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Screening-Related Factors in Anal Canal Lesions in HIV-Positive Patients

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Abstract

The incidence of anal dysplasia in HIV-positive men who have sex with men (MSM) is increasing. This dysplasia is described as either high-grade anal intraepithelial (HAIN) or low-grade anal intraepithelial (LAIN). Anal squamous cell carcinoma (ASCC) is more frequent in HIV-positive patients and is related to human papillomavirus (HPV) infections, conditions of low systemic immunity and long use of highly active antiretroviral therapy. The anal Pap smear protocol and high-resolution anoscopy seem to be effective for screening for anal dysplasia and early anal cancer. The aim of this study was to identify some factors that could raise the risk of HAIN and LAIN among HIV-positive patients. We evaluated 134 HIV-positive and HIV-negative patients who had previously had anal intercourse, at CRT/AIDS São Paulo from October 2011 to March 2012. HIV-negative patients and HIV-positive patients without recent CD4 lymphocyte counts were excluded. All of the patients underwent the anal Pap smear protocol and high-resolution anoscopy. The anal lesions were treated with 90% trichloroacetic acid (TCA) and 5% imiquimod for 12 weeks. The patients with residual lesions were operated. The statistical analysis was performed using Student's t test and the significance level was set at less than 5%. 101HIV-positive patients were evaluated (91 males and 10 females), among whom 31 had warts and 3 had LAIN and warts. One patient was operated after the treatment with 90% TCA and 5% imiquimod. In conclusion, HIV-positive patients with anal warts presented low CD4 lymphocyte counts and anal dysplasia.

Keywords: Acquired immunodeficiency syndrome; Anal canal; Carcinoma; Squamous cell; HIV; Human papilloma virus; Trichloroacetic acid; Imiquimod

Introduction

The incidence of anal squamous cell carcinoma (ASCC) and anal dysplasia is currently increasing significantly. This dysplasia is described as either high-grade anal intraepithelial (HAIN) or low-grade anal intraepithelial (LAIN). Factors that are important in such cases include epidemic infection caused by the human immunodeficiency virus (HIV), conditions of low systemic immunity, long use of highly active antiretroviral therapy (HAART), coexistence with preceding anal intercourse (i.e. among men who have with men, MSM) and infection caused by the human papillomavirus (HPV) [1].

Infection caused by HPV is more common in HIV-positive patients than in HIV-negative patients [2]. HIV infection has changed the epidemiological profile of ASCC [3]. Previously, ASCC was evident in elderly female patients, whereas nowadays young male patients with this cancer are seen [3]. ASCC is now considered to be directly related to sexual transmitted disease, since it has been shown to have a close relationship with HPV infection [4].

Thus HIV-positive patients have less tolerance and lower response to chemotherapy and radiotherapy prescribed for ASCC [5] than do HIV-negative patients. However the data is controversial, Abramowitz et al. found similar rates of toxicity during chemotherapy for HIV-positive and negative patients with ASCC [6]. The HAART administration during chemotherapy may enable the development of antitumor responses and does not appear to reduce cancer risk overall [7].

The screening procedures for LAIN and HAIN include anal Pap smears and high-resolution anoscopy [8]. These seem to be effective in screening for anal dysplasia and early anal cancer. Recent studies have suggestedthat screening programs are cost-effective among the population that previously has had anal intercourse [9,10]. The aim of the present study was to identify some factors that could raise the risk of HAIN and LAIN among HIV-positive patients [1].

Material and Method

The study is a cross-sectional descriptive case detection, conducted at two study groups: HIV-positive and HIV-negative patients. We evaluated 134 HIV-positive and HIV-negative patients who had previously had anal intercourse, at CRT/AIDS São Paulo-Brazil from October 2011 to March 2012. The study was approved by the Board of Ethics in Research of CRT/AIDS São Paulo.All of these patients had been referred to undergo the anal lesion screening protocol by infectologists. These patients had previously had anal intercourse and either were or were not infected with HIV. The HIV-positive patients were under treatment with HAART.

The screening consisted firstly of a general clinical evaluation and this was followed by an anal Pap smear and high-resolution anoscopy. The anal Pap smear was collected from the anal canal using a cytobrush, the swab was rotated in a cone-shaped arc against the anal canal for 30 seconds smeared onto a glass side and immediately fixed in ethanol for cytological evaluation [2,11]. The specimens were analyzed by a pathologist and reviewed by a senior pathologist.They were classified as normal, atypical squamous cells of undetermined significance (ASCUS), LAIN and HAIN [12].

The high-resolution anoscopy was performed after topical

application of 3% acetic acid in the anal canal for 2 minutes. It was observed the presence of the lesions, location in anal canal, tinctorial characteristics, aspect, surface and vascular profile according to a modified version of the Barcelona classification [13].

The patients with anal warts that were identified during the anoscopy were treated with 90% trichloroacetic acid (TCA) and 5% imiquimod for 12 weeks [14,15]. One patient had a residual lesion after the clinical treatment and was operated. His biopsy showed low-grade dysplasia and his evolution was good, without any recurrence.

Results

11 women and 123 men were evaluated. The mean age among the HIV-negative patients was 34.48 years and the mean age among the HIV-positive patients was 44.51 years. One woman was HIV-negative and ten were HIV-positive. We had 32 HIV-negative men and 91 HIV-positive men. One HIV-negative man showed LAIN and warts during high-resolution anoscopy. Nine HIV-negative men had normal Pap smears but showed warts during high-resolution anoscopy.

31 HIV-positive patients had warts during high-resolution anoscopy. Three of them were women and 28 were men. Three HIV-positive male patients had LAIN and warts during high resolution anoscopy; one of them had a residual lesion after the clinical treatment and was operated. The pathologist confirmed that the latter case consisted of LAIN, after the surgery. Two HIV-positive patients had LAIN, but the high-resolution anoscopy was normal. These patients were subsequently reevaluated every six months.

We did not identify any cases of ASCUS, HAIN or ASCC. All of the results were analyzed by means of the Student's t parametric test, using the SPSS software in its version 9.0, and were found to be statistically significant at the 5% level.

Analysis on the HIV-positive individuals showed that low serum CD4 T lymphocyte counts were a determining factor for the appearance of anal warts. The mean CD4 T lymphocyte count for the patients without warts was 701 and the mean CD4 T lymphocyte count for the patients with anal warts was 454.67 (p=0.002). There was no statistical difference among the patients with anal warts with regard to the HIV viral load (p=0.196). There was no statistical difference among the mean CD4 T lymphocyte counts with regard to the Pap smear results (p=0.408). The evaluation of LAIN among HIV-positive patients with anal warts was statistically significant (p=0.004).

We estimated the statistical relationships between the variables in order to analyze the impact of CD4 T lymphocyte count on the presence of anal warts. The presence of anal warts was a binary variable, i.e. the values were taken to be 0 or 1, such that 0 was defined as absence of anal warts and 1 as presence of anal warts. Because of the nature of this variable, it was decided to model the relationship between the variables using a set of models of discrete dependent variables, which in a special case applies to binary values. Probit and logit are models of this nature.

One big problem when estimating a simple linear relationship for binary dependent variables is that the forecasted value may lie outside of the interval from 0 to 1. The abovementioned models solve this problem by using the independent variable within a cumulative distribution function for the independent variable. Probit assumes a cumulative distribution function for the standard normal distribution, and its functional form is defined as:

$$\Pr(y_{i=1}|x_i',\beta) = 1 - \Phi(-x_i'\beta) = \emptyset(x_i'\beta) \tag{1}$$

On the other hand, logit assumes a cumulative distribution function for the logistic distribution [16], and its functional form is given by:

$$\Pr(y_i = 1|x_i',\beta) = 1 - \frac{e^{-x_i'\beta}}{1+e^{-x_i'\beta}} = \frac{e^{x_i'\beta}}{1+e^{x_i'\beta}} \tag{2}$$

These two models were estimated (i.e. the values for β that best fitted the data were determined) with and without a constant term, using the STATA software in its version 12. When present, the constant term represented the minimum level for the dependent variable. The latter was dubbed logit1 and probit 1 and the former, logit 2 and probit 2. The results from the estimations are presented in Table 1.

The most important result in Table 1 is that there was a negative and statistically significant relationship between CD4 T lymphocyte count and the presence of anal warts. Hence, higher CD4 T lymphocyte counts should diminish the chances of observing warts in the patient. The presence of a constant was statistically significant at the level of 5% in the logit model and at the level of 6% in the probit model (shown in the column labeled "P-value"). Since in both cases the constant seems to add explanatory power, we deem the models to be valid. Another important point to take into consideration is that their values do not translate directly into the values of the dependent variable, but rather, should be plugged into equations (1) and (2) to produce the probability that the patient will present anal warts.

In this regard, since the functions appear on the right-handside of equations (1) and (2), the impact of the independent variable (anal warts) on the dependent variable (CD4 T lymphocyte count) does not follow directly from the estimated coefficients.In these cases, the traditional approach for determining the impact is to calculate the marginal effects, which is done by deriving each function in relation to the independent variable.

It is important to remember that the numerical result from this depends on the point estimate of the independent variable. Here, we took two values: 454.67, which was the mean serum CD4 T lymphocyte value found in HIV-positive patients with anal warts; and 625.17, which was the mean serum CD4 T lymphocyte value found in the HIV-positive patient group. These outcomes are presented in Table 2.

Model	Coefficient	Estimate	Standard error	P-value	McFadden R-squared
Logit1	CD4	-0.0017	0.00	0.00	-
Logit2	Constant	1.1768	0.61	0.05	0.117
	CD4	-0.0036	0.00	0.00	
Probit1	CD4	-0.0010	0.00	0.00	-
Probit2	Constant	0.6765	0.36	0.06	0.115
	CD4	-0.0021	0.00	0.00	

Table 1: Estimation of probit and logit models for CD4 T lymphocyte count estimates in HIV-positive patients

Model	Marginal effect	Standard error	P-value	Point estimate
Logit1	-0.0003682	0.00	0.000	454.67
	-0.0003249	0.00	0.000	625.17
Logit2	-0.0008504	0.00	0.002	454.67
	-0.0006838	0.00	0.000	625.17
Probit1	-0.0003729	0.00	0.000	454.67
	-0.0003371	0.00	0.000	625.17
Probit2	-0.0008016	0.00	0.001	454.67
	-0.0006834	0.00	0.000	625.17

Table 2: Marginal effects of probit and logit models on CD4 T lymphocyte values in HIV-positive patients.

The interpretation of the marginal effect in each model is direct, in terms of the effect of the independent variable (CD4 T lymphocytes) on the dependent variable (presence of anal warts). For example: in the logit 1 model (without constant), using a value of 454.67 for CD4 T lymphocytes, an increase of one unit of CD4 T lymphocytes reduced the independent variable by 0.0003682. If the chosen point estimate was 625.17, the reduction would be 0.0003249. The same interpretation was valid for the other models, and all the results were statistically significant at the 5% level. Obviously, the independent variable did not assume values different from 0 or 1, but the estimation of the marginal effects quantified the inverse relationship between CD4 T lymphocytes and the presence of anal warts.

Discussion

We identified low CD4 T lymphocyte count as an important factor relating to appearance of anal warts. Presence of this finding stimulated a broad statistical analysis that showed its impact on anal warts. Other factors were not statistically different, such as HIV viral load in relation to presence of anal warts; and CD4 T lymphocyte count in relation to Pap smear results.

It has been suggested that prolonged administration of HAART provides long-term protection against the development of anal dysplasia [17]. Only one patient had a residual anal lesion after the topical treatment, and this patient then underwent surgery. This patient could have had HAIN or ASCC, as described by Schlecht et al. but fortunately this did not happen [18]. We did not find ASCUS in our sample.

Use of 5% imiquimod for 12 to 16 weeks had regressive action on anal dysplasia, decreased recurrence after treatment and had anti-HPV action. Our data showed better results from treatments using 5% imiquimod than reported in the literature: previous data showed that the expected clinical response would be around 48% [19].

The incidence of HAIN and LAIN on Pap smears from MSM has been described as high, but this was not found in our study. For all that, we had a senior pathologist reviewing our entire sample and the technique performed for the swab was the same as described in the literature [1,2,11]. The common factor found was that all HIV-positive patients should be screened for anal lesions [2] and that, if available, hybrid capture data for HPV should also be gathered [20,21]. Recent studies have elucidated that detection of HPV oncogenes could be specific for detection of HAIN and LAIN [22].

Alterations seen on Pap smears and through high-resolution anoscopy, and presence of HPV infection, are known to be the major predictive factors for HAIN and justify screening for these patients [23]. High-resolution anoscopy provides additional evaluation even for patients with negative Pap smears. One of the possible reasons for the high frequency of findings of negative Pap smears in our study is that the samples were not collected under direct viewing, as performed in cervical cytological evaluations, for which the screening programs are well-established [24-27].

Conclusion

In conclusion, the low serum CD4 T lymphocyte counts were an important factor in relation to the presence of anal dysplasia and warts in HIV-positive patients.

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