



Short Communication

Association between cervical lesion grade and micronucleus frequency in the Papanicolaou test

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Abstract

The aim of this study was to evaluate the association between the frequency of micronuclei (MN) and the cellular changes detected in the conventional Papanicolaou test. One hundred and seventy-four Papanicolaou test smears with cellular changes were examined. MN screening was done in cytopathological smears by counting 1,000 cervical cells in a light microscope. MN frequencies were significantly higher in the group with cellular changes compared to the control group ($p < 0.001$). The mean MN frequencies were 0.95 ± 1.12 (mean \pm SD) in the control group ($n = 223$), 2.98 ± 1.20 in individuals with atypical squamous cells of undetermined significance (ASC-US) ($n = 50$), 4.04 ± 1.45 in cervical intraepithelial neoplasia (CIN) I ($n = 52$), 5.97 ± 1.83 in CIN II ($n = 30$), 7.29 ± 1.55 in CIN III ($n = 17$) and 8.64 ± 1.55 in invasive cancer ($n = 25$). These findings suggest that MN monitoring should be included as an additional criterion for the early detection of cytogenetic damage in routine examinations. This monitoring should be done in the same smear as used for cytopathological examination. More specific and systematic studies are necessary to confirm this proposal.

Keywords: cervical cancer, cervical lesions, micronucleus, Papanicolaou test.

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Cervical cancer is the third most common cancer among women worldwide (Jemal *et al.*, 2011) and the second cause of cancer mortality in Brazilian women (WHO/ICO, 2013). Among all known types of cancer, cervical cancer has some of the best prospects in terms of prevention and cure: when diagnosed early, cervical cancer has up to a 100% chance of cure. However, about 80% of cervical cancer cases occur in developing countries, where neither population-based screening nor optimal treatment is available (NIH report, 2010).

The Papanicolaou (Pap) test is the most common and inexpensive screening method for cervical cancer and has

played an effective role in reducing the prevalence and mortality rates of this cancer among women (Peirson *et al.*, 2013). Given the slow progression of cervical cancer, correct diagnosis is possible in asymptomatic women. In Brazil, the Pap test is the screening strategy recommended by the Federal Health Ministry, with priority for women 25-59 years old (INCA, 2006). Although Brazil was one of the first countries to adopt the Pap test, the disease remains a serious public health problem. From 2003 to 2008, ~87% of Brazilian women were covered by the Pap test (BRATS, 2011). In a recent study of 2,030 women in southern Brazil, 10.9% had never been tested and 12.2% had not been tested in the last three years (Bairros *et al.*, 2011).

Human papillomavirus (HPV) is the etiological agent of cervical intraepithelial neoplasia (CIN) and cervical cancer. Some studies have shown that women infected with

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HPV strains 16 or 18 have a higher rate of progression from CIN to cancer (Bernard *et al.*, 2013). Although HPV infection is responsible for virtually all cervical cancers, in some situations the presence of the virus is not linked to the cellular changes visualized by microscopy. Indeed, one of the most important deficiencies of the Pap test is its limited sensitivity in detecting cervical cancer precursors, coupled with the subjective interpretation of results. As a result, false negatives account for 10-50% of Pap test results (Dehner, 1993). Complementary methods aimed at increasing the sensitivity of screening for cervical cancer have been described, including high-risk HPV testing (Abreu *et al.*, 2012) and micronucleus (MN) identification (Aires *et al.*, 2011; Gayathri *et al.*, 2012). Although HPV testing is considered the gold standard for diagnosing this viral infection and has the potential to improve the effectiveness of screening programs, its implementation must still be kept low-cost in order to be used in low-income populations (Abreu *et al.*, 2012).

The presence of MN is a biomarker that has been successfully used to screen populations at risk of cancer and is a sensitive indicator of genetic damage (Samanta and Dey, 2012). MN are small, additional nuclei formed by the exclusion of chromosomal fragments (clastogenesis) or whole chromosomes that are not incorporated into the main nuclei because of mitotic malfunction (aneuploidy) (Fenech *et al.*, 2011).

Since the evolution from CIN I to CIN III is accompanied by enhanced genetic instability (Hopman *et al.*, 2004), the presence of MN has been used, in combination with cytological findings, as a biomarker of the risk of cervical cancer (Pandey *et al.*, 2011). Previous studies have shown that the frequency of MN increases in parallel with the severity of phenotypic changes in the transition from low-grade to high-grade lesions (Guzmán *et al.*, 2003; Gayathri *et al.*, 2012). In this study, we examined the association between MN frequency and cellular changes detected in the Pap test in a large cohort of women from southern Brazil.

Three hundred and ninety-seven smears were analyzed in the Cediclin Laboratory (Canoas, RS, Brazil) from October 2008 to November 2009. The smears were stained using standard Pap methodology and classified using the criteria described by the Brazilian nomenclature for cervical reports (INCA, 2006). Smears were selected based on the respective original cytological examination report and the results were classified as normal, atypical squamous cells of undetermined significance (ASC-US), CIN I, CIN II, CIN III and invasive cancer (IC). Normal smears, *i.e.*, those in which no cellular change was observed, were used as the control group. The classification ASC-H was not included because of the lack of smears with this diagnosis. This work was approved by the Committee for Ethics in Research at ULBRA/Canoas (protocol no. CEP-2008-601H).

MN screening was done in all selected cytopathological (CP) smears (conventional Pap test) by counting 1,000 cervical squamous cells with a light microscope at a magnification of 1000x (100x objective and 10x eyepiece), according to the criteria defined by Tolbert *et al.* (1992). The MN identification criteria were: (a) a chromatin structure similar to and color intensity similar or lighter than that observed in the main nucleus, (b) an evident edge, similar to a nuclear membrane, (c) a round shape, (d) an intracytoplasmic location and (e) a diameter < 20% of the main nucleus.

The smears evaluated at the Cediclin Laboratory were sent to the Biological Research Institute of the Central Laboratory of Rio Grande do Sul (IPB/LACEN-RS) for external monitoring of quality control. The Brazilian Ministry of Health advocates that a minimum of 10% of the performed exams should be revised (INCA, 2002). The results for MN detection were compared with those for the Pap test of the same smear.

The relationship between MN frequency and cellular changes detected in the Pap test was examined using the software GraphPad PRISM 5.0 (GraphPad Inc., San Diego, CA). All results were expressed as the mean \pm standard deviation. Analysis of variance (ANOVA) was used to compare the MN frequencies among the different lesion classes (ASC-US, CIN I, CIN II, CIN III and invasive cancer) and Dunn's *post-hoc* test was used to compare the groups. A non-parametric ANOVA (Kruskal-Wallis test) was used to analyze the data when they were not homoscedastic or normal. P-values < 0.05 were considered statistically significant.

Of the smears analyzed, 223 had normal CP results (control group) and 174 had cellular changes (case group). The smears with cellular changes consisted of 50 cases of ASC-US, 52 of CIN I, 30 of CIN II, 17 of CIN III and 25 of cervical cancer. Micronuclei were observed in all groups, even in the controls (Figure 1). The MN frequencies in the different groups were 0.95 ± 1.12 (n = 223) in the control

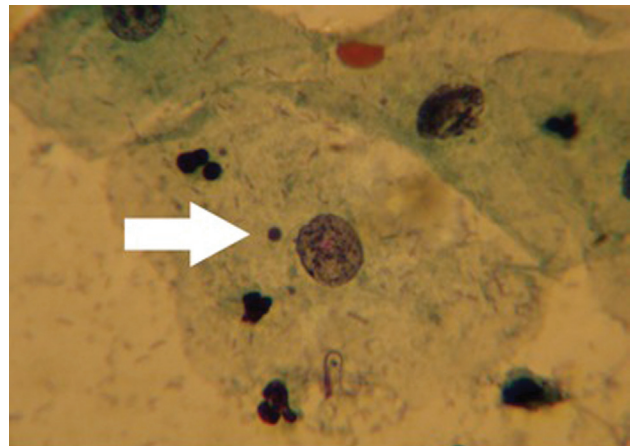


Figure 1 - Cervical smear showing the morphology of a squamous cell with micronuclei (arrow). Magnification: 1000x.

group, 2.98 ± 1.20 ($n = 50$) in individuals with ASC-US cellular changes, 4.04 ± 1.45 ($n = 52$) in CIN I, 5.97 ± 1.83 ($n = 30$) in CIN II, 7.29 ± 1.55 ($n = 17$) in CIN III and 8.64 ± 1.55 ($n = 25$) in cervical cancer. These frequencies were significantly higher in groups with cellular changes compared to the control group ($p < 0.001$) (Figure 2).

The increase in MN frequency observed here could be related to both clastogenic and aneugenic events. The genetic instability caused by HPV infection involves the expression of viral oncogenes E6 and E7 (Duensing *et al.*, 2000; Duensing and Munger, 2004). Whereas E6 expression may result in failed cytokinesis, E7 expression may uncouple centrosome duplication from cell division. Although alterations in centrosome number are random and unpredictable and can lead to chromosomal instability, centrosome status has become a useful parameter for monitoring neoplastic progression and for assessing patient prognosis for some tumor types. In addition, in the absence of a functional TP53 gene, cells may become aneuploid (Braakhuis *et al.*, 2013).

Few studies have scored MN in cervical precancerous and cancerous conditions. To our knowledge, the present study involves one of the largest samples and is the first to report on a population of women from southern Brazil. The MN frequencies observed here were significantly higher in the groups with cellular changes compared to the control group, in agreement with previous case-control studies (Leal-Garza *et al.*, 2002; Gandhi and Kaur, 2003; Cortés-Gutiérrez *et al.*, 2010; Gayathri *et al.*, 2012).

As shown here, there was a positive linear correlation between the number of MN and the risk of cervical cancer. Women with CIN III alterations had higher MN frequencies than those with CIN II lesions, who in turn had higher MN frequencies than CIN I patients. In a study of exfoliated cells from 101 patients the MN frequency was found to be

higher in women with some grade of CIN lesions (CIN I, II and III) and increased with the severity of cellular damage (Campos *et al.*, 2008). A high frequency of MN that increased with the severity of the lesions was also observed in a study of 221 slides involving CIN I, CIN II, CIN III and invasive cancer (Gayathri *et al.*, 2012). Guzmán *et al.* (2003) confirmed the existence of a relationship between lesion grade and MN frequency in epithelial cells of patients who had undergone CP. Although there was no significant increase in MN frequencies in the progression from mild to severe lesions, there was a marked increase in the frequency of micronucleated cells compared to healthy subjects. In contrast, other studies have reported no significant difference between the number of MN and the stages of cervical cancer (Leal-Garza *et al.*, 2002; Gandhi and Kaur, 2003).

One of the challenges of the Pap test is to detect lesions with a high risk of progressing to cancer, particularly since most cellular lesion regress spontaneously without treatment. In this context, it is reasonable to assume that the definition of an MN cell ratio could be useful in discriminating between patients with a low risk and a high risk of developing cervical cancer. The sensitivity of the Pap test in detecting high grade lesions (CIN II and CIN III) has been shown to be only 55.4%, with a specificity of 96.8% (Mayrand *et al.*, 2007). The usefulness of the MN assay for detecting other types of cancer has also been investigated. A recent meta-analysis revealed a significant increase in the basal MN frequency in relation to breast cancer, leading to the suggestion that this test be used to screen women with a family history of this cancer (Cardinale *et al.*, 2012).

The results described here suggest that MN monitoring could be incorporated into routine screening procedures as an additional criterion for the early detection of cytogenetic damage based on the same smear as used in cytopathological analyses. Such monitoring would improve the sensitivity and specificity of the screening and reduce the rate of false-negatives. However, additional detailed, systematic studies are needed to confirm this suggestion.

Acknowledgments

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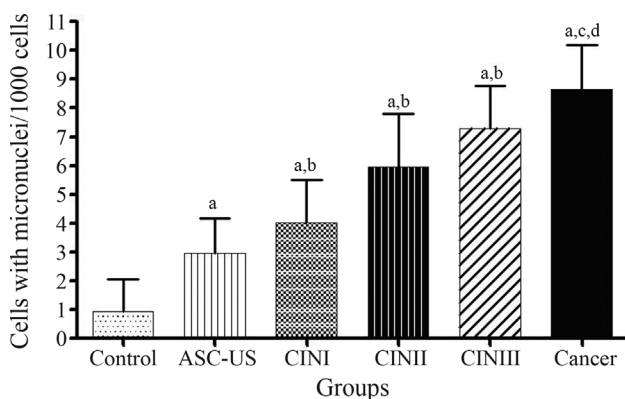


Figure 2 - Frequency of micronuclei in each of the groups evaluated. ^{a,b,c,d}Significant compared to the control ($p < 0.001$), ASC-US ($p < 0.01$), ASC-US ($p < 0.001$) and CIN I ($p < 0.01$) groups, respectively. In all cases, the significance was determined using the Kruskal-Wallis test. ASC-US – atypical squamous cells of undetermined significance, CIN – cervical intraepithelial neoplasia (grades I, II and III), and IC – invasive cancer. The columns are the mean \pm SD of total individual analyzed per group.

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Internet Resources

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