIN [32]. In their study, Dursun *et al.* found that HPV was positive in 36% of women with abnormal cervical cytology [18]. All these studies confirm the existence of a significant relationship between positive HPV DNA test and CIN. Similarly in this study, 32.5% of women with abnormal cervical cytology had a positive HPV test.

Conclusion

There are only a limited number of studies on the prevalence of cervical HPV in Turkey. The present study shows that HPV infection is more prevalent than expected among Turkish women, which emphasizes the importance of awareness-raising among the society about cervical cancer screening programs and HPV vaccines.

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