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Alpha, beta and gamma Human Papillomaviruses in the anal canal of HIV-infected and uninfected men who have sex with men

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Summary *Objectives:* Anal infection by cutaneous Human Papillomaviruses (HPV) has been rarely investigated. We aimed to assess the prevalence, genotype diversity, and determinants of mucosal (alpha) and cutaneous (beta and gamma) anal HPV infection in men who have sex with men (MSM).
Methods: Anal samples were collected with a Dacron swab. Alpha HPVs were detected using the Linear Array HPV genotyping test, while beta and gamma HPVs using a PCR combined with Luminex technology.
Results: A total of 609 MSM (437 HIV-uninfected and 172 HIV-infected, most of which were undergoing cART) were enrolled. Alpha, beta, and gamma HPVs were detected in 78.0%, 27.6% and 29.3% of the participants. Only alpha HPV prevalence was significantly higher among HIV-infected compared to uninfected MSM (93.0% vs. 72.1%, $p < 0.0001$). Beta2 and gamma10 represented the most frequent cutaneous HPV species, with no significant differences between HIV-infected

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and uninfected individuals. The most common alpha, beta, and gamma genotypes were HPV16, HPV111, HPV121, respectively. Alpha HPV infection was significantly associated with lifetime number of partners, receptive anal sex, and HIV status. Beta and/or gamma HPV infection showed no significant association with HIV status, socio-demographic or sexual behavioral factors.
Conclusions: A wide spectrum of mucosal and cutaneous HPV types is present in the anal canal. Only mucosal HPV prevalence increased significantly in cases of concomitant HIV infection.

Introduction

More than 170 different HPV genotypes have been identified to date.[1] Based on the epithelial tissue tropism, HPVs have been classically subdivided into mucosal and cutaneous types. While the former types belong to the genus *Alpha-papillomavirus* only, cutaneous types are represented in the genera *Alpha-papillomavirus*, *Beta-papillomavirus*, *Gamma-papillomavirus*, *Mu-papillomavirus*, and *Nu-papillomavirus*. Mucosal HPV types have been typically found at mucosal sites (anogenital, oral and oropharyngeal epithelia) while cutaneous HPVs have been mainly isolated from the skin.

Anal infections caused by mucosal HPV types have been detected in both men and women, but they are particularly frequent among men who have sex with men (MSM), especially those with HIV-1 infection. Differently, cutaneous HPV types are ubiquitous in the general population.[2–5] Due to their frequent detection in healthy skin and hair follicles, hypotheses about their commensal nature have been formulated. Recently, cutaneous HPV types have been detected in the anal canal of MSM,[6] in the male genital area,[7] as well as in male external genital lesions.[8,9] Additionally, beta and gamma HPVs have been found in the oral[10] and nasal mucosa.[11] These data support the idea that cutaneous HPVs may be widely distributed in anatomical sites other than the skin.

Many epidemiological and biological studies have provided evidence for the role of certain beta HPV types in the development of non-melanoma skin cancer (NMSC) in association with UV exposure. Beta HPV types were first isolated in individuals suffering from the genetic disorder epidermodysplasia *verruciformis*, which is characterized by a high susceptibility to beta HPV infections, development of confluent skin warts and NMSC at UV-exposed sites.[12,13] Studies on alpha HPVs demonstrated that the oncogenic potential of individual types is determined by their ability to promote cellular transformation as well as deregulating immune-related pathways. Impairment of immune surveillance strongly facilitates viral persistence and, subsequently, HPV-induced carcinogenesis. Accordingly, the most oncogenic type, HPV16, can persist in the host much longer than the other mucosal HPV types. At present, very little is known about the possible efficiency of beta and gamma HPV types in evading the immune system and establishing a persistent infection. Moreover, limited data are available on the presence of cutaneous HPVs at anogenital level, especially among HIV-infected individuals.

In this study, we aimed to assess the prevalence and genotype-specific distribution of alpha, beta and gamma HPV types in the anal canal of both HIV-infected and uninfected MSM, and to evaluate whether HIV status influences these infections.

Materials and methods

Study population

HIV-infected and uninfected MSM were enrolled among the attendees of the STI/HIV Unit of the San Gallicano Dermatological Institute (Rome, Italy) between August 2009 and March 2014. HIV-uninfected MSM, whose enrollment criteria have been described elsewhere,[14] were recruited among participants in an enhanced screening program for HIV and other STIs (COROH Project). Based on the same criteria, HIV-infected MSM were recruited among patients attending the STI/HIV Unit for the clinical management of HIV infection and antiretroviral therapy administration. Data on medical history, socio-demographic factors and sexual behavior were collected through face-to face interviews conducted by trained operators. Clinical, virological and immunological parameters of HIV-infected patients were retrieved from the medical records, considering the values closest to the date of anal sample collection. The study was cleared by the local ethics committee (CE/564/11 and CE/436/14) and performed in accordance with the principles of the Declaration of Helsinki.

Anal sample collection and nucleic acid extraction

Anal specimens were collected using a Dacron swab. Cells were dislodged in 20 ml of PreservCyt (Hologic, Pomezia, Italy). Nucleic acids were extracted from 250 μl of the PreservCyt sample using the Amplilute Liquid Media Extraction Kit (Roche Molecular Diagnostics, Milan, Italy), following the manufacturer's instructions.

Alpha, beta and gamma HPV detection and genotyping

Samples were tested with the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics), which detects 37 mucosal HPV types, following the manufacturer's instructions. Cutaneous HPVs were identified using type-specific multiplex genotyping (TS-MPG) assays (IARC, Lyon, France), which combine a multiplex PCR[15] with a bead-based Luminex technology.[16,17] The TS-MPG assay for beta HPVs detects 43 genotypes (species beta1: 5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93; beta2: 9, 15, 17, 22, 23, 37, 38, 80, 100, 104, 107, 110, 111, 113, 120, 122, 145, 151; beta3: 49, 75, 76, 115; beta4: 92; beta5: 96, 150). TS-