Distribution of HPV genotypes in Slovenian patients with anal carcinoma: preliminary results

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KEY WORDS

anal carcinoma, HPV, prevalence

ABSTRACT

The aim of the present study was to obtain first data on the distribution of human papillomavirus (HPV) genotypes in patients with anal cancer (AC) in Slovenia. A total of 21 samples of AC (16 archival FFPE samples and 5 fresh-frozen tissue samples) collected from the same number of patients were analysed. All samples were tested for the presence of HPV DNA using a consensus GP5+/GP6+ PCR and HPV genotypes determined by the INNO LiPA HPV Genotyping Extra test, capable of recognizing 28 different alfa-HPV genotypes. All 21 AC samples were HPV DNA positive. The most frequent HPV genotype, found in 19/21 AC samples, was HPV-16. Only low-risk HPV-6 was detected in one sample and infection with high-risk HPV-52 and low-risk HPV-61 was identified in one sample. Prophylactic HPV vaccination with currently available vaccines could potentially prevent the great majority of anal cancers in Slovenia.

Introduction

Although the association of high-risk HPV infection with cervical intraepithelial neoplasia and cervical carcinoma is well known, it is now evident that several other anogenital squamous cell carcinomas and their precursors are also attributable to HPV (1). The detection rates of HPV in anal cancers in published studies range from 70 to 100% (2). As with cervical carcinoma, HPV-16 and HPV-18 are the most common genotypes associated with anal dysplasia and anal cancer (1). Infection with multiple HPV genotypes is common in anal cancer and anal intraepithelial neoplasias grade 3 (3). Additional risk factors for the development of anal cancer are HIV infection, a history of anogenital warts and tobacco smoking (4, 5).

The purpose of the present study was to obtain first data on the distribution of HPV genotypes in patients with anal cancer (AC) in Slovenia.

Materials and methods

Sixteen formalin-fixed paraffin-embedded (FFPE) tissue samples and 5 fresh-frozen tissue samples obtained at the time of anal biopsy or surgery were collected from 21 Slovenian patients (10 female, 11 male) with anal cancer. Seventeen samples were histologi-

cally characterised as squamous cell carcinoma (SCC) and 4 samples as carcinoma *in situ*. Three to five tissue sections (10um thick) were cut from each FFPE sample. The microtome blade was changed after each use. DNA extraction was done within 1 hour after FFPE sample cutting. Fresh-frozen tissue samples were kept frozen at -80°C until analysis.

DNA was extracted from fresh-frozen tissue and FFPE samples using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) and the protocol for nucleic acid purification from mammalian tissue, following the manufacturer's instructions. The quality of extracted DNA was verified in all specimens by real-time PCR amplification of a 268-bp fragment of human beta-globin gene, as described previously (6).

All specimens were tested for the presence of HPV with consensus GP5+/GP6+ PCR targeting approximately 150 bp fragments of the L1 HPV gene, as described previously (7). HPV genotypes were determined using the commercially available assay INNO LiPA HPV Genotyping Extra (Innogenetics, Gent, Belgium), capable of recognizing 28 different alfa-HPV genotypes. The INNOLiPA HPV Genotyping Extra test based on PCR using SPF10 primers amplifies a very short fragment in the L1 HPV gene, so it is appropriate for HPV genotyping in FFPE samples in which the quality of DNA is poor.

Results

The 268-bp fragment of human beta-globin gene was successfully amplified from all 21 AC samples, indicating that the DNA was adequate for further analysis and that DNA isolates contained no apparent PCR inhibitors. The presence of HPV DNA was detected in all 21 AC samples (Table 1). Infection with a single HPV genotype was found in 20/21 of tested AC samples. Among 20 samples with a single HPV infection, HPV-16 was found in 19/21 AC samples and only low-risk HPV-6 was detected in one AC sample. Infection with both high-risk HPV-52 and low-risk HPV-61 was detected in one sample (Table 1).

Discussion

In order to obtain first data concerning HPV prevalence and genotype distribution in patients with anal cancer in Slovenia, 21 AC samples collected from the same number of patients were tested for the presence of HPV DNA. High-risk HPV genotypes were detected in 20 of 21 AC tissue samples, which is in agreement with results of similar recent studies (1, 8). In a 33-year old female patient with anal squamous cell carcinoma, only low-risk HPV-6 was identified. Histological examination of this case showed well differentiated squamous cell carcinoma with no evidence of previous anogenital warts. Although HPV-6 is associated in the majority of cases with anogenital warts and low grade anal intraepithelial neoplasia, the progression of HPV-6 induced condylomatous lesions to dysplasia or invasive anal carcinoma has been well documented (9).

In conclusion, although anal cancer is a relatively rare disease, its incidence is increasing, mainly in the population of men who have sex with men (5, 10, 11). Since the great majority of anal cancers in Slovenia are etiologically linked with HPV-16, it seems that prophylactic HPV vaccination with currently available HPV vaccines could potentially prevent the great majority of anal cancers in this country.

Table 1. HPV genotype distribution in anal cancer.

Patient	Gender	Sample type	Localization	Histological type	HPV genotype
1	M	fresh	perianal	Ca in situ	16
2	F	fresh	perianal	Ca in situ	16
3	F	FFPE	perianal	SCC	16
4	M	FFPE	anal	SCC	16
5	M	FFPE	anal	SCC	16
6	F	FFPE	anal	SCC	16
7	F	FFPE	anal	SCC	16
8	M	FFPE	anal	SCC	16
9	F	FFPE	anal	SCC	16
10	F	FFPE	anal	SCC	16
11	F	FFPE	anal	SCC	16
12	M	FFPE	anal	SCC	16
13	F	FFPE	anal	SCC	6
14	F	FFPE	anal	SCC	16
15	M	FFPE	anal	SCC	16
16	M	FFPE	anal	SCC	16
17	M	FFPE	anal	SCC	16
18	F	FFPE	anal	SCC	16
19	M	fresh	perianal	Ca in situ	16
20	M	fresh	perianal	SCC	16
21	M	fresh	perianal	Ca in situ	52, 61

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