Diversity of human papillomavirus in the anal canal of men: the HIM Study

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Abstract

Human papillomavirus (HPV) infections are associated with the development of anogenital lesions in men. There are no reports describing the distribution of non-α HPV types in the anal canal of a sexually diverse group of men. The HPV Infection in Men (HIM) Study is a multicentre study on the natural history of HPV infection in Brazil, Mexico, and the USA. At baseline, 12% of anal canal PCR HPV-positive specimens were not typed by the Roche Linear Array, and were considered to be unclassified. Our goals were to characterize HPVs among these unclassified specimens at baseline, and to assess associations with participant socio-demographic and behavioural characteristics. Unclassified HPVs were typed by sequencing of amplified PGMY09/11 products or cloning of PGMY/GP + nested amplicons followed by sequencing. Further analysis was conducted with FAP primers. Of men with unclassified HPV in the anal canal, most (89.1%) were men who have sex with women. Readable sequences were produced for 62.8% of unclassified specimens, of which 75.2% were characterized HPV types. Eighteen, 26 and three different α-HPV, β-HPV and γ-HPV types were detected, respectively. α-HPVs were more commonly detected among young men (18–30 years) than among older men (45–70 years), whereas β-HPVs were more frequent among mid-adult men (31–44 years). β-HPVs were more common among heterosexual men (85.0%) than among non-heterosexual men. All β-HPVs detected among non-heterosexual men were β2-HPV types. The high prevalence of β-HPV in the anal canal of men who do not report receptive anal sex is suggestive of other forms of transmission that do not involve penile–anal intercourse.

Keywords: Anal canal, cutaneous HPV, HIM Study, human papillomavirus, males, prevalence, unclassified types
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Introduction

Although anal canal cancer is an uncommon disease, the incidence is increasing in developed countries [1]. Human papillomavirus (HPV) causes most cases of anal cancers (80–90%), and HPV-16 is the most prevalent type in these tumours (~80%) [2]. Among men, the incidence of HPV-associated anal cancer is highest in men who have sex with men (MSM) and in human immunodeficiency virus (HIV)-infected males [3].

The HPV genome consists of a circular double-stranded DNA molecule of ~8000 bp. HPV types differ by ≥10% in the complete *L1* gene sequence [4]. To date, 199 different HPV types have been fully sequenced, and nearly all cluster into three genera: α-HPV, β-HPV, and γ-HPV. Whereas α-HPVs have been mainly isolated

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from mucosal and genital lesions, and are thus categorized as mucosal types, β-HPVs and γ-HPVs have been mostly isolated from the skin, and have been grouped together as cutaneous HPV [4]. The β-HPV and γ-HPV genera have more genotypes than the α-HPV genus, and partial DNA sequence information points to the existence of hundreds of putative novel HPV types of both viral genera [5,6]. Recent data indicate a high prevalence of cutaneous HPV at diverse anatomical sites that are different from those from which they were isolated [7–11].

The HPV Infection in Men (HIM) Study is an ongoing, prospective anogenital HPV natural history study of >4000 men aged 18–70 years residing in Brazil, Mexico, and the USA. The baseline anal canal genotype-specific HPV prevalence in this population was 16.3%; another 12.4% of specimens were positive for HPV DNA, but could not be classified as any of the 37 genotypes identified by the Roche Linear Array, and were grouped as unclassified HPV [12]. Our aims were to characterize HPV types among anal canal unclassified HPV specimens collected at baseline, and to evaluate associated socio-demographic and behavioural risk factors.

Materials and methods

Clinical samples and study design

Men were enrolled in Brazil (São Paulo), Mexico (Cuernavaca) and the USA (Tampa) between 2005 and 2009, reported no prior diagnosis of anogenital warts or cancers, and had no recent symptoms of or treatment for a sexually transmitted infection, including HIV/AIDS. Men completed a pre-enrolment (baseline) visit, and were enrolled on completion of their second (enrolment) visit 2 weeks post-baseline; they were then followed up every 6 months for up to 4 years. Details of the HIM Study are described elsewhere [12,13]. This cross-sectional analysis included the 3524 men who completed their baseline visit between September 2005 and June 2009 and consented to collection of anal canal exfoliated cells. The ethics committees of participating hospitals and institutions approved all study procedures, and participants provided written informed consent to the study protocol.

At baseline, men completed an 88-item computer-assisted self-interview covering information about demographic characteristics, substance use, and sexual behaviours. Specimens were obtained from the genital area with Dacron swabs (Digene, Gaithersburg, MD, USA). With a separate swab, exfoliated cells from between the anal verge and the dentate line were collected. All staff collecting anal canal samples were trained to avoid touching the perianal skin with the swab. All samples were placed in standard transport medium and stored at −80°C until HPV testing.

HPV detection

DNA extraction was conducted with the QIAamp Media MDx Kit (Qiagen, Valencia, CA, USA). Samples were tested for PCR amplification with PGMY09/11 primers, and HPV genotyping was conducted with the Roche Linear Array (Roche Molecular Diagnostics, Alameda, CA, USA), which is able to discriminate 37 α-HPV types (oncogenic types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66; non-oncogenic types 6, 11, 26, 40, 42, 53, 54, 55,61, 62, 64, 67–73, 81–84, IS39, and CP6108) [14]. Samples that tested PCR-positive and Linear Array-negative with all specific HPV probes were considered to be unclassified and included in the present study.

Unclassified HPV characterization

Purified HPV DNA was initially genotyped by direct sequencing of PGMY09/11 PCR amplimers or cloning of these fragments followed by sequencing. Next, 1 μL of PGMY09/11-negative products was used in a nested PCR with GP5+/6+ primers [15]; positive samples were sequenced following cloning. Finally, nested PCR-negative samples were subjected to a novel amplification reaction employing FAP59/64 primers [16], and positive samples were analysed exclusively by direct sequencing. AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA, USA) was used in all PCRs. Purification of the amplimers with the EXO SAP-IT (GE Healthcare, Little Chalfont, UK) was performed before sequencing. Sequencing was conducted in an ABI 3130XL Genetic Analyzer (AB Applied Biosystems, Carlsbad, CA, USA) with the BigDye Terminator v3.1 Cycle Sequencing kit (AB Applied Biosystems). Sequence identity was determined through comparison with the BlastN database.

Statistical analysis

Men were categorized as men who have sex with women (MSW), MSM, men who have sex with both men and women, and men who denied having any sex, based on their self-reported recent (previous 3–6 months) and lifetime penetrative sexual behaviour [17]. Specimens were classified in one of five categories: HPV-negative, HPV-positive for a characterized type, HPV-positive for an uncharacterized type, untyped (i.e. inconclusive), or inadequate (human β-globin PCR-negative). Characterized HPV types were additionally classified according to genus and species. Differences in socio-demographic and behavioural risk factors considered to be associated with the presence of a specific HPV genus (α, β, or γ) and species (β1 or β2) were evaluated with Fisher's exact test. All statistical tests were two-sided, and attained statistical significance at $\alpha = 0.05$. Analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA).

