

Distribution of human papillomavirus types in Turkish women

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Summary

Purpose of investigation: Since oncogenic types of human papillomavirus (HPV) are associated with a higher risk of cervical cancer and certain types can be controlled by a vaccine, a study has been performed to determine the HPV genotype distribution among Turkish women. **Methods:** The study included patients with abnormal cytology or in the follow-up for cervical intraepithelial neoplasia between 2002 and 2009 at Hacettepe University Hospital. The results of 1,797 consecutive cervical samples were analyzed retrospectively. INNO-LiPA HPV genotyping, HPV-Typing and Seeplex HPV 18-plex genotyping tests were used to determine the types of HPV. **Results:** HPV was detected in 404 (22.4%) of 1,797 samples studied. HPV DNA was identified in 194 cases by using HPV-Typing test but the specific genotype was not available. The most frequent genotype was HPV 16 which was observed in 103 cases (49.0%). **Conclusion:** HPV 16 was the most common genotype observed among Turkish women with abnormal cytology. It suggests that HPV vaccination may be useful for prevention of cervical cancer in this population.

Key words: Human papillomavirus; Cervical cancer; Pap test; Genotyping.

Introduction

Carcinoma of the uterine cervix continues to be one of the most common female genital cancers worldwide despite availability of effective screening [1]. Infection by human papillomavirus (HPV) has long been recognized as a main causal factor for almost all cases of cervical cancer. More than 100 genotypes of HPVs are characterized to date and over 30 types that infect the anogenital tract have been described [2, 3]. Tumorigenicity of the virus differs markedly among genotypes and several high-risk types have been implicated in cervical carcinogenesis. HPV 16 is the most common type observed in women with normal cervical cytology and in those with cervical neoplasia [4]. The pattern of HPV type distribution may vary among countries and regions. With the advent and routine application of HPV vaccines, data on regional HPV genotype distribution would be more useful to predict the potential benefits. However, data regarding community based distribution of HPV genotypes are still limited in Turkey. Determination of distribution of HPV genotypes will also allow monitoring of the impact of vaccination on HPV type replacement and enhancing further research.

Since oncogenic types of HPV are associated with a higher risk of cancer and certain types can be controlled by a vaccine, a study has been performed to determine the HPV genotype distribution among Turkish women.

Methods

A study including 1,797 cases between 2002 and 2009 was carried out in Hacettepe University Hospital, Ankara, Turkey. The research protocol was approved by the institutional ethics committee. The study included patients with abnormal cytology or in follow-up of cervical intraepithelial neoplasia at the Department of Obstetrics and Gynecology, Gynecologic Oncology Unit. The mean age of the study population was 36.8 years (± 9.3). The mean values for gravidity and parity were 3.0 and 2.0, respectively. The mean ages of menarche and first sexual intercourse were 13.5 years (± 1.4) and 20.5 years (± 4.1), respectively.

The results of 1,797 consecutive cervical samples were analyzed retrospectively. INNO-LiPA HPV Genotyping Test (Innogenetics, USA) was used between 2002 and 2005 while the HPV-typing test (GenID, Germany) was used between 2005 and 2007; and the Seeplex HPV 18-plex genotyping test (Seegene, Korea) was used between 2007 and 2009 to determine the types of HPV.

The INNO-LiPA HPV Genotyping Test is based on the reverse hybridization principle. Part of the L1 region of the HPV genome was amplified and denatured biotinylated amplicons were hybridized with specific oligonucleotide probes immobilized on the strip. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase was added and binded to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen gave a purple/brown precipitate and results were interpreted visually. Amplification and detection methods were performed according to instructions of manufacturer.

The HPV-typing test is based on the reverse hybridization assay for the differentiation of high- and low-risk genotypes. After DNA was isolated from clinical samples, PCR and subsequent reverse dot blot hybridization with sequence-specific oligonucleotide probes that represented particular HPV genotypes immobilized on nitrocellulose membranes were applied according to the manufacturer's instructions.

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The Seeplex HPV 18-plex Genotyping Test utilizes amplification of target DNA by multiplex PCR based on dual priming oligonucleotide technology and detects 18 different HPV DNA genotypes. Following DNA extraction with the Nucleospin DNA extraction kit (Macherey-Nagel, Germany) per instructions of the manufacturer, amplification was carried out in GeneAmp PCR Systems 9700 (Applied Biosystems, USA). The PCR amplicons were analyzed by a capillary electrophoresis-ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). Typing of HPV was achieved by separation of the amplicons according to their sizes. Evaluation of this data was performed by using Applied Biosystems GeneMapper Software. Amplification and detection methods were performed according to manufacturer's instructions.

ThinPrep system was used for liquid-based cytological analysis. Pap test results were evaluated in terms of the Bethesda III classification.

Results

HPV was detected in 404 (22.4%) of 1,797 samples studied. HPV DNA was identified in 194 cases by using the HPV-typing test but the specific genotype was not available. In the remaining 210 cases analyzed by INNO-LiPA HPV and Seeplex HPV 18-plex genotyping tests, 22 different HPV types were detected. While 68 (32.3%) of the samples had infection with multiple genotypes, 142 (67.7%) had single genotype infection. The most frequent genotype was HPV 16 which was observed in 103 (49.0%) cases (Table 1). The following common types were HPV 51 (19.5%), HPV 31 (17.1%), HPV 6 (10.0%), HPV 42 (8.0%), HPV 33 (7.6%), HPV 68 (5.2%), and HPV 18 (4.7%). Among HPV 16 positive samples, 62 (60.1%) had single type of HPV infection. HPV 66 and HPV 54 were observed as only single infections whereas HPV 35, HPV 40, HPV 44 and HPV 45 were detected only in multiple infections.

Of the 404 cases with HPV infection, Pap smear results were negative in 243 (60.1%) cases (Table 2). The most common epithelial cell abnormality observed at Pap smear was LSIL in 67 (16.6%) cases. Among 103 cases with HPV 16 infection, Pap tests were negative in 59 (57.3%) patients while the remaining 44 had various cytological abnormalities.

Discussion

The present study on 1,797 consecutive patients from Turkey undergoing HPV testing for abnormal cytology or follow-up of cervical intraepithelial lesion shows a 22.4% prevalence of HPV infection. Worldwide prevalence of HPV infection is estimated to be approximately 10% among women with normal cytology [4]. Figures from Europe and Asia (where Turkey belongs) are even lower reaching approximately 8%. A review of several previous studies including approximately 3,000 cases from Turkey reported a 5% HPV prevalence among low-risk women [5]. The study population of the current work represents a high-risk group for HPV infection since they had either an abnormal Pap test or in the follow-up cervical intraepi-

Table 1. — HPV genotype distribution.

	Total (n, %)	Multiple type (n, %)	Single type (n, %)
HPV 16	103 (49%)	41 (39.8%)	62 (60.19%)
HPV 51	41 (19.5%)	23 (56.09%)	18 (43.90%)
HPV 31	36 (17.1%)	22 (61.1%)	14 (38.89%)
HPV 6	21 (10%)	11 (52.38%)	10 (47.61%)
HPV 42	17 (8.09%)	10 (58.82%)	7 (41.17%)
HPV 33	16 (7.6%)	9 (56.25%)	7 (43.75%)
HPV 68	11 (5.2%)	8 (72.2%)	3 (27.27%)
HPV 18	10 (4.7%)	6 (60%)	4 (40.0%)
HPV 43	8 (3.80%)	6 (75.0%)	2 (25%)
HPV 56	6 (2.8%)	4 (66.6%)	2 (33.33%)
HPV 39	6 (2.8%)	4 (66.6%)	2 (33.33%)
HPV 52	6 (2.8%)	5 (83.3%)	1 (16.66%)
HPV 58	5 (2.38%)	4 (80%)	1 (20.0%)
HPV 11	5 (2.38%)	1 (20%)	4 (80.0%)
HPV 59	3 (1.42%)	2 (66.6%)	1 (33.3%)
HPV 70	3 (1.42%)	2 (66.6%)	1 (33.3%)
HPV 35	3 (1.42%)	3 (100%)	0 (0.0%)
HPV 66	2 (0.95%)	0 (0.0%)	2 (100%)
HPV 40	2 (0.95%)	2 (100%)	0 (0.0%)
HPV 54	1 (0.47%)	0 (0.0%)	1 (100%)
HPV 44	1 (0.47%)	1 (100%)	0 (0.0%)
HPV 45	1 (0.47%)	1 (100%)	0 (0.0%)

Table 2. — Pap smear results in HPV positive cases.

Pap test	n	%
Negative	243	60.1
Atypical glandular cells (AGC)	7	1.7
Atypical squamous cells (ASC)	63	15.5
ASC-US	57	
ASC-H	6	
Low-grade squamous intraepithelial lesion (LSIL)	67	16.6
High-grade squamous intraepithelial lesion (HSIL)	24	6.1
Total	404	100.0

thelial neoplasia. Thus, this figure is definitively higher than the global crude HPV prevalence as expected.

HPV prevalence rates are reported to be greater in high-risk populations. Menegazzi *et al.* reported 45.9% rate of HPV infection in an Italian group of patients undergoing opportunistic screening and evaluation of HPV associated lesions [6]. Tsao *et al.* found 32.4% of HPV infections among 343 Taiwanese women who visited clinics for screening or for follow-up of cervical intraepithelial neoplasia [7]. HPV infection rates of 22.4% in a high-risk population of Turkish women may be evaluated to be lower than the figures reported from similar study groups in the literature.

HPV 16 was found to be the most common genotype in this study. According to a meta-analysis including 48 studies from different regions of the world that provided type-specific information, the most common genotype was HPV 16 followed by HPV 18, HPV 31, HPV 58 and HPV 52 [4]. Determination of HPV 16 as the most common genotype in the current study is in agreement

with worldwide genotype distribution of HPV. However, the second most commonly reported HPV 18 genotype was found to be less common in this series. Distribution of individual HPV genotypes varies across geographic areas and ethnic groups [2, 4]. Determination of HPV 51 as one of the most common types of infection has been reported in studies from Italy, Taiwan, Germany and Denmark [6-9]. The finding of HPV 51 as the second most common type of infection in this series is in accordance with these studies. HPV 31 was found to be the third most common type of HPV infection which correlates well with the literature [4].

Current vaccines protect against HPV 16 and HPV 18 infections. Furthermore, cross reactivity for HPV 31, HPV 33 HPV 45 and HPV 52 suggest an even higher percentage of efficacy [10]. Observation of HPV 16 as the most common genotype as well as the other common HPV types profiled among Turkish women suggests that HPV vaccination may be useful for prevention of cervical cancer in this population. HPV vaccination should be a national priority in light of the findings in this study. However, besides genotypes which are targeted by current vaccines, other types should also be taken into consideration in the development of enhanced vaccines with larger coverage.

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