No clinical predictors of intraepithelial neoplasia in HIV-positive patients with external condilomata acuminata

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Abstract

To identify clinical parameters in association with human papilloma virus (HPV) genotypes and histopathology diagnosis in HIV-positive patients with external condylomata acuminata (ECA), 400 Chilean HIV-positive patients were included in the study. Forty-seven patients presented ECA. Clinical parameters and socio demographic data were recorded. Histopathology study and HPV linear array genotyping assay were performed. Intraepithelial neoplasia (IEN) grade 2 or 3 was found in 8.5% of patients, associated to HPV-16. Patients were mainly single, MSM, with history of sexually transmitted disease (STD), multiple sexual partners, receiving antiretroviral therapy and with recurrent lesions. All ECA were mainly perianal, grey or pink colored, exophytic with less than two years evolution. No clinical parameter could predict the development of high grade IEN in HIV patients with ECA. It seems necessary to perform biopsy and genotype all HIV positive patients with ECA.

Key words: Condylomata acuminata, HIV, human papilloma virus

INTRODUCTION

Condylomata acuminata (CA) is one of the most common sexually transmitted diseases (STD) worldwide.[¹,²] In Chile, it is the major STD representing 31% of STD diagnoses, mainly in men and women aged 20 to 24 years. In men who have sex with men (MSM), this figures rise to 42%.[³] In HIV/AIDS patients, mainly in MSM, CA is the most common anal disease, affecting 25% to 76%.[¹,⁴-⁶] Among this population, CA show rapid growth, resistance to conventional therapies, and a high rate of recurrence.[⁷,⁸] The anatomical location of the CA seems to be an important factor in determining their course.[⁹] External CA (ECA) are those located in areas visible to the clinician, without the need of an instrument for their inspection, and are easier to diagnose and treat.[¹⁰] CA are caused by human papilloma virus (HPV), who belong to the Papillomaviridae family and are classified according to its phylogeny. The alpha genus includes the genotypes that primarily infect the mucosa.[¹¹] Among these genotypes, some are considered “low risk” (LR) and others “high risk” (HR) or “probable high risk” for cancer.[¹²] The increasing number of HPV cases in HIV-positive patients is highly relevant, as this population shows a greater frequency of intraepithelial neoplasia (IEN)
on the anus, even among patients under HAART therapy. The clinical and socio epidemiological features associated with the increased risk of neoplasia in HIV patients varied according to the revision. Furthermore, prior studies have detected the presence of multiple HPV genotypes and a large proportion of oncogenic types, in HIV-positive patients. However, these studies used dissimilar methods, some of which do not differentiate among genotypes or identify only a very limited number of them. Currently, there are genomic array methodologies that allow for identification of multiple and different HPV genotypes from a single clinical sample. Our aim was to identify clinical parameters associated to HPV genotypes and IEN in HIV patients with ECA.

MATERIALS AND METHODS

This study evaluated 400 HIV-positive patients seen over the course of one year at the North Metropolitan Public Health Service, which attended about 20% of the inhabitants of Santiago de Chile. Forty-seven patients who presented external genital or perianal CA were enrolled. Exclusion criteria comprised age younger than 18 years, pregnancy, and sex workers. The study was approved by the local ethics committee, and each patient provided informed consent.

A physical examination was completed by a dermatologist, who recorded the following parameters: CA location (perianal, genital, or multiple), color of lesion (pink, grey, or hyperpigmented), and pattern of presentation (exophytic, endophytic, flat, or tumoral). Using a self-report questionnaire, patients answered about gender, age, civil status, education level, sexual transmitted infections (STI) history, sexual orientation, current consumption of tobacco and other 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 (hemorrhoids, fissures, or fistulas), condom use, number of days CA have been present, and history of previous outbreaks. History from the clinical files was obtained, including HIV stage according to the 1992 CDC classification, use of antiretroviral therapy, LTTc4 level, and HIV viral load over the past 3 months.

Under local anesthesia, an incisional biopsy of ECA was taken and divided in two for histopathology (hematoxylin-eosin stain) and viral study (maintained at ~80°C).

All samples were analyzed by two independent pathologists and classified as one of the following diagnoses: 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 identification and genotyping, we extracted DNA from samples using the "AmpliTute Liquid Media Extraction Kit" (Roche®), according to manufacturer instructions, with SDS and Proteinase K; lysates were loaded into columns, washed and eluted in aqueous buffer. HPV amplification and genotyping was performed with "Linear Array HPV Genotyping Test" (Roche®), which includes a PCR reaction specific for HPV DNA including biotinylated primers; amplified DNA was then hybridized to the array strips which include probes for 37 different HPV genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 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.

The continuous variables are presented as averages with SD, and the categorical variables as frequency distributions. The between-group differences were studied using Fisher’s exact test. The significance level was set at 5%. All analyses were completed using the statistical software Stata 10.1.

RESULTS

A total of 47 HIV-positive patients with ECA were included according to study criteria. Histopathology analysis confirmed the diagnosis of CA in 42 (89.4%) samples and four (8.5%) biopsies showed IEN grade 2 or 3. Only one (2.1%) was a non-specific chronic and acute inflammation. Viral analysis identified HPV genotype in 42 (89.4%) patients. The main genotypes were HPV-6 (38.3%), HPV-11 (27.7%), HPV-16 (19.15%), and HPV-61 (10.04%). Twenty-one (44.7%) positive patients had multiple genotypes with an average number of 3.3 genotypes. IEN cases were statistically associated to HPV-16 (P = 0.001). [Table 1].

Three cases clinically classified as giant perianal condylomata were confirmed by histopathology and two of them had HR-HPV genotypes (HPV-18 and HPV-45).

Clinical lesions were localized mainly perianal (78.7%), had a grey or pink color (80.8%), were exophytic (66%) and had an evolution lower than 2
There was no statistical association with IEN cases [Table 2].

Forty-four (93.6%) patients were male, 80.8% were MSM, 83% practiced anal sex, 44.7% used drugs, 68% smoked tobacco, 74.5% had history of STD, 52.6% were under 30 years of age, 78.7% single, 51.6% had finished school, 59.57% had more than 10 sexual partners and 49% had history of anal pathology. Sixty percent of 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, 70.2% had over 200 cells/ mm³ and 53% had undetectable viral loads.

### DISCUSSION

The proportion of IEN found in this study is smaller than that observed in the literature in HIV/AIDS patients, which identified 24% rate of grade 2 and 3 anal intraepithelial neoplasia (AIN) and 34% rate of low-grade AIN. This may be explained because we did not sample tissue from the anal canal, which is the site in which the greatest number of neoplasia develops. Also, it is possible that the rates would have been higher if the lesions had been followed for longer time. However, our finding of IEN in 8.5% of the study sample is higher than the 3.5% reported in the general population with perianal condylomata.

Only HPV genotype 16 was statistically associated to neoplasia and HPV-18 did not play an important role in either the ECA group or the IEN patients. The low percentage of patients infected with HPV-18 is similar to reports in French population. It is also consistent with studies of cervical HPV genotype prevalence in the Chilean population, which may suggest that this genotype does not circulate to a significant degree among our population. This finding is important considering the available vaccines and the increasing frequency of anal cancer and mortality in the HIV positive patients. The high proportion of genotype 61 (LR-HPV) found in HIV-positive patients has not been previously reported. It has been identified only in HIV negative men with condylomata. However, it is important to consider that different studies have often used dissimilar methodologies for genotyping, so results

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**Table 1: Characteristics associated to Intraepithelial neoplasia cases**

| Sex | Age* | Sex Or | HIV† | LTcd4** | Viral load† | Stage | NIE | HPV genotype |
|-----|------|--------|------|---------|-------------|-------|-----|---------------|
| M   | 42   | MSM    | 10 y | 171     | ND          | C3    | 3   | 18, 26        |
| M   | 33   | MSM    | 12 y | 58      | 760         | A3    | 3   | 16            |
| M   | 36   | MSM    | 7 y  | 284     | ND          | C3    | 3   | 16, 31, 58, 59, 53 |
| M   | 29   | MSM    | 2 y  | 61      | 66,000      | A3    | 2   | 16, 52, 56, 66 |

M, Masculine; MSM, Men who has sex with men; Sex Or, Sexual orientation; y, Years; Neg, Negative. Age*, Age in years, HIV†, Years from conversion, LTcd4**, cells/ mm³, viral load†, copies/ml

**Table 2: Clinical parameters according to histopathology diagnosis**

| Location                                      | ECA N (%) | IEN N (%) | P Value |
|------------------------------------------------|------------|------------|---------|
| Recurrence of ECA                            | 19 (44.2)  | 3 (75)     | 0.257   |
| Location                                      |            |            |         |
| Perianal                                      | 33 (76.7)  | 4 (100)    | 0.370   |
| Genital                                      | 5 (11.6)   | 0 (0)      | 0.628   |
| Multiple locations                            | 5 (11.6)   | 0 (0)      | 0.628   |
| Pattern of presentation                       |            |            |         |
| Exophytic (wart)                              | 16 (37.2)  | 1 (25)     | 0.541   |
| Endophytic (flat)                             | 19 (44.2)  | 2 (50)     | 0.610   |
| Giant condyloma                               | 8 (18.6)   | 1 (25)     | 0.586   |
| Time patient has had the ECA                  |            |            |         |
| <2 years                                      | 34 (79.1)  | 4 (100)    | 0.414   |
| >2 years                                      | 9 (20.9)   | 0 (0)      | 0.414   |
are not always fully comparable.[25]

The high proportion of patients with multiple infections reported here may be a result of the sexual behaviour of these patients and/or possibly of the pathogenesis of this infection, which includes re-infections, viral persistence, and genomic insertion in some cases.

Our HIV-positive patients had a profile consistent with national reports.[26] Most of them received antiretroviral therapy and had good levels of LTcd4+ and low viral loads. The recurrent ECA were among the most common STD.

No differences in clinical parameters of the lesion were identified in HIV-positive patients with ECA that progressed to IEN. Among the factors associated with neoplasia studied here, we did not find significant differences, possibly due to the number of cases. Currently there are no clinical markers that would allow an early diagnosis of IEN with a first symptom of ECA, and it seems necessary to perform biopsy and genotype all HIV positive patients with ECA before treatment.

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Source of Support: O.A.I.C. Hospital J.J. Aguirre and Virology Program, I.C.B.M., Faculty of Medicine, University of Chile.

Conflict of Interest: None declared.