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Research Article

# Human Papilloma Virus Genotypes and Intraepithelial Neoplasia in HIV Positive and Negative Patients with External Condylomata Acuminata

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**Keywords**

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**Abstract**

In order to identify differences in clinical, histopathology and virology data of patients with external condylomata acuminata (ECA) by HIV status, 47 HIV positive patients and 42 HIV negative patients were enrolled. Socio-demographic and clinical data was recorded. Histopathology study and HPV detection (Roche Linear Array genotyping assay) were performed. HIV positive patients were mainly single, MSM, with history of STI, multiple sexual partners, receiving antiretroviral therapy and with recurrent peri-anal ECA. HPV-16 and HPV-61 were associated to this group while HPV-6 was found in HIV negative patients with ECA. Intraepithelial neoplasia (IEN) was found in 5.6% of patients, associated to HIV and HPV-16. We found differences in socio-demographic characteristics and HPV genotypes in patients with ECA by HIV status. The association of high grade IEN with HIV infection makes evident the need of defined parameters as markers for early cancer diagnosis.

## ABBREVIATIONS

CA: Condylomata Acuminata; STI: Sexually Transmitted Infection; MSM: Men Who Have Sex with Men; ECA: External Condylomata Acuminata; HPV: Human Papilloma Virus; HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; LR: Low Risk; HR: High Risk; PHR: Probably High Risk; IEN: Intraepithelial Neoplasia; HAART: Highly Active Antiretroviral Therapy; VIN: Vulvar Intraepithelial Neoplasia

## INTRODUCTION

Condylomata acuminata (CA) represents the most common sexually transmitted infection (STI) worldwide [1]. In Chile, they correspond to 31% of all STI diagnoses, mainly in young men and women and rise to 42% in men who have sex with men (MSM) [2]. In HIV/AIDS patients CA is the most common anal disease, with statistics between 22.8% to 75.6% [3,4]. Among them, CA show rapid growth, a high rate of recurrence and the anatomical location seems to be an important factor in determining their course [5-7]. The external condylomata acuminata (ECA) are located in visible areas, without the need of an instrument for their inspection [8]. CA are caused by human papilloma virus (HPV), from the *Papillomaviridae* family and according to its phylogeny, the alpha genus includes the genotypes that primarily infect the mucosa [9] some of these genotypes are considered "low risk" (LR) for cancer development and others "high risk" (HR) or "probable high risk" (PHR) [10]. The number of HPV cases in HIV positive patients is increasing and this population shows a greater frequency of Intraepithelial Neoplasia (IEN) of the anus, even among patients under HAART therapy [11].

Former studies with dissimilar methods have detected multiple HPV genotypes and a substantial proportion of oncogenic types [11-13]. Today, the emergence of genomic arrangement methodologies allow for identification of multiple HPV genotypes from a single lesion. Our aim was to identify differences in clinical, histopathology and virology characteristics in patients with ECA, by HIV status.

## MATERIALS AND METHODS

Over the course of one year at the North Metropolitan Santiago Health Service, San Jose Hospital, all HIV patients presenting ECA (genital or peri-anal) were referred to the STI center for treatment and study enrolment. HIV negative patients meeting the same clinical diagnosis and inclusion criteria were randomly selected for enrolment. Exclusion criteria were age younger than 18 years, pregnancy, and sex workers. The study was approved by the local ethics committee, and each patient provided written informed consent.

Participants recorded in a self-report questionnaire the following information: gender, age, education level, sexual orientation, civil status, current consumption of drugs, number of sexual partners in lifetime and over the past year, age at first sexual encounter, practice of anal sex and presence of anal pathology, STI history, condom use, number of days CA have been present, and history of previous outbreaks. Data from the clinical files was recorded, including HIV stage, use of antiretroviral therapy, LTCD4 level, and HIV viral load over the past 3 months. A physical examination was completed, recording CA location, colour and pattern of presentation.

An Incisional biopsy of the ECA was performed under local anesthesia and divided in two for histopathology and viral studies. One sample was preserved in formaldehyde and processed with haematoxylin-eosin stain, and the other one was sent to the virology laboratory and maintained at -80°C.

Two independent pathologists analyzed all samples. They classified CA, IEN grade 1, 2, or 3 (defined by the presence of atypical cells in 25, 50, or 100% of the epithelium, respectively), invasive carcinoma, or other diagnosis. For HPV diagnosis, DNA extraction was performed using the "Amplitude Liquid Media Extraction Kit" (Roche) according to manufacturer instructions. Amplification for 37 different HPV genotypes was performed with "Linear Array HPV Genotyping Test" (Roche), which include probes for: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108. Strips were developed with the "Linear Array Detection Kit" (Roche) based on HRP-Streptavidin conjugate binding to the biotinylated DNA.

Continuous variables are presented as averages with standard deviation and the categorical variables as frequency distributions. The between-group differences were analyzed using the Student t-test, chi square, and Fisher's exact test. The significance level was set at 5%. All analyses were performed using the statistical software Stata 10.1.

## RESULTS AND DISCUSSION

A total of 89 patients with ECA were included according to study criteria; 47 HIV positive and 42 HIV negative. In Table (1) socio-demographic characteristics associated to HIV status are shown. Significant STI history in HIV positive patients included previous ECA episodes (42.5%), syphilis (36.2%), clinical genital herpes (21.9%) and hepatitis B virus infection (12.8%). Sixty percent of HIV positive patients had been controlled for more than 5 years, 78.2% were under antiretroviral therapy and 78.7% were in stage A or B of AIDS progression. Regarding LTCD4 count,

**Table 1:** Baseline Characteristics associated to HIV status.

| | HIV + | | HIV - | | |
|---|---|---|---|---|---|
| | N | (%) | N | (%) | p-value |
| **Gender** | | | | | |
| **Male** | 44 | -93.62 | 11 | -26.19 | <0.0001 |
| **Female** | 3 | -6.38 | 31 | -73.81 | |
| **Age in years** | | | | | |
| **<30** | 20 | -52.63 | 18 | -47.37 | 0.573 |
| **>30** | 27 | -52.94 | 24 | -47.06 | |
| **Civil Status** | | | | | |
| **Single** | 37 | -78.72 | 11 | -26.19 | <0.0001 |
| **Other** | 10 | -21.28 | 31 | -73.81 | |
| **Education level** | | | | | |
| **High school or less** | 24 | -51.06 | 31 | -73.81 | 0.023 |
| **Higher Education** | 23 | -48.94 | | | |
| **Sexual orientation** | | | | | |
| **Heterosexual** | 9 | -19.15 | 40 | -95.24 | <0.0001 |
| **Homosexual** | 35 | -74.47 | 1 | -23.81 | |
| **Bisexual** | 3 | -6.38 | 1 | -2.38 | |
| **STI history** | | | | | |
| **None** | 12 | -25.53 | 31 | -73.81 | <0.0001 |
| **One** | 14 | -29.79 | 10 | -23.81 | |
| **Two or more** | 21 | -44.68 | 1 | -2.38 | |
| **Practice of anal sex** | | | | | |
| **Yes** | 39 | -82.97 | 19 | -45.23 | <0.0001 |
| **Condom use** | | | | | |
| **Always** | 21 | -44.68 | 4 | -9.52 | <0.0001 |
| **Sometimes** | 21 | -44.68 | 25 | -59.52 | |
| **Never** | 5 | -10.64 | 13 | -30.95 | |
| **Drugs use** | | | | | |
| **Yes** | 21 | -44.68 | 7 | -16.67 | 0.004 |
| **N° sexual partners in past year** | | | | | |
| **0** | 11 | -23.4 | 4 | -9.52 | 0.005 |
| **1** | 16 | -34.04 | 25 | -59.52 | |
| **4-Feb** | 12 | -25.53 | 13 | -30.95 | |
| **> or = 5** | 8 | -17.02 | 0 | | |
| **N° total sexual partners** | | | | | |
| **1** | 1 | -2.13 | 5 | -11.9 | <0.0001 |
| **4-Feb** | 6 | -12.77 | 24 | -57.14 | |
| **9-May** | 12 | -25.53 | 7 | -16.67 | |
| **>10** | 28 | -59.57 | 6 | -14.29 | |
| **Tobacco use** | | | | | |
| **Yes** | 32 | -68.08 | 25 | -59.52 | 0.401 |
| **No** | 15 | -31.92 | 17 | -40.48 | |
| **Anal Pathology** | | | | | |
| **Hemorrhoids** | 6 | -12.76 | 9 | -21.42 | <0.001 |
| **Fissure** | 13 | -27.65 | 14 | -33.33 | |
| **Fistulas** | 4 | -8.51 | 0 | | |
| **Age at first sexual encounter** | | | | | |
| **<18** | 17 | -36.17 | 15 | -35.71 | 0.964 |
| **>18** | 30 | -63.82 | 27 | -64.28 | |

70.2% had over 200 cells/ mm$^3$ and 53% had undetectable viral loads. Correlations between the clinical parameters of the ECA and HIV status are shown in (Table 2). Virology analysis identified HPV genotype in 81 (91%) of patients (Table 3). The main genotypes identified were HPV-6 (50.56%), HPV-11 (28.08%), HPV-16 (12.35%) and HPV-45 (8.98%). There were significant differences in LR genotypes by HIV status, specifically for HPV-6, which correlated with HIV negative patients. On the other hand, HPV-16 (HR genotype) and HPV-61 (LR genotype) were associated with HIV positive patients. No statistical difference was found in single or multiple infections. Histopathology analysis confirmed the diagnosis of CA in 81 (91%) samples. Five (5.61%) biopsies showed IEN, mainly grade 2 or 3 associated to multiple HR-HPV in HIV positive patients. Only one confirmed Vulvar IEN grade 1 (VIN 1) was found in an HIV negative woman. In 3 (3.40%) samples the diagnosis was other: non-specific chronic and acute inflammation in an HIV positive patient, and fibro epithelial papiloma and dermal melanosis in two HIV negative patients.

**Table 2:** Clinical Parameters according to HIV status.

| | HIV+ | | HIV- | | |
|---|---|---|---|---|---|
| | N | (%) | N | (%) | p Value |
| **Recurrence of ECA** | 24 | 51.6 | 10 | 23.81 | 0.008 |
| **Location** | | | | | |
| **Peri-anal** | 37 | 78.72 | 9 | 21.43 | <0.0001 |
| **Genital** | 5 | 10.64 | 26 | 61.9 | |
| **Multiple locations** | 5 | 10.64 | 7 | 16.67 | |
| **Colour** | | | | | |
| **Pink** | 17 | 36.17 | 17 | 40.48 | 0.82 |
| **Grey** | 21 | 44.68 | 16 | 38.1 | |
| **Hyperpigmented** | 9 | 19.15 | 9 | 21.43 | |
| **Pattern of presentation** | | | | | |
| **Exophytic (wart)** | 31 | 65.96 | 34 | 80.95 | 0.264 |
| **Endophytic (flat)** | 13 | 27.66 | 7 | 16.67 | |
| **Giant condyloma** | 3 | 6.38 | 1 | 2.38 | |
| **Time patient has had the ECA** | | | | | |
| **< 2 years** | 38 | 80.85 | 36 | 85.71 | 0.541 |
| **> 2 years** | 9 | 19.15 | 6 | 14.29 | |

**Abbreviations:** ECA: External Condilomata Acuminata

**Table 3:** Major HPV genotypes identified, by HIV status.

| | HIV+ | | HIV- | | | |
|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | Total N | p-Value |
| **LR-HPV** | | | | | | |
| **6** | 18 | -40 | 27 | -60 | 45 | 0.012 |
| **11** | 13 | -54.17 | 11 | -45.83 | 24 | 0.534 |
| **61** | 5 | -100 | 0 | 0 | 5 | 0.037 |
| **LR-HPV≥1** | 32 | -47.06 | 36 | -52.94 | 68 | 0.043 |
| **HR-HPV** | | | | | | |
| **16** | 9 | -81.82 | 2 | -18.18 | 11 | 0.039 |
| **18** | 3 | -60 | 2 | -40 | 5 | 0.554 |
| **45** | 4 | -50 | 4 | -50 | 8 | 0.578 |
| **59** | 4 | -57.14 | 3 | -42.86 | 7 | 0.563 |
| **HR-HPV≥1** | 19 | -59.38 | 13 | -40.63 | 32 | 0.24 |
| **Negative** | 5 | -62.5 | 3 | -37.5 | 8 | 0.422 |

Our data show notable differences in both the socio-demographic characteristics and HPV genotypes of patients with ECA, by HIV status. The HIV negative patients studied were mainly heterosexual women, with fewer than 5 total lifetime sexual partners and lesions located primarily on the genitals. They had a low rate of prior STI history, and most presented ECA for the first time. We identified in these patients primarily LR-HPV infections, especially genotype 6, which is consistent with a previous report in a French population with external anogenital condylomata [14]. Although, the second most common genotype was HPV-11, it was equally common in HIV positive and negative patients. This data differs from studies on other continents, where HPV-11 has not been identified, or has been detected only in a very low percentage of HIV positive patients [15,16]. Only one patient had a histopathology diagnosis of VIN 1, and she had multiple LR-HPV infections including HPV-11, which is consistent with data from a North American female population [17] The Chilean female population has been found to have a low rate of infection with HPV-6 and HPV-11 in cervical samples [18], in contrast to our results in anal-genital samples. Considering that, our group of women ranged in age from 19 to 55 years, with an average age of 33 years, which situates them in the lower end of the bimodal curve for LR-HPV prevalence for cervical samples [18], it is possible that the biological pathogenesis of these infections differs, with a greater persistence for LR infections in the external anal-genital mucosa and/or a greater immunological response to HR genotypes.

The high proportion of patients with multiple infections reported here may be a reflection of the sexual behaviour of these patients or their partners (data not collected) as the majority of the HIV negative patients were married or cohabiting women, who may have been unconcerned about STI. On the other hand, the pathogenesis of this infection, which includes re-infections, viral persistence, and genomic insertion in some cases, facilitates the viral multiplicity. It is noteworthy that there was no correlation found between HIV status and the presence of multiple HPV genotypes. The HIV positive patients´ profile is consistent with national statistics [19]. The clinical correlations between condylomata located primarily in the peri-anal area and recurrent outbreaks are coherent with this group's self-reports of high number of sexual partners and unsafe behaviour. We have demonstrated a correlation between HIV positive status and the presence of HPV-16, but HPV-18 did not play an important role in either the HIV positive group or the IEN patients. This finding is important considering the available vaccines and the increasing frequency of anal cancer and mortality in the HIV positive patients [20,21]. Though LR-HPV 61 is usually identified in HIV negative men with condylomata in other regions of the world, different studies often used dissimilar genotyping methodologies so results are not always fully comparable [22]. Our data about IEN is lesser than other reports but it has to be considered that we did not sample tissue from the anal canal [23,24]. Nevertheless our finding of 5.6% of the total samples is higher than the 3.5% in general population´s peri-anal condylomata [25]. Among the factors associated with neoplasia we did not find significant

differences among the clinical characteristics. We believe that this is important to analyze more deeply in the future, in order to define specific parameters according to HIV serological status that may predict when an ECA is in risk of becoming an IEN. The present study stands out the most common STI reason for consultation as patients usually only notice the ECA. As we utilized a genomic array, we were able to research multiple HPV infections at the same time in one CA and thus our results contribute to the scarce Latin American genotypes data and emphasis the world regional differences. The main limitations were the number of HIV negative patients with ECA enrolled during the limited time and the data obtained from a self-report questionnaire and not from a professional interview.

## CONCLUSION

Socio demographic characteristics are notably dissimilar in between patients with ECA according to HIV serological status. In the clinical examination, only location of ECA differs but there is no predictable characteristic for EIN. Taking into account the high proportion of multiple infections and some specific HPV genotypes involved, extended regional genotyping studies are needed, as new vaccines are available and others in development.

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