The incidence of HPV infection in anal cancer patients in Greece

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Abstract

Background and aim of the study : **Although anal cancer represents a relatively uncommon malignancy, its incidence over the last five decades, has been reported as increased for both sexes, worldwide. Human papillomavirus (HPV) infection has been shown to be a major cause for its development. The aim of the present study is to report on clinical, epidemiological and virological data of squamous anal cancer in Greek patients.**

Patients and Method : **Between January 2002 and December 2010, 11 Greek patients (6 females) who were diagnosed as suffering from squamous cell anal or perianal cancer, were treated in our Hospital. Formalin fixed paraffin embedded tissue samples, obtained at the time of the anal biopsy or surgery, were analyzed by PCR in order to identify the presence as well as the type of HPV infection.**

Results : **Overall, the presence of HPV DNA was detected in 6 out of the 11 patients (54.5%). The "highrisk" HPV DNA was detected in 3 of them (2 women and 1 man), while the "lowrisk" HPV DNA was detected in the remaining three (2 women and 1 man).**

Conclusion : **The incidence of HPV infection in squamous cell anal cancer Greek patients, is lower than other Western countries, probably reflecting differences in sexual habits in the Greek population.** (Acta gastroenterol. belg., **2014**, 77, **213-216**).

Key words : HPV, PCR, anal cancer, Greece.

Background and aim of the study

Anal and perianal squamous cell carcinoma is a relatively uncommon malignancy, accounting for about 99.000 newly diagnosed cases per year worldwide in 2002 (1). Although, it is generally accepted that there is a slight female predominance (60% women versus 40% men), this female to male ratio varies between countries, ranging from 0.7 in Japan to 2.2 in Denmark (2). Despite the rarity of the disease, an increasing incidence has been reported for both men and women in the general population of the United States, Europe and South America over the last five decades (1-3). For example, data from the National Cancer Institute have revealed an incidence of 1.6 new cases per year per 100.000 population, during the 2002-2006 period (4). Although the peak incidence of the disease is during the sixth decade of life, this incidence has markedly increased in younger males also, over the last thirty years (5).

The aim of the present study is to report on clinical, epidemiological and virological data of squamous cell anal cancer in Greek patients.

Patients and method

Patients

Between January 2002 and December 2010, 11 Greek patients (6 females, 5 males) with anal or perianal cancer, histologically characterized as squamous cell carcinomas (SCCs), were treated in our hospital. Patients' data including gender, age, smoking and alcohol habits, tumor localization (anal canal, perianal region) and histology, were available in all cases (Table 1). Six out of the 11 patients who received treatment for squamous cell anal cancer in our Hospital, were submitted only to chemoradiotherapy. The remaining 5 patients underwent salvage abdominoperineal resection since they experienced local recurrence of their disease after initial chemoradiotherapy.

Histopathology

Eleven formalin fixed paraffin embedded tissue samples obtained at the time of the anal biopsy or surgery were retrieved from the tissue archive of the pathology department. The samples were fixed in buffered formalin for no longer than 24 h. Four or five tissue sections of 5 μm were cut from each FFPE sample and stored in a 1.5 ml sterile tube. The microtome blade was changed after each use and the working areas were cleared thoroughly with a 10% diluted disinfectant. DNA was extracted from FFPE samples using a CLART Human Papillomavirus 2 kit (AT110424MT, Genomica) and the respective protocol for nucleic acid purification, according to the manufacturer's instructions was followed. HPV genotyping was performed by PCR amplification of a 450 bp fragment within a highly conserved L1 region of the virus. This highly conserved sequence presents slight variations among each individual HPV type that allows the genomic identification of each type through the recognition of the vital DNA by specific probes. This method of detection of the virus type through the identification of viral DNA variations carries an excellent

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Table 1. — **Profiles of the patients with anal and perianal squamous cell carcinomas**

Patient	Gender	Age	S/A	Sample Type	Localization	Histological type	HPV genotypes	Type of HPV infection
1/91751	F	89	N/N	FFPE	PERIANAL	SCC	61,73	HR
2/27197	M	67	Y/Y	FFPE	ANAL	SCC	6,51	HR
3/40708	F	53	Y/N	FFPE	ANAL	SCC	16	HR
4/77235	F	86	N/N	FFPE	ANAL	SCC	61	LR
5/23998	M	52	Y/Y	FFPE	PERIANAL	SCC	6	LR
6/42176	F	63	Y/N	FFPE	ANAL	SCC	6,44	LR
7/11106	M	31	Y/Y	FFPE	ANAL	SCC	NEGATIVE	
8/31279	M	52	Y/Y	FFPE	ANAL	SCC	NEGATIVE	
9/124125	F	57	Y/N	FFPE	ANAL	SCC	NEGATIVE	
10/31682	M	46	N/N	FFPE	ANAL	SCC	NEGATIVE	
11/32419/33787	F	73	N/Y	FFPE	ANAL	SCC	NEGATIVE	

S : Smokers.
A : Alcohol consumers, more than a unit a day.
N : No.
Y : Yes.

accuracy record with its specificity being as high as 100%, and its sensitivity respectively 99%.

The kit used for HPV genotyping (AT1204, CLART HPV 2, Genomica) detects the most clinically relevant HPV types, which are classified according to their malignant potential as follows (6) :

– Low oncogenic risk type HPVs (LR) : 6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84 and 89
– High oncogenic risk type HPVs (HR) : 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 and 85.

In cases of multiple HPV species detection, if at least one HR genotype was present, the sample was considered as HR, while if only LR genotypes were present, the sample was considered as LR.

Results

The presence of HPV DNA was detected in 6 out of the 11 patients (54.6%). The mean age of the patients was 60.8 years. Five out of the 11 of patients were consuming more than one unit of alcohol per day and 4 of them were non-smokers.

Among the six HPV positive patients, three of them had a high risk genotype HPV infection and the remaining three had infection only with low risk genotype of HPV.

Among the high risk genotype HPV infection patients (n = 3), two were women, two had double HPV infection (with one high and one low risk genotype of HPV), while the high risk HPV16 type was detected in only one patient (9.1%).

Among the low risk type HPV infection patients (n = 3), two were women and only one had double HPV infection, with two low risk HPV genotypes (Table 1).

None of the patient was HIV positive. Five out of the six women with anal cancer were tested for cervical HPV infection and only one was found positive for low risk HPV type infection. The distribution of HPV types did not differ between anal or perianal tumors.

As a result, overall three patients had infection with HR types (27.3%), three of them were infected with LR types (27.3%) and five of them (45.4%) were negative for any HPV infection.

Discussion/Conclusion

Squamous cell anal cancer is now considered as a sexually transmitted disease, since human papillomavirus (HPV) infection has been shown to be strongly involved in the development of this entity. The prevalence of perineal HPV infection among men ranges widely worldwide, varying from 0% to 73% in the general population (7), and it has been increased nearly 3 fold over the last 30 years (8). The prevalence of anal HPV infection among heterosexual men has been reported as high as 24.8%, while in one third of these infections oncogenic HPV types are involved (9). On the other hand, the prevalence of anal HPV infection among asymptomatic women is estimated between 2% and 44% (10).

About 130 different HPV genotypes have been identified up today (11). Forty of them have been isolated from infections of the lower genital tract, while 15 of them (because they are involved in the development of squamous cell cancers), have been characterized as oncogenic (12). HPV genotypes are usually classified as high or lowrisk, depending on their oncogenic potential (10). ‘‘Lowrisk’’ types (genotypes 6, 11, 40, 42, 43, 44) are primarily associated with genital warts and respiratory papillomatosis, while ‘‘highrisk’’ types (genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52) are associated with

lowgrade and highgrade squamous intraepithelial lesions and invasive cancer (13). Especially types 16 and 18 are considered as "high risk" since they are isolated from the majority of cervical and anal cancer specimens (14).

The presence of HPV DNA in squamous cell anal cancers is estimated to be as high as 90% (15) and numerous reports have linked anal and cervical cancer in women with HPV infection. Specific highly oncogenic HPV types, have been detected in more than 99% of invasive cervical cancer specimens, using sensitive polymerase chain reaction (PCR) assays (16).

Apart from the HPV infection, immunosuppression, HIV infection, history of receptive anal intercourse, history of cervical, vulvar, or vaginal cancers, or even smoking have been recognized as potential risk factors for the development of anal cancer (17,18,19).

Some tobacco components seem to induce damages in the anal epithelium, thus increase the risk for anal cancer development in smokers (20). In our study 7 out of the 11 (63.6%) patients were smokers at the time of diagnosis. The respective frequency of smoking was 60% for the HPV negative patients, while 67.7% of the HPV positive patients had a history of smoking.

Promiscuous sexual life increases the risk for both HPV and HIV infection. Nearly 40% of homosexual men carry one or more high-risk oncogenic HPV genotypes (21), which further increases the likelihood for acquiring HIV infection (22). It is believed that HPV infection causing disruption of the epithelium integrity and the mucosal immune system, predisposing to HIV infection (23).

In a crossectional cohort study Darwich *et al*. (24), examined the prevalence and distribution of various HPV genotypes detected in samples from anal canals of HIV positive homosexual and heterosexual men. They concluded that specimens with cytologically detected abnormalities were positive for HPV infection, in 92% of the homosexual group and in 65% of the heterosexuals. All high grade squamous intraepithelial lesions were positive for HR-HPV in both groups, in the homosexual group there was a greater diversity of the genotypes and HPV-16 was the most prevalent HR genotype in both groups, although other genotypes were also expressed. The hypothesis behind that was that under the same immune conditions, HPV-16 is innately better than other HPV types to avoid immunosurveilance. However, in immunosupressed population all HPV types can predispose to anal cancer development. The authors concluded that anal cancer screening should be offered to all HIV-infected men, regardless their sexual orientation. Since HIV-positive individuals treated with new therapeutic agents, have longer life expectancy, HR-HPV infections can predispose to anal cancer development (25).

All of our patients have been self-identified as straight heterosexual, and only one of the women reported a history of anal intercourse. Data about the number of sexual partners were not available for this study. Also none of the women in this study-population had any history of cervical cancer, while 5 out of 6 were regularly examined for genital HPV infection and only one of them was found positive for a low risk HPV species.

Finally patients under immunosuppressive therapy carry a higher risk of developing squamous cell anal cancer (19). Only one patient in this report was under immunosuppression, since he was a renal transplant recipient.

In the present study, HPV infection was detected in 6 out of 11 squamous cell anal cancer patients. Among the six HPV infected anal cancer patients, the "highrisk" HPV DNA was detected in 3 of them (2 women and 1 man), while the "lowrisk" HPV DNA was detected in the remaining three (2 women and 1 man). The above results suggest that the prevalence of HPV infection in Greek squamous cell anal cancer patients is lower than the reported in other Western countries (17,26). In agreement to the previous observation, data deriving from Greece examining the prevalence of HPV infection among general female population as well as among cervical cancer female patients, suggest a lower prevalence of HPV infection in Greek female population compared to other Western countries (27). A cumulative consideration of these data suggests an overall lower prevalence of HPV infection in Greece. This phenomenon might be very well random, but on the other hand it might also imply that Greek population has "safer" sexual habits, a fact which prohibits the wider spread of sexually transmitted diseases.

In conclusion, it seems that HPV infection in Greek squamous cell anal cancer patients is lower than the respective incidence in other Western countries. This might be related to differences in sexual habits. On the other hand, other risk factors such as smoking and immunosuppression were also present in our small group of patients. Our virological data support relevant data from other countries which mandate the development of refined screening programs for the detection of HPV infection, and for strict surveillance strategies for HPV positive patients, leading to an adequate treatment of potentially premalignant lesions.

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