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Betapapillomaviruses in the anal canal of HIV positive and HIV negative men who have sex with men☆

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ABSTRACT

Background: Betapapillomaviruses (β-PV) are etiologically associated with epidermodysplasia verruciformis and a proportion of skin precancerous lesions and cancer, mainly in immunocompromised individuals.
Objectives: The prevalence and persistence of anal β-PV infection and β-PV type distribution were determined in a cohort of men who have sex with men (MSM). A correlation with HIV-1 infection status and selected demographic and behavioral risk factors were additionally established.
Study design: A total of 181 anal swabs (135 initial and 46 follow-up swabs) obtained from 135 Slovenian MSMs (17.0% HIV-1 positive) were tested for the presence of 25 different β-PV types using Diassay RHA Kit Skin (beta) HPV assay and, if negative, with an in-house nested M^a/H^a PCR.
Results: β-PVs were detected in 88/135 (65.2%) initial anal swabs. Infection with multiple β-PV types was found in 26 samples; the number of β-PVs ranged from 2 to 9. A total of 29 distinct β-PVs were detected: HPV-36 and HPV-38 were the most prevalent, followed by HPV-23, HPV-24, and HPV-93. HIV-1 positive status, promiscuity and use of alkyl nitrites were significantly associated with a higher prevalence of anal β-PV infection. Three partial DNA sequences suggesting putative new HPV types were identified.
Conclusion: To the best of our knowledge, this is the first study to investigate and characterize β-PV infections in the anal region. We showed that anal β-PV infection is highly prevalent in the MSM population and that β-PVs can establish persistent infection in the anal region for up to 4.8 years.

1. Background

Papillomaviruses (PV) are remarkably heterogeneous DNA viruses that infect a wide variety of vertebrate species and are causally involved in the etiology of various neoplastic changes of the skin and mucosa. They are highly species specific, or at least infect closely related animal species [1]. Based on the nucleotide similarity of the L1 gene, PVs are hierarchically classified into genera, species and types, and PV types are those that are typically associated with specific disease(s) [1,2].

At present, over 250 PV types have been completely characterized and officially recognized, of which 170 have been detected in humans [2]. HPVs are currently classified into five major genera: *Alphapapillomavirus* (α-PV), predominantly found in anogenital lesions, and *Betapapillomavirus* (β-PV), *Gammapapillomavirus* (γ-PV), *Mupapillomavirus* (μ-PV) and *Nupapillomavirus* (ν-PV), typically isolated from skin and hair follicle specimens [1,2]. β-PVs are etiologically associated with epidermodysplasia verruciformis, a rare autosomal recessive hereditary disorder that is characterized by chronic β-PV infection and subsequent development of cutaneous squamous cell carcinoma. An etiological role of β-PV in the development of precancerous lesions and cancer of the skin in organ-transplant patients, as well as in immunocompetent individuals, has also been recently suggested [3–7]. Moreover, we recently co-characterized a novel β-PV type (HPV-120; ENA

☆ Partial L1 nucleotide sequences of these isolates are available in the ENA database under the following acc. nos.: SIBX12 (HF937426), SIBX13 (HF937427), and SIBX14 (HF937428).

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accession number FN598907) from an anal swab specimen, which surprisingly reacted with the commonly used broadrange α-PV specific PGMY09/PGMY11 primer set. Using HPV-120 type specific real-time PCR, this particular HPV type was subsequently found in 3.3% of the 210 tested anal swab specimens obtained from the same number of Slovenian men having sex with men (MSM) [8], suggesting that β-PVs may be commonly found at this anatomical location.

2. Objectives

In this study, we determined the prevalence and persistence of anal β-PV infection and β-PV type distribution in a cohort of MSM and established a correlation with subjects' HIV-1 infection status and selected demographic and behavioral risk factors. To the best of our knowledge, this is the first study to investigate and characterize β-PV infections in the anal region.

3. Study design

The study included 135 Slovenian males with a history of receptive anal sexual intercourse, recruited from January 2007 to December 2008 through two proctologic outpatient offices in the Rožna dolina Surgical Centre, Ljubljana, Slovenia and in the Surgical Centre Zdrav Splet, Maribor, Slovenia. At enrolment, each participant provided written, informed consent and completed questionnaire regarding sexual behavior. The study has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) and has been approved by the National Medical Ethics Committee of the Republic of Slovenia (consent number: 131/06/07). Of 135 subjects, 123 originated from our previous study investigating the prevalence of α-PV in the anal region [9] and 12 were newly enrolled. The study subjects aged from 17 to 81 years (median age = 31 years) and 23/135 (17.0%) were HIV-1 positive. Of the 133 MSM for whom clinical data were available, more than two-thirds (90/133; 67.6%) had a clinically evident anal pathology, including anal warts (77/133; 57.9%), hemorrhoids (5/133; 3.8%) and anal fissure (3/133; 2.3%). Altogether, a total of 181 anal swab DNA samples were included in the study: 135 initial samples obtained from all study subjects at the first visit and 46 follow-up samples obtained from 30 subjects at the control visit(s). The number of follow-up specimens per male ranged from 1 to 3.

Detection and typing of β-PVs was initially performed using a commercially available RHA Kit Skin (beta) HPV assay (RHA; Diassay BV, Rijswijk, The Netherlands), which enables simultaneous identification of 25 different β-PV types by reverse-line blot hybridization technique, as described previously [10]. All β-PV negative samples or samples with undetermined β-PV type (HPV-X) were additionally tested with an *in-house* nested M^a/H^a PCR, targeting an approximately 450-bp L1 gene fragment of various β-PVs, as described previously [11]. For all PCR amplifications, 5 μl of each sample was used per 25 μl reaction. M^a/H^a PCR amplicons were gel-purified and further processed for sequence analysis and HPV type determination, as described previously [11]. Detection and typing of α-PVs in anal swab samples of the newly included MSM was performed, as described previously [9].

All statistical calculations were done with SPSS 21.0 software (SPSS Inc., Chicago, IL). Univariate analysis of variance was used for testing the differences in variance between groups. Mean values were expressed with a standard error of the mean value. A null hypothesis was disproved with $P < 0.05$. The correlation of parametric and non-parametric variables was tested with the Pearson and Spearman correlation test.

4. Results

Overall, β-PV DNA was detected in 88/135 (65.2%) initial anal swab samples. Infection with a single β-PV type was found in 53 samples and infection with multiple β-PV types was found in 26 samples; the number of identified β-PVs in multiple infections ranged from 2 to 9 (average = 3 types, median = 2 types) (Supplementary Table 1). Nine samples, three from HIV-1 positive and six from HIV-1 negative subjects, contained unknown HPV type(s). As shown in Fig. 1, a total of 29 distinct β-PVs were detected among the 135 initial samples, including potentially novel HPV type SIBX14 (most closely related to HPV-122, partial L1 gene nucleotide identity 82%). Overall, HPV-36 and HPV-38 were the most prevalent types, followed by HPV-23, HPV-24 and HPV-93; together, these five types accounted for 33.8% (48/142) of identified β-PV infections; we considered each identified HPV type or HPV-X as one β-PV infection. As summarized in Supplementary Table 1, infection with α-PV DNA was detected in 109/135 (80.7%) initial anal swab samples. Of these, 19 samples contained a single α-PV type and 90 samples contained multiple β-PV types. The number of identified α-PVs in multiple infections ranged from 2 to 10 (average = 5 types, median = 4 types).

Supplementary table related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2014.07.009.

A significantly different prevalence of anal infection with β-PV types was found among HIV-1 negative (66/112; 58.9%) and HIV-1 positive men (22/23; 95.7%) ($P < 0.005$). The prevalence of multiple β-PV infections was similar in HIV-1 negative and HIV-1 positive subjects (19/106 vs. 7/20, $P = 0.085$), while a significant difference in the average number of β-PV types was observed between these two groups (0.95 ± 0.13 vs. 1.60 ± 0.29, $P < 0.05$). Infection with α-PV types was detected in 87/112 (77.7%) HIV-1 negative and 22/23 (95.7%) HIV-1 positive MSM ($P < 0.05$); infection with β-PV types was more common among α-PV positive than among α-PV negative subjects ($P < 0.001$).

Of the 135 studied subjects, 94 completed the questionnaire about sexual behavior. The reported duration of homosexual/bisexual relationship (anal sex practice) ranged from 0 to 36 years (mean = 12.1 years; median = 11.0 years). Fourteen (15.1%) MSM reported 1–5 lifetime sexual partners, 19 (20.4%) reported 6–10 partners, 12 (12.9%) reported 11–20 partners, 25 (26.9%) reported 21–50 partners, 14 (15.1%) reported 51–100 partners and 9 (9.7%) MSM reported more than 100 lifetime sexual partners. β-PV prevalence correlated positively with the number of homosexual partners during a lifetime ($P < 0.05$). β-PV infection was more frequent (63/92 vs. 0/2, $P < 0.05$) among subjects who had practiced homosexual/bisexual intercourse for more than one year than among those with a shorter period of practice. Similarly, β-PV infection was more frequent (59/82 vs. 4/12, $P < 0.01$) and the average number of detected HPV types was higher (1.18 ± 0.16 vs. 0.27 ± 0.14, $P < 0.05$) among subjects who had practiced homosexual/bisexual intercourse for more than three years than among those with a shorter period of practice. Of the 135 MSM, 96 completed the questionnaire about drug use during the sexual intercourse. Specifically, 35 (39.6%), 51 (53.1%), 21 (21.9%), 4 (4.2%), 30 (31.3%), 1 (1.0%) and 7 (7.3%) MSM reported to use tobacco, alcohol, marijuana, crystal methamphetamine, alkyl nitrite (poppers), ketamine and ecstasy, respectively. Interestingly, β-PV infection was more prevalent (27/30 vs. 36/66, $P < 0.005$) only among MSM who used alkyl nitrites (poppers) during sexual intercourse than among those who did not, and the average number of detected HPV types was higher in the former group (1.68 ± 0.36 vs. 0.75 ± 0.11, $P < 0.005$).

One to three follow-up samples were available for 30 enrolled subjects (18 HIV-1 negative and 12 HIV-1 positive). The follow-up period ranged from 1 to 65 months (average = 28 months,