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Antinuclear antibodies in patients with cervical lesions and invasive cervical cancer

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ABSTRACT

Background: Antinuclear antibodies (ANA) have been found in several types of cancer although the meaning of its presence is not completely known.
Aim: To study the prevalence of ANA in patients with cervical intraepithelial lesion and invasive cervical cancer.
Methods: A total of 205 women who underwent screening for cervical cancer or treatment at the Erasto Gaertner Cancer Hospital in Curitiba - Brazil, were enrolled in the study. Based on their latest cervical colposcopy-guided biopsy results, they were divided into four groups: CIN-I: 19.4%; CIN-II: 24.0%; CIN-III: 24.0%; and invasive cancer: 32.4%. As control were studied 68 healthy controls. ANA was searched by immunofluorescence in Hep-2 cells evaluating the pattern and titer.
Results: Controls had 4/68 (5.8%) of ANA positivity and patients with CIN and invasive cancer had 15.1% ($p = 0.001$). Patients with CIN-I and CIN-II had the same prevalence of ANA as controls ($p = 1.0$ and $p = 0.11$ respectively), but not those with CIN-III ($p = 0.03$) and invasive cancer ($p = 0.05$). The most common ANA immunofluorescence pattern was fine speckled pattern (38.7%) and fine dense speckled pattern (38.7%); the mean titer was 1:160.
Conclusion: ANA is more common in invasive cervical lesions than in controls or non invasive lesions. To understand the meaning of this finding more studies are needed.

1. Introduction

Cancer and autoimmunity can influence each other. If on the one hand, the immune system surveillance plays a crucial role avoiding the appearance of neoplastic cells, on the other, cancer cells, that suffer mutagenesis, display several antigenic proteins unknown to the immune system. Those, not being recognized as self, may lead to appearance of autoimmune phenomena [1].

Several authors have described the occurrence of autoantibodies classically found in systemic rheumatic diseases, such as antinuclear antibodies (ANA) in cancer patients [2]. They have been found in patients with lymphomas, thymomas, breast, renal and lung cancer [2–5]. Curiously, some studies have shown that autoantibodies in cancer patients may appear prior to the diagnosis, in early stages of tumorigenesis [6].

Cervical cancer (CC) is one of the leading causes of morbidity and mortality among women in developing countries. This form of cancer is preceded by a pre-invasive phase known as cervical intraepithelial neoplasm (CIN) that is categorized in CIN I, II and III, depending on the proportion of the epithelium thickness affected [7]. CIN-I lesions may reverse to normal [8] but CIN II and III are considered cancer precursors [9].

Studies in the prevalence of ANA in cervical cancer are rare. Nelson et al [10], studying the sera of patients with adenocarcinoma and squamous cell carcinoma of the cervix, found that 25% of them had detectable ANA.

In the present study, we aimed to know the prevalence of ANA antibodies in a female sample with cervical lesions and to analyze if its prevalence differs in patients with CIN from invasive cancer.

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2. Methods

This is a cross sectional study approved by the local Committee of Ethics in Research with a convenience sample of 205 patients and 68 auto declared healthy controls. All participants signed consent. This study was carried out at the Erasto Gaertner Cancer Hospital (HEG) in Curitiba, southern Brazil, which is a reference center for the treatment of gynecological malignancies.

All subjects were followed at the out-patient clinic of the HEG and were consecutively included from a period of one year (from July 2014 to June 2015). Inclusion criteria were cervical cancer screening and treatment at HEG. We also collected historical data on disease progression from patients' medical records. Exclusion criteria were pregnancy, HIV-positivity, systemic infection, autoimmune disease, and blood transfusion within the last 60 days. Written informed consent was obtained from all patients. Controls were obtained from the hospital staff.

Five ml of venous blood was obtained for each patient and control; serum aliquots were stored at −80 °C until the assays were performed. ANA were screened with indirect immunofluorescence on HEp-2 cells, using the commercially available ImunoCon ANA HEp-2 (WamaDiagnóstica, São Paulo, Brazil) as recommended by the manufacturer. Values from 1:40 to 1:80 were considered positive in low titer, from 1:160-1:320 as medium and > 1:640 as high titers [11]. The fluorescence patterns were classified as fine speckled, coarse speckled, homogeneous, peripheral and cytoplasmic pattern [12]. A single individual, blind for clinical data, read all tests.

A group of 205 women was included in this study. Based on their latest cervical colposcopy-guided biopsy, the subjects were divided into four groups: low grade CIN-I: n = 39; moderate CIN-II: n = 49; CIN-III: n = 49; and invasive cancer (Ca): n = 68. The loop electrical excision procedure or cold-knife cone excision was used to confirm the previous biopsy results. The prevalence of ANA was compared among them.

Comparison testes were done with the Fisher test (nominal data) and Mann Whitney test (numerical data). The significance adopted was of 5%.

3. Results

Among the 205 women the mean age of cervical lesion patients was of 36 years (range 15.0–78.0) and controls of 41.5 years (range 23.0–60.0) with p = 0.20.

In the control sample 4/68 (5.8%) and in the cervical lesion group of patients, 31/205 (15.1%) were ANA positive (p = 0.05; OR = 2.85; 95%CI = 0.96–8.39).

The cervical lesion group of patients had 39/205 (19.0%) of CIN-I; 49/205 (23.9%) of CIN-II; 49/205 (23.9%) of CIN-III and 68/205 (33.1%) of patients with invasive carcinoma. The ANA prevalence, patterns and titers in controls and patients according to CIN or presence of invasive carcinoma is on Table 1.

When each group of cervical lesion patients was compared to controls, the following results were obtained: controls (5.8%) vs CIN-I (5.1%) with p = 1.0; controls (5.8%) vs CIN-II (16.3%) with p = 0.11; controls (5.8%) vs CIN-III (18.3%) with p = 0.03 (OR = 3.3; 95%CI = 1.07–10.4) and controls (5.8%) vs invasive carcinoma (17.6%) with p = 0.05 (OR = 3.4; 95%CI = 1.04–11.2).

The comparison of ANA prevalence between the CIN-I sample with CIN-II and with CIN -III showed no differences (p = 0.17 and 0.10 respectively). The comparison of CIN-I with invasive carcinoma showed a tendency to differ (p = 0.07) as well as of CIN-I with CIN-II + III (p = 0.09). No differences were found in the comparison of CIN-III and invasive carcinoma group (p = 1.0).

In the cervical lesion sample with ANA positive, 13/31 (41.9%) had fine dense speckled pattern; 12/31(38.7%) had fine speckled, 5/31 (16.1%) had cytoplasmic and 1/31 (3.2%) had nucleolar pattern.

4. Discussion

The present study shows that patients with cervical lesions had a higher prevalence of ANA than controls. When the sample was divided in those with CIN or invasive cancer, it was possible to note that females with CIN-I and II did not differ from controls. However, those with CIN-III and invasive carcinoma had a higher rate of positivity than control group and similar prevalence among themselves, showing that this autoantibody is more common in those with more invasive lesions.

The meaning of ANA presence in cancer is not completely understood. Three hypothesis try to explain it. The first is that they are just epiphenomena secondary to appearance of an aberrant tumoral autoantigen [13]. According to this theory, they may not participate in the tumorigenesis process but they could be useful as biomarkers of tumor presence. Autoantibodies such as p62, Koc and CENP-F have already been used as an indicator for hepatocarcinoma [14,15]. Guffoy et al [16] noted that the appearance of ANA with anti-pseudo-PCNA type 1 pattern should lead to the search of solid or hematologic cancers instead of rheumatic diseases. The second hypothesis states that these autoantibodies may play a protective role against neoplastic cells. ANAs have anti-tumor activity that could be mediated by antibody-dependent cell-mediated cytotoxicity [1]. Some antibodies such as the 3E10 (an anti-DNA antibody) are capable of penetrating into living cells nuclei, binding to DNA and impairing its repair pathways. This promotes apoptosis in pre-cancer cells with DNA abnormalities such as those with BRCA2 deficiency but not in normal cells [17,18]. Clinical data on this role is controversial [4,5,19]. Blaes et al [19] studying non-small cell lung cancer, found that the presence of ANA in the serum was associated with a better prognosis. Another study [4], in diffuse large B cell lymphoma found that ANA positive patients had a better outcome than

Table 1
Prevalence, titer and pattern of ANA in cervical lesion patients (n = 205) and controls (n = 68).

Sample	Mean Age (Years)	N of positive ANA patients	Median titer (range)	ANA pattern
Controls	41.5 ± 11.8	4/68 (5.8%)	1:80 (1:80-1:320)	FDSP -50% Fine speckled -25% Homogeneous -25%
CIN-I (n = 39)	30.0 ± 8.7	2/39 (5.1%)	1:120 (1:80-1:160)	FDSP = 50% Fine speckled -50%
CIN-II (n = 49)	34.8 ± 11.1	8/49 (16.3%)	1:80 (1:80-1:160)	FDSP- 37.5% Fine speckled -37.5% Cytoplasmic -12.5% Nucleolar-12.5%
CIN-III (n = 49)	36.6 ± 11.9	9/49 (18.3%)	1:80 (1:80-1:160)	FDSP-22.2% Fine speckled- 44.4% Cytoplasmic- 33.3%
Invasive carcinoma (n = 68)	48.5 ± 15.5	12/68 (17.6%)	1:80 (1:80-1:160)	FDSP- 58.3% Fine speckled-33.3% Cytoplasmic- 8.3%

CIN = intraepithelial carcinoma; ANA = anti-nuclear antibodies; n = number; FDSP = fine dense speckled pattern.

those without it. On the other hand, the presence of autoantibodies in patients with breast cancer was associated with a higher risk of recurrence or metastases [5]. A third possible role is that these autoantibodies are active players in the process of carcinogenesis. Madrid et al [20], studying ANAs in breast cancer has observed that infiltrating B and T cells are present in the tumor tissue. These authors proposed that the immune reaction triggered by antibodies against tumoral antigens results in chronic inflammation that would help in the neoplasm establishment (20).

ANA is the hallmark of systemic lupus erythematosus (SLE) where it is present in 90–100% of the patients [21]. A special link between this disease and cervical cancer has been found, as cervical cancer are more common in lupus patients than in general population [22]. This has been considered secondary to the high prevalence of HPV infection in this population [23]. Klumb et al. [24] showed that SLE women, despite having lower number of sexual partners and higher age at first sexual activity, have been found to have 7.2 times (95% CI = 2.9–7.8) more HPV infections than controls. As SLE patients are immunosuppressed individuals, infections such as HPV are more common [25]. The role of ANA in this context is not known.

The most common immunofluorescence patterns found in the present sample were the fine speckled and fine dense fine speckled. The latter is not associated with rheumatic diseases and it is directed to DFS70/LEDGFp75 that is a multifunctional protein present almost universally in all tissues of the body [26]. DFS70/LEDGFp75 plays important protective role in the signaling of cell apoptosis, in cellular stress survival and DNA repair. It is overexpressed in malignant lesions promoting cell survival, proliferation and migration resulting in tumor growth [25]. Therefore, this protein may be considered a tumor-associated antigen and a stress oncoprotein relevant to several types of cancer. Anti DFS70/LEDGFp75 antibodies may have cytotoxic properties and some authors speculate if the autoantibody-mediated removal of DFS70/LEDGFp75 would confer protective advantage to cancer patients [26].

Our study has several limitations: we did not characterize the antigens causing the ANA positivity. We also do not have HPV serology that could be informative in this context. In addition, this is a cross sectional study and the follow-up of such patients, with prognostic observations would be of interest. However, it does highlight the high presence of ANA in cervical lesions and the association of its presence with invasive lesions.

Concluding, in our sample ANA positivity was higher than controls in patients with invasive cervical lesions. ANA speckled pattern (fine and dense fine) were the most common.

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Conflict of interests

None.

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