Angiogenesis in squamous intraepithelial neoplasia of the uterine cervix in HIV-seropositive women

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Summary

Objectives: This study aimed to quantify angiogenesis in squamous intraepithelial lesions of the uterine cervix in seropositive HIV patients as well as to establish a relationship between vascular density and variations in the CD4+ lymphocyte titer and the viral load of human immunodeficiency virus (HIV). *Methods:* 125 patients, 55 HIV seropositive and 70 seronegative, were allocated with respect to grade of squamous intraepithelial lesion (SIL). The obtained samples were stained with an immunohistochemical marker for CD34 antigen and vessel counts were performed in ten consecutive fields at 400x magnification. The seropositive HIV patients were distributed into groups according to the CD4+ index and HIV viral load. *Results:* Seropositive HIV patients presented a higher mean vascular density (MVD) than the control group, even in the absence of cervical intraepithelial lesions. High- and low-grade lesions in the presence of HIV seropositivity presented higher MVD than that found in seronegative HIV patients. There was no significant variation in the MVD and CD4+ count ratio or viral RNA-HIV load, except for high-grade (H)SIL. *Conclusions:* Infection with HIV influenced angiogenesis of uterine cervix in the presence of squamous intraepithelial lesions and more significantly in HSIL.

Key words: Angiogenesis; HIV; CIN; SIL.

Introduction

Since the first description of acquired immunodeficiency syndrome (AIDS), infection with the human immunodeficiency virus (HIV) has reached alarming proportions, especially in the female population. In Brazil, from 1980 to 2003 a 10.2% increase in notifications of male individuals was observed while for women there was a 75.3% increase during the same period [1, 2]. Several studies relate infection with human papillomavirus in immmunosuppressed patients to high rates of intraepithelial neoplasia and cervical carcinoma [3-11]. The existence of cervical neoplasia in HIV-infected women represents one of the most serious challenges to oncologic treatment of immunosuppressed patients. Recent data suggest that immune response could play a more prominent role in HPV replication and in the development of early disease such as low-grade intraepithelial neoplasia [12].

On colposcopy, neoplasias of the uterine cervix are shown as alterations in the vascular net and in columnar and squamous epithelia [13].

Concomitance of HIV infection and cervical intraepithelial lesions shows clinical presentations which are more aggressive and resistant to treatment [14, 15]. This fact aroused our interest regarding the eventual significance of angiogenesis in HIV-seropositive patients with cervical intraepithelial lesions, which is the main purpose of this study.

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Patients and Methods

One hundred and twenty-five patients from the Colposcopy and Transmissible Sexual Diseases and AIDS Services of Amapá state and from the Pathology Sector of the Outpatient Clinic of the Inferior Genital Tract, and from the Department of Gynecology of UNIFESP-EPM during the period from November 2001 to May 2004 were included. All patients signed their free and informed consent, previously approved by the Ethics in Research Committee of UNIFESP-EPM.

The age of these patients ranged from 17 to 62 years, with a mean of 34.30 years. The patients were divided into two groups according to HIV-seropositivity as follows: HIV-seropositive group and HIV-seronegative group. Of the 55 HIV-seropositive patients, 20 a had a high-grade squamous intraepithelial lesion (HSIL – group A), 20 low-grade squamous intraepithelial lesion (LSIL – group B) and 15 had no intraepithelial lesion (group H). The 70 HIV-seronegative patients constituted the following subgroups: 30 HSIL (group D), 20 LSIL (group E) and 20 patients with a normal cervix (group C). The four groups, A,B,D and E were further divided into classes 1 and 2, according to paired colposcopic directed biopsies performed on the same patient, from areas at the site of a lesion and at the site of normal mucosa, respectively.

Histopathological processing of the biopsy products followed the standard protocol of the Pathology Service of the Hospital of Amapá and the Department of Pathology of UNIFESP-EPM. The same was applied to cervical fragments obtained from surgical samples of the patients submitted to hysterectomy due to uterine leiomyoma in the control group (C).The material for immunohistochemical processing consisted of one fragment for each patient from groups C and H and two from each participant in groups A,B,D and E, totaling 215 slides.

In order to identify the vascular structures, cells containing CD34 antigen were marked by brownish colored staining using

monoclonal mouse antibodies at a 1:50 dilution (Monoclonal Mouse Anti-Human CD34, class II, clone QBEnd-10, Code M 7165 – Dakocytomation). A positive control was performed for the evaluation of primary antibody efficacy using tissue fragments of human amygdalae.

Vessel count was performed using a light microscope with 10x and 40x objectives and 10x ocular to select the area of greatest vascular density. Vessel count was performed at 400x magnification with the help of the Kontron Imaging System KS 300 computer program.

Ten consecutive histological fields were evaluated in each slide, taking care to mark the last vessel as a reference point to avoid counting the same element twice. It was established that the total vessel number would be the result of the sum of the number of vessels counted in each of the ten fields. The vascular unit also had to show presence of brown-stained cells bordering the lumen space. Partially identified vessels were discarded.

For the quantification of CD4+ lymphocytes, flow cytometry was applied to the total blood sample. In this study a cutoff value of 200 cells/mm^3 was applied [15-17].

HIV load was determined by the RT-PCR (Reverse Transciptase-Polymerase Chain Reaction – Real Time Roche Molecular Systems). The cutoff value count used was 10,000 viral copies/ml plasma, higher values being considered high viral load [15, 18].

The Student's t-test was used for comparison of variables regarding vessel number in relation to independent groups described as HIV and high-grade (A1 and A2) and low-grade (B1 and B2) lesions; HIV-seronegative patients with high-grade (D1 and D2) and low-grade (E1 and E2) lesions; as well as controls (C) and seropositive women without intraepithelial lesions (H). The paired t-test was used to compare different colposcopic sites in the same patient of each group in relation to the variable: number of vessels.

Pearson's chi square test (X^2) was applied for comparison between groups A1, A2, B1, B2 and H in relation to the variables categorized as HIV load and CD4+ lymphocyte levels. The SPSS version 8.0 software was used for statistical analysis. Level of significance was set at 5% ($\alpha = 0.05$, $p \leq 0.05$).

Results

The mean number of vessels present in the studied patients as related to HIV seropositivity and diagnosis of a squamous intraepithelial lesion was compared with the control group (C) (Figure 1). Mean vascular density (MVD) found in all groups showed to be significantly higher than that of the control group.

On analyzing the different high- and low-grade intraepithelial lesion groups regarding the presence or not of a colposcopic alteration and varying only in HIV-seropositivity, the parametric Student's t-test showed that HIV-seropostive patients had a higher MVD than HIV-seronegative, except for groups with low-grade intraepithelial lesions in areas described as being normal on colposcopy (B2 and E2) (Table 1).

The parametric paired t-test was applied to evaluate vascularization in areas with colposcopic alterations (Table 2). A statistical difference was confirmed between values of group A ($p < 0.001$), but this did not occur in group D ($p = 0.062$). A similar evaluation was applied to patients with LSIL (groups B and E). A significant difference was detected only for group B seropositive patients ($p = 0.002$).

After morphologically dividing high-grade lesions into CIN III and CIN II, it was observed that vessel count in areas of colposcopic alterations significantly differed between CIN III and CIN I both in the HIV-seropositive ($p = 0.04$) and the HIV-seronegative groups ($p = 0.04$).

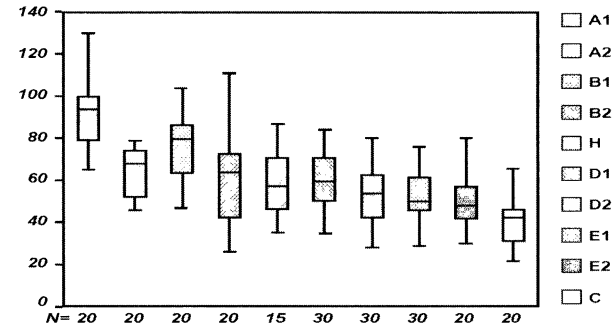

Figure 1. — Means of vascular densities in HIV-seropositive and HIV-negative patients and controls with or without high- and low-grade squamous intraepithelial lesions.

Student's t-test
A1 X C: $p < 0.001$*
A2 X C: $p < 0.001$*
B1 X C: $p < 0.001$*
B2 X C: $p = 0.001$*
D1 X C: $p < 0.001$*
D2 X C: $p = 0.001$*
E1 X C: $p = 0.001$*
E2 X C: $p = 0.01$*
C X H: $p < 0.001$*

Legend:
A1 - HSIL/with image/ HIV +
A2 - HSIL/without image/ HIV +
B1 - LSIL/with image/HIV +
B2 - LSIL/without image/ HIV +
H - absence of SIL/HIV +
D1 - HSIL/with image/ HIV –
D2 - HSIL/without image/ HIV –
E1 - LSIL/with image/ HIV –
E2 - LSIL/without image/ HIV –
C - control

Table 1. — *Distribution of the means of vascular densities in HIV-seropositive and HIV-seronegative patients with high- and low-grade squamous intraepithelial lesions.*

Group	A1	D1	A2	D2	B1	E1	B2	E2
Mean	92.00	59.36	63.35	52.63	75.80	52.70	60.05	50.05

Student's t-test

A1 X D1: $p < 0.001$*	CI 95%: 23.74 – 41.51
A2 X D2: $p = 0.003$*	CI 95%: 3.78 – 17.64
B1 X E1: $p < 0.001$*	CI 95%: 14.40 – 31.79
B2 X E2: $p = 0.083$	CI 95%: 0 – 21.38

Legend:
CI - confidence interval
A1 - HSIL/with image/ HIV +
A2 - HSIL/without image/ HIV +
B1 - LSIL/with image/HIV +
B2 - LSIL/without image/ HIV +
D1 - HSIL/with image/ HIV -
D2 - HSIL/without image/ HIV –
E1 - LSIL/with image/ HIV –
E2 - LSIL/without image/ HIV –

Table 2. — *Distribution of the means of vascular densities in HIV-seropositive and HIV-seronegative patients.*

	HIV positive	HIV negative		
HSIL subgroups	MVD	MVD	CI 95%	p value
A1	92.00		21.21-36.08	< 0.001*
A2	63.35			
D1		59.36	0-13.82	0.062
D2		52.63		
LSIL subgroups				
B1	75.80		6.28-25.21	0.002*
B2	60.05			
E1		52.70	0-9.86	0.451
E2		50.05		

Paired t-test

Legend:
CI - confidence interval
HSIL - high-grade squamous intraepithelial lesion
LSIL - low-grade squamous intraepithelial lesion
MVD - mean vascular density
A1 - HSIL/with image/ HIV +
A2 - HSIL/without image/ HIV +
B1 - LSIL/with image/HIV +
B2 - LSIL/without image/ HIV +
D1 - HSIL/with image/ HIV –
D2 - HSIL/without image/ HIV –
E1 - LSIL/with image/ HIV –
E2 - LSIL/without image/ HIV –

However, comparison between CIN II and CIN I showed significance only for HIV-seropositive patients (p = 0.02). This relationship was not replicated when evaluating vascularization in the areas described as normal on colposcopy.

No significant difference (p = 0.760) was observed on comparing the different HIV-seropositive groups according to variation in CD4+ lymphocyte number. Confronting CD4+ lymphocyte indices and MVD in HIV-seropositive patients, no significant difference could be observed between the evaluated groups, with the exception of patients with high-grade intraepithelial neoplasia in the presence of CD4+ lymphocyte count higher or equal to 200 cells/mm^3.

The hypothesis of the association of histopathological diagnoses in HIV-seropositive groups with variation in plasma HIV-RNA load was verified by applying the chi square test. No such association could be evidenced (p = 0.112). No relationship between vascular density and variations in plasma HIV-RNA load was detected, except for HIV-seropositive HSIL patients in areas of colposcopic alterations (group A1) and viral load less than 10,000 copies of HIV-RNA/ml plasma.

No statistical difference between vascular density and the use of antiviral therapy was found.

Discussion

When areas of the same histopathological diagnosis are compared, differing only in HIV serology, we noted that HIV-positive cases of patients with both high- and low-grade intraepithelial lesions presented a statistically significant increase in vascular density. This fact is probably due to factors related to the presence of HIV, such as repercussion of a local inflammatory response.

In our material, through biopsies from areas with vascular alterations visible on colposcopy and areas described as being free of alterations on colposcopy, it was possible to observe greater vascular densities in the connective stroma. Statistical significance, however, could only be confirmed for HIV-seropositive patients (groups A1 p = 0.001 and B1 p = 0.002). HPV-HIV interaction could result in greater expression of angiogenensis in seropositive patients at sites of clinical squamous intraepithelial lesions (groups A1 and B1). This fact seems to be due to exacerbated metabolic activity and subsequent increase in vascular supply.

The difference between the angiogenic values in areas with colposcopic alterations histopathologically diagnosed as CIN III and CIN I was statistically significant, independent of HIV-seropositivity (p = 0.04). The finding of more pronounced neovascularization in the more severe lesions could be considered an important prerequisite for the development of invasive clones.

Quantification of CD4+ lymphocytes below 200 cells/mm^3 was present in 40% of the cases with high- and low-grade intraepithelial lesions, but was not statistically significant between the groups. In our study, a little more than half of the patients with low-grade intraepithelial lesions presented indices higher than 10,000 copies of HIV-RNA/ml plasma. On the other hand, among the patients with high-grade squamous lesions the same viral indices occurred in eight (40% of the cases). It is curious to observe that the same fact occurred in only 20% of the cases (3 patients) among the 15 HIV-seropositive patients without any intraepithelial lesions. From what has been presented we can deduce that high-grade lesions in HIV-seropositive patients do not depend only on HIV load.

We did not observe a significant correlation between the MVD variation and plasma HIV-RNA load, except for patients with high-grade lesions with a viral load less than 10,000 HIV-RNA copies/ml plasma.

In our study, variation in MVD in the different groups of HIV-seropositive women, with or without antiretroviral therapy, was not significant between the groups, as described by Ellerbrock *et al.* [19] and Conley *et al.* [20].

Conclusions

It can be concluded that HIV-infected patients with cervical intraepithelial neoplasia present a greater vascular density, more significantly in those with HSIL. There was no significant variation in the relationship between vascular density and CD4+ lymphocyte count which also did not significantly vary among HIV-seropositive patients regarding viral load. We believe that further studies are required in order to be able to understand interrelations between viral infection, immunosuppression, angiogenesis and neoplasia in HIV-seropositive patients, in addition to the effect of antiretroviral therapy.

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