Prevalence of HPV Types in a Cohort of Greeks with Clinical Indication of Infection

CHRISTOS YAPIJAKIS[1,2], MARIA ADAMOPOULOU[1,2], GEORGIOS ANTONOPOULOS[3], NIKOS KOUFALIOTIS[2] and ELEFTHERIOS VAIRAKTARIS[1]

[1]*Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Attikon Hospital, Athens;*
[2]*Department of Molecular Biology and Genetics, Bioerevna Medical Center, Athens;*
[3]*Department of Gynecology, Gennimatas Oncological Hospital, Athens, Greece*

Abstract. *Background: Several types of human papilloma virus (HPV) have been associated with increased risk for epithelial malignancies. In light of a recently available vaccine that protects against persistent infection by certain HPV types (6, 11, 16, 18) and resulting neoplasias, the prevalence of HPV types was investigated in a cohort of people with a suspected viral infection. Patients and Methods: The studied material consisted of genital or oral scrapings obtained from 263 consecutively referred Greeks (aged 18-64 years) with a clinical indication of HPV infection. DNA samples isolated from scrapings were tested by PCR, using consensus primers for at least 50 HPV types. In cases of detected viral DNA sequence, HPV typing was performed by restriction analysis using 4 enzymes and confirmed by DNA sequencing. Results: 215/263 (81.7%) of the samples were HPV-positive. HPV types associated with high risk for neoplasias were detected in 91/215 (42.3%), intermediate risk types in 64/215 (29.8%) and low-risk types in 60/215 (27.9%) of the positive samples. A total of 85/215 (39.5%) were positive for one of the vaccine-related types. Furthermore, types 16 and 18 comprised about the same proportion of the high-risk types detected in this study (35/91, 38.5%). Conclusion: The observed high prevalence rate of high-risk types underlines the importance of testing individuals with an indication of a possible HPV infection. In addition, there is a need for prevention strategies, such as the annual Pap smear screening of women, as well as wider use of HPV molecular screening and vaccines targeted at common HPV types.*

Accumulating epidemiological evidence supports a strong association between human papillomavirus (HPV) and neoplastic lesions in epithelial tissue of the genital tract, anus and the oral region (1-3). Furthermore, HPV infection is more prevalent and more persistent in women than men (3). Specific types of the virus have been considered as the most significant etiological factor in cervical cancer, which is the second most common cause of cancer-related death in women worldwide and the first in developing countries (1-3). Clinical indications for HPV testing include the presence of *condylomata acuminata* and pre-cancerous lesions referred as squamous intraepithelial lesions (SIL) in the genital tract and oral region (2, 4, 5). HPV types which are associated with anogenital cancer have also been detected in oral and oropharyngeal squamous cell carcinoma (OSCC), suggesting a possible sexual transmission of HPV based on epidemiological and molecular means (5, 6).

There are over 70 different HPV types, classified into three categories according to their transforming properties and risk for cancer induction. High-risk types, such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66, have been frequently detected in cervical cancer, while HPV types 16, 18 and 31 have also been detected in oral squamous cell carcinomas (1-5, 7, 8). Low-risk types HPV 6, 11, 34, 40, 42, 43 and 44 have been usually related to benign lesions, such as *condyloma acuminata,* while the intermediate risk types HPV 32, 49, 53, 54, 55, 57 61, 64, 67 and 69 have frequently been detected in low- and high-grade SIL (2, 4, 7, 8).

The high frequency of HPV presence in dysplastic or neoplastic lesions and its oncogenic potential necessitates detection and typing of the virus, which have important prognostic and therapeutic implications. HPV typing provides clinicians with valuable information in the decision-making process for the best available treatment, which ranges from chemotherapy (using imiquimod 5% as an effective treatment in reducing the viral load), to cryotherapy, laser therapy, electrosurgery and surgery for removal of larger lesions which have not responded to other methods of treatment (9, 10).

Until recently, the best strategy for prevention of HPV-related neoplasias included viral DNA testing and typing in individuals with a clinical indication of HPV infection.

Correspondence to: Dr. Eleftherios Vairaktaris, MD, DDS, Ph.D., DMD, MS, Ph.D., Department of Oral and Maxillofacial Surgery, Attikon Hospital, Rimini 1, Athens 12462, Greece. Tel: +30 2106443035, Fax: +30 2106443803, e-mail: elvairakt@med.uoa.gr

Key Words: HPV detection, HPV typing, Greek population.

The development of vaccines targeted at rather common HPV types is a relatively new prophylactic measure that promises better prevention by immunization of young girls and boys before the onset of sexual activity (11). Nevertheless, the currently available vaccines protect against persistent infection by a few HPV types only (6, 11, 16 and 18).

Epidemiological studies regarding the distribution of HPV types in various populations are imperative in order to estimate the protective efficiency of these vaccines in any given population. Interestingly, although there are dozens of reports regarding the prevalence of HPV types in patients with cancer, very few studies so far have assessed their prevalence in the general population or in individuals at risk. In light of the need to collect data on populations at risk, we report here the findings of a viral detection and typing investigation in a cohort of 263 Greeks with a clinical indication of HPV infection.

Patients and Methods

Study group. In this study, 263 Greeks of upper and middle socioeconomic status, who resided in the metropolitan area of Athens, participated after informed consent. They included 250 women aged 18-64 years (median age 32 years) and 13 men aged 27-60 years (median age 43 years). The women were referred because of abnormal Pap test with or without clinical lesions (N=183, 73.2%), clinically observed lesions in the vagina only (N=56, 22.4%), or clinically observed lesions in the oral cavity (N=11, 4.4%). The men had lesions in the oral cavity (N=7, 53.8%) and condylomata on the penis (N=6, 46.2%). Collection of genital and oral specimens was performed by experienced specialists: a gynecologist and an oromaxillofacial surgeon.

Molecular analysis. Total DNA was extracted from genital or oral scrapings using the NucleoSpin™ Tissue DNA isolation kit (Macherey-Nagel, Germany), following the protocol supplied by the manufacturer. Isolated DNA samples were used for a polymerase chain reaction (PCR), using the consensus primers MY09/MY11 located at the L1 open reading frame of several HPV types, as described elsewhere (12). A positive control (HPV type 16 DNA) and a negative control (blank sample) were used in each DNA amplification attempt and the PCR products were visualized in an agarose gel containing ethidium bromide. In case of amplified HPV DNA fragment, viral typing was performed by restriction fragment length polymorphism (RFLP) analysis, after digesting the amplification products, separately, with the restriction enzymes DdeI, HaeIII, HinfI and RsaI, according to the manufacturer's instructions (Takara, Bio Inc. Kyoto, Japan). The digested products were analyzed by 4% Metaphor agarose gel electrophoresis and the HPV types were determined according to previously published restriction patterns (13). The accuracy of HPV typing was also examined by DNA sequencing of several samples using the same primers. All nucleotide sequences were sought in GenBank and each one was considered a match if it was found to have more than 85% nucleotide similarity to a known HPV type sequence, as previously suggested (14).

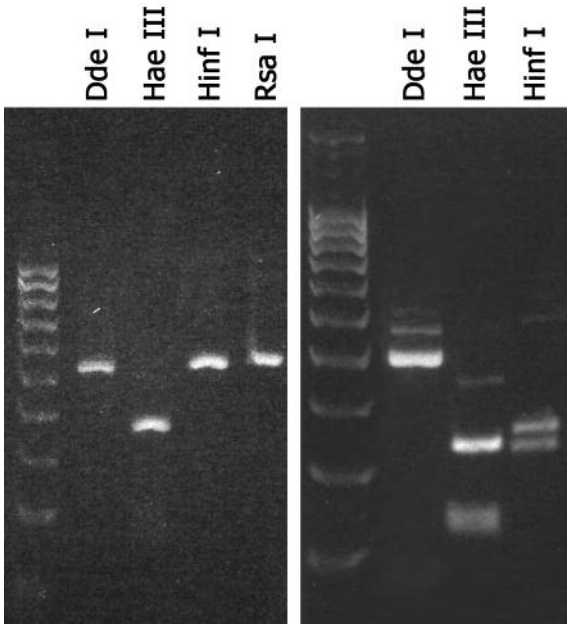

Figure 1. *Electrophoretic patterns of HPV type 59 (left) and 6 (right). A 100 bp ladder was used as molecular weight marker.*

Results

The molecular investigation of DNA samples from 263 individuals revealed that 215 of these were positive for the presence of HPV (81.7%) and 48 were negative (18.3%). The use of three restriction enzymes (DdeI, HaeIII, HinfI) was sufficient for typing of one third of the HPV-positive samples (69/215, 32.1%), while for the remaining samples, additional digestion with RsaI was necessary (Figure 1). DNA sequencing confirmed the HPV typing with a sequence similarity of 93-98% (Figure 2). High-risk types were detected in 42.3% of the HPV-positive individuals (91/215), while intermediate and low-risk types were found in 29.8% and 27.9%, respectively (Table I).

Among the high-risk types, the most frequent were HPV 16, 31 and 33 (14.4%, 6.1%, 5.6% respectively, Table I). The most frequent intermediate-risk types included HPV 53 and 64 (12.6% and 4.7% respectively), while the most common low-risk types were 6 and 11 (16.8% and 6.5% respectively, Table I).

In the 197 genital region lesions that were positive for HPV, the most frequently observed types included the high-risk 16, 31 and 33 (11.7%, 5.6%, and 5.6% of the genital samples, respectively), the intermediate-risk 53 and 64 (12.7% and 5.1%, respectively), as well as the low-risk types 6 and 11 (18.3% and 7.1%, respectively). On the other hand, all 18 examined oral cases were HPV-positive and the detected viral types included high-risk types 16 (N=8, 44.4%), 18 (N=5, 27.8%), 31 (N=2, 11.1%) and intermediate-risk type 53 (N=2, 11.1%).

Interestingly, 85/215 (39.5%) samples were positive for one of the vaccine-related types 6, 11, 16 and 18. Furthermore, 16 and 18 comprised about the same proportion of the high-risk types that were detected in this study (35/91, 38.5%).

Discussion

The rate of HPV detection and type distribution in samples of individuals of Greek origin, referred because of a possible HPV infection, were analyzed in this study. The impressively high percentage of HPV-positive samples (81.7%) was probably due to the fact that the samples were selected and sent for molecular investigation by experienced specialists due to clinical or cytological signs of epithelial dysplasia, which is often an HPV-related lesion. Additionally, the collection of samples was performed by the same clinicians in order to standardize the procedure and reach the highest concordance of findings with possible viral infection.

High-risk types were detected in about 40% of the positive samples followed by about 30% of intermediate- and 30% low-risk types. Most frequent HPV types were high-risk 16, 31 and 33, intermediate-risk 53 and 64, while the most common among low-risk types were 6 and 11. The five most frequent types observed in genital lesions included 6, 53, 16, 11 and 31. These findings, concerning a group of upper and middle class individuals with a spectrum of indications for a possible HPV infection, somewhat differ in prevalence of some types in comparison to earlier reports of Greek patients with highly aggressive and rapidly progressive cancer. According to those studies, HPV 16 was the predominate type (36%) followed by HPV 18 (12%), HPV 33 (6%), 31 (6%) and 51 (4%) (15-17). Finally, mixed HPV infections were previously reported to be at a very low frequency in high-grade SIL and carcinomas; in accordance with this, the present study revealed only single infections (16).

Studies in other populations, using molecular methodology that allows detection of a broad spectrum of HPV genotypes, report a variation in the relative prevalence rates of HPV types, depending on geographic regions. Specifically, in Germany and Sweden, HPV 16 and 31 seem to be the dominant types, followed by types 6, 58, 61, 53, 42 and 51 at lower frequencies (5, 18). The study in Germany suggests a complex distribution of HPV types as well as the detection of uncommon types 34, 53, 66, 73, 82, and 83 in significant rates (18). In Italy, correspondingly, HPV types 16 and 18 predominate and the frequency rate increases with the severity of genital lesions (19). In the United States, high-risk type 16 was found in 47% of high-grade SIL and cancer, while types 18, 45, 56 were found at high rates in invasive carcinomas (9). On the other hand, HPV 31, 33, 35 and 51 were found mostly in high-grade SIL but with lower frequency in cancer (9). In Japan, HPV types 16, 31, 33, 39 51, 52 and 56 were most frequently detected in high-grade SIL in contrast to types 18, 30, 43 and 54 which were identified in low-grade SIL (18).

Compared to the aforementioned studies, our findings differ on the low prevalence rate of HPV 18. This could be attributed to the fact that our specimens were obtained from middle and upper socioeconomic population group, mainly with abnormal Pap tests or low-grade genital abnormalities, compared to the individuals of the studies mentioned above, who had serious clinicopathological findings such as high-grade SIL and invasive cancer (5, 8, 9, 15, 16). In addition, most of the above studies tested for fewer HPV types within the high- and low-risk groups, while the methodology used in the present study is capable of identifing a broader spectrum of HPV types. In accordance with this notion, a German study of 2,916 cytological samples screened for many HPV types reported that the most frequent high-risk types were 16 and 31, which our findings are in agreement with (16).

This study revealed the prevalence of viral types associated with high risk for cancer in almost half of the cases referred because of a possible HPV infection. Therefore, in order to reduce the burden of genital or oral cancer, prevention strategies are needed which should be targeted to both women and men. Women should be encouraged to undergo annual Pap smear screening, and to control cofactors such as cigarette smoking or other viral infections like herpes virus simplex 2 (20, 21). Men should be encouraged to use condoms (22, 23). Furthermore, specialists should make better use of available molecular technology for HPV detection and typing because their cancer predictive value is important for accurate decision-making in treating epithelial lesions. Finally, the availability and wide use of HPV vaccines targeted at common HPV genotypes, based on knowledge of the epidemiological distribution of viral types in different populations, may prove to be highly effective (11, 24). This hopeful notion is underlined by the present study in a Greek population in which the high-risk viral types associated with the currently available vaccine were detected in about 40% of the total samples with HPV high risk-types.

Acknowledgements

The authors thank Georgia Stamati for technical assistance in DNA extraction.

References

1 Bosch FX, Manos MM, Munoz N, Sherman M, Jansen A, Peto J, Schiffman M, Moreno V, Kurman R and Shah K: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. J Natl Cancer Inst *87*: 796-802, 1995.

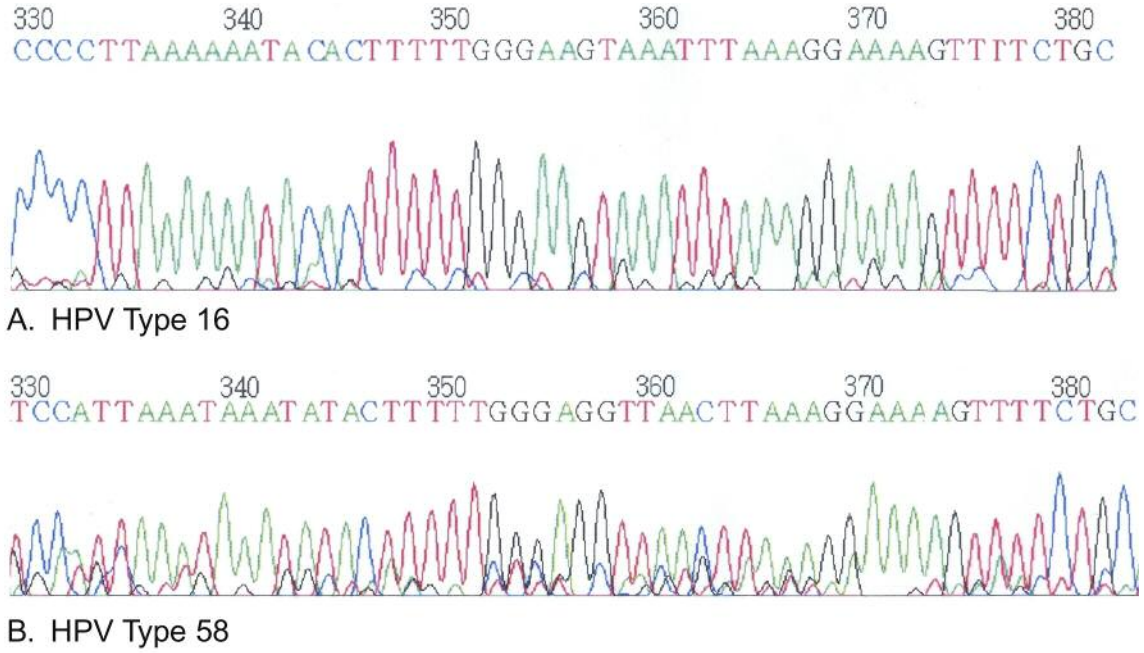

Figure 2. *DNA sequence of HPV types 16 and 58.*

2 Bosch FX, Lorincz A, Munoz N, Meijer CJ and Shah KV: The causal relation between human papillomavirus and cervical cancer. J Clin Pathol *55*: 244-265, 2002.

3 Thompson DL, Douglas JM Jr, Foster M, Hagensee ME, Diguiseppi C, Baron AE, Cameron JE, Spencer TC, Zenilman J, Malotte CK, Bolan G, Kamb ML and Peterman TA: Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. J Infect Dis *190*: 1563-1574, 2004.

4 Moberg M, Gustavsson I and Gyllensten U: Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. Int J Cancer *112*: 854-859, 2004.

5 Ha PK and Califano JA: The role of human papillomavirus in oral carcinogenesis. Crit Rev Oral Biol Med *15*: 188-196, 2004.

6 Scully C: Oral squamous cell carcinoma; from a hypothesis about a virus, to concern about possible sexual transmission. Oral Oncol *38*: 227- 234, 2002.

7 Matsukura T and Sugase M: Identification of genital human papillomaviruses in cervical specimens: segregation of specific virus types in specific clinicopathologic lesions. Int J Cancer *61*: 13-22, 1995.

8 Lorincz O, Red R, Jenson B, Greenberg M, Lancaster W and Kurman R: Human papillomavirus infection of the cervix relative risk associations of 15 common anogenital. Obst and Gynecol *79*: 328-337, 1992.

9 Cox JT, Petry KU, Rylander E and Roy M: Using imiquimed for genital warts in female patients. J Womens Health *13*: 265-271, 2004.

10 Zanotti KM and Belinson J: Update on the diagnosis and treatment of human papillomavirus infection. Cleve Clin Med *69*: 951-955, 2002.

Table I. *Frequency of detected HPV types in 215 positive samples.*

Cancer risk types frequency (%)	HPV types	N	%
High	16	31	14.4
91/215 (42.3%)	18	4	1.8
	31	13	6.1
	33	12	5.6
	35	3	1.4
	39	3	1.4
	51	7	3.3
	52	5	2.3
	56	1	0.4
	58	4	1.8
	59	4	1.8
	66	2	1.0
	68	2	1.0
Intermediate	32	2	1.0
64/215 (29.8%)	53	27	12.6
	54	1	0.4
	55	7	3.3
	61	6	2.8
	63	1	0.4
	64	10	4.7
	67	5	2.3
	69	5	2.3
Low	6	36	16.8
60/215 (27.9%)	11	14	6.5
	34	6	2.8
	44	4	1.8

11 Arbyn M and Dillner J: Review of current knowledge on HPV vaccination: an appendix to the European Guidelines for Quality Assurance in Cervical Cancer Screening. J Clin Virol *38*: 189-197, 2007.
12 Zheng PS, Li SR, Iwasaka T, Song J, Cui MH and Sugimori H: Simultaneous detection by consensus multiplex PCR of high and low-risk and other types of human papilloma virus in clinical samples. Gynecol Oncol *58*: 179-183, 1995.
13 Bernard HU, Chan SY, Manos MM, Ong CK, Villa LL, Delius H, Peyton CL, Bauer HM and Wheeler CM: Identification and assessment of known and novel human papillomavirus by polymerase chain reaction amplification, restriction fragment length polymorphisms nucleotide sequence, and phylogenetic algorithms. J Infect Dis *170*: 1077-1085, 1994.
14 Vernon SD, Unger ER and William S: Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. J Clin Microbiol *38*: 651-655, 2000.
15 Labropoulou V, Diakomanolis E, Dailianas S, Kalpaktsoglou K, Rodolakis A, Beaudenon S, Kakkanas A and Mavromara P: Genital papillomavirus in Greek women with high-grade cervical intraepithelial neoplasia and cervical carcinoma. J Med Virol *48*: 80-87, 1997.
16 Vassilandonopoulou G, Panotopoulou E, Fotiou S, Tserkezoglou A, Machera E and Kottaridis S: Human papillomaviruses in cervical cancer I. HPV-16 and 18 predominate in the Greek population. Anticancer Res *17*: 117-120, 1997.
17 Prokopakis P, Sourvinos G, Koumantaki E and Spandidos DA: K-*ras* mutations and HPV infection in cervicitis and intraepithelial neoplasias of the cervix. Oncol Rep *9*: 129-133, 2002.
18 Speich N, Schmit C, Bollmann R and Bollmann M: Human papillomavirus (HPV) study of 2916 cytological samples with PCR and DNA sequencing: genotype spectrum of patients from the west German area. J Med Microbiol *53*: 125-128, 2004.
19 Venuti A, Badaracco G,. Sedati A, Carbini R and Marcante ML: Determinants of human papillomavirus types 16 and 18 infections in the lower female genital tract in an Italian population. Eur J Gynaecol Oncol *15*: 205-210, 1994.
20 Daling JR, Madeline M, McKnight B, Carter JJ, Wipf GC, Ashley R, Schwartz SM, Beckmann AM, Hagensee ME, Mandelson MT and Galloway DA: The relationship of papilloma-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiol Biomarkers Prev *5*: 541-548, 1996.
21 Ho G, Kadish A, Burk R, Basu J, Palan PR, MiKhail M and Romney SL: HPV 16 and cigarette smoking as a risk factor for high grade cervical-intraepithelial neoplasia. Int J Cancer *78*: 281-285, 1998.
22 McPartland TS, Weaver BA, Lee SK and Koutsky LA: Men's perceptions and knowledge of human papillomavirus (HPV) infection and cervical cancer. J Am Coll Health *53*: 225-230, 2005.
23 Hogewoning CJ, Bleeker MC, van den Brule AJ, Voorhorst FJ, Snijders PJ, Berkhof J, Westenend PJ and Meijer CJ: Condom use promotes regression of cervical intraepithelial neoplasia and clearance of papillomavirus: a randomized clinical trial. Int J Cancer *107*: 811-816, 2003.
24 Harro C, Pang Y, Roden R, Hildesheim A, Wang Z, Reynolds MJ, Mast TC, Robinson R, Murphy BR, Karron RA, Dillner J, Schiller JT and Lowy DR: Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like vaccine. J Natl Cancer Inst *93*: 284-292, 2001.

Received October 16, 2008
Revised May 21, 2008
Accepted May 26, 2008