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Squamous anal cancer: Patient characteristics and HPV type distribution

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ABSTRACT

Background: Infection with high risk human papillomavirus (HPV) is strongly associated with anal cancer. However, detailed studies on HPV type distribution by gender and age are limited.
Methods: Retrospective study of 606 invasive anal cancers diagnosed between 1990 and 2005 in two large urban areas of the province of Québec, Canada. Cases were identified from hospitalization registry. Patient characteristics were collected from medical files. Archived anal squamous cancer specimens were available from 96 patients and were tested for HPV DNA and typing. Variant analysis was performed on 16 consecutive and 24 non-consecutive HPV16-positive samples to assess potential contamination during amplification.
Results: Among the 606 patients with anal cancers, 366 (60%) were women. Median age at diagnosis was 63 years. HPV was detected in 88/96 (92%) of cases. HPV16 was the most frequent type detected in 90% of HPV-positive specimens. Other types including 6, 11, 18, 33, 52, 53, 56, 58, 62 and 82 were also found. HPV 97 was not detected. HPV prevalence was associated with female gender and younger age. No contamination occurred during amplification as shown by the subset of 41 HPV16-positive samples, as 37, 2 and 1 isolates were from the European, African and Asian lineages, respectively. The most frequent variants were G1 ($n = 22$) and the prototype ($n = 12$).
Conclusions: Women with anal cancer are at higher risk for anal HPV infection, and HPV infection, especially HPV16, is strongly associated with squamous anal cancer. Therefore, HPV vaccine could potentially prevent the occurrence of anal cancer in both men and women.

1. Introduction

Anal cancer is a relatively rare disease compared to cervical cancer. Its annual incidence is <2/100,000 persons in the general population worldwide [1], but has been reported to increase in both men and women in some countries [2–6]. In the province of Québec, Canada, the incidence of anal squamous carcinomas increased significantly among men and women over the last two decades [7]. The annual age-standardized incidence increased from 0.4/100,000 in 1984 to 0.7/100,000 in 2007 among men and from 0.3 to 1.0/100,000 among women [7]. Anal cancer is more common in high-risk populations, such as HIV-seropositive individuals, men who have sex with men (MSM) [8–10], women with cervical or vulvar cancer or precancer [11] and immunocompromised individuals [12]. In North America, the unadjusted annual anal cancer incidence rates were 131/100,000 in HIV-seropositive MSM and 46/100,000 in heterosexual HIV-seropositive men, corresponding to a relative incidence ratio of 80.3 (95% CI: 42.7–151.1) and 26.7 (95% CI: 11.5–61.7), respectively, compared with HIV-seronegative men [13].

Anal cancer shares clinical and histological similarities with cervical cancer [14]. Both have been causally linked to high-risk human papillomaviruses (HPV) infection, particularly to HPV 16 [15,16]. HPV infection have been detected in >70% of invasive anal cancers [17,18] with substantial variation according to histological type, PCR primers used, study year, case source (hospital-based vs.

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population-based studies) and geographical area. A retrospective multicenter study in France indicated a 97% HPV prevalence in anal cancer and HPV 16 was the most prevalent genotype (75%) [19].

Considering that few studies have investigated the rate of HPV infection in anal cancer and that HPV prevalence and genotyping in anal cancer has not been documented in Canada, we undertook this study to characterize and estimate the prevalence of type-specific HPV involved in anal cancer in men and women in the province of Québec.

2. Materials and methods

A total of 33 eligible hospitals (with a least 5 new anal cancer cases reported during the study period and located within a 100 km radius of one of the two most densely populated urban areas; Montréal or Québec city) were selected from the Québec hospital discharge database (*Med-Echo*). Cases were identified from the register of hospitalization in each participant hospital using the International Classification of Diseases, 9th revision, ICD-9 (ICD-9 1542, 1543 and 1548), including squamous and adenocarcinomas morphologies. A total of 606 invasive anal cancer cases admitted between 1990 and 2005 were thus identified from the participating institutions. Demographic and clinical characteristics, including age at diagnosis, gender, marital status, HIV status, smoking habits, tumor grade and treatment modality were obtained from the medical file of each reported case by one of three of the investigators (ED, CR, NO). Presence and type of symptoms was tabulated if noted in the medical file. Genital warts were defined as any history of warts as noted in the charts.

The study protocol was approved by the Research Ethics committees of *Université de Montréal* and McGill University and permission to access hospital medical records was obtained from the Directors of Professional Services of each participating institutions.

2.1. Specimen selection

Anal biopsies samples were available for HPV genotyping for 96 (16%) individuals with squamous carcinoma. These samples were made available from the pathology archives of one of the participating hospital center and were diagnosed from 1996–2005. Older samples were not available. Adenocarcinomas were excluded for a lack of agreement between researchers about the definition of anal adenocarcinomas as well as the lack of clinical information on the exact location of the tumor in the anorectal area. Histological revision of specimens was performed by a certified pathologist. Each patient provided written informed consent.

2.2. HPV DNA detection and genotyping

Archived biopsies (up to 10 years) embedded in paraffin were minced with a scalpel. Slices were stored in xylene for one day and were crushed in 200 μl of Tris–EDTA buffer pH 7.4 with tween 20 at 0.8% (v/v). HPV DNA was extracted with xylene treatment followed by nucleic acid extraction, as described previously [20]. Five hundred nanograms of DNA were tested by PCR with co-amplification of HPV and β-globin DNA sequences using PGMY primers in the Linear Array HPV genotyping assay (LA-HPV, Roche Molecular Systems) that detects 36 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89) [21]. Co-amplification of a β-globin DNA sequence permitted determination of whether the specimens had adequate cellularity. Samples reactive in the Linear Array with the cross-reactive probe for HPV52 were further tested with a HPV52-specific real-time PCR assay [22]. Controls for contamination were included in each amplification run.

Samples that tested negative for HPV DNA with LA-HPV were further tested with AmpliTaq gold and GP5+/6+ L1 consensus primers [23,24]. HPV amplicons of 145 bp were purified with the QIAquick gel extraction kit protocol (Quiagen Inc., Mississauga, Ontario) and sequenced with direct double-standard PCR-sequencing using a fluorescent cycle-sequencing method (BigDye terminator ready reaction kit, Perkin-Elmer Waltham, Massachusetts) on an ABI Prism 3100 Genetic Analyzer system. HPV sequences were compared with sequences of known types and novel putative types. Isolates sharing >90% homology in the partial nucleotide L1 sequence were considered to belong to the same type. Samples that were both β-globin and HPV-negative were considered inadequate. HPV97 is a recently characterized genotype related genetically to genotypes 18 and 45 and classified in the high-risk alpha 7 species. All samples were tested for the presence of HPV97 DNA with PCR as previously published [25]. Negative, weakly positive (10 HPV97 DNA copies), and strongly positive controls (1,000,000 HPV97 copies) were included in each run.

All samples were also tested for the presence of cutaneous HPV types. Five microliters of processed sample was amplified with 5 U of AmpliTaq Gold DNA polymerase (Roche Diagnostic Systems, Mississauga, Ontario), with primer pairs FAP59/64 (detects Epidermodysplasia-related cutaneous HPV genotypes), HVP2/B5 (detects all HPV genus except types in the Gamma-papillomavirus genus) and a nested PCR with CP62/CP69 and CP65/CP68 primer pairs (detects all cutaneous HPV genotypes in the Beta and Gamma-papillomavirus genus), under conditions described elsewhere [26]. For each PCR run, amplification buffer and human fibroblast DNA negative controls were included. HPV5 or HPV8 plasmids served as positive controls. HPV amplicons were purified and submitted to direct double-stranded PCR-sequencing (see above).

2.3. HPV16 variant analysis

Biopsies positive for HPV16 by PCR and that had been consecutively tested in PCR runs ($n = 16$) were further tested by HPV16 molecular variant analysis to investigate if consecutive HPV16-positive samples contained the same HPV16 variant. A similar number ($n = 24$) of non-consecutive HPV-16-positive biopsies were randomly tested for HPV16 variant analysis. A PCR-sequencing method with primers flanking a segment of the LCR of HPV was used as described previously [27]. Contamination would be suspected if the proportion of the same variant was significantly higher among the consecutive than the non-consecutive HPV-16-positive biopsies.

2.4. Statistical analysis

Demographic and clinical characteristic of all anal cancer patients were compared according to gender using Mantel–Haenszel chi-square test or Fisher exact test when appropriate. Distributions of age were compared with Wilcoxon rank sum non-parametric test. Overall HPV prevalence was estimated as percentage of all cases tested for HPV DNA. Type-specific prevalence was estimated as percentage of all cases tested and as percentage of only HPV-positive cases. Statistical analyses were performed using SAS software, version 9.2 (SAS institute). A two-sided p-value of <0.05 was considered as statistical significant.

3. Results

3.1. Baseline characteristics

The study population consisted of 606 anal cancer patients. Majority of cases ($n = 366$, 60%) were women (Table 1). Median age at diagnosis was 63 years for both men and women. From patients