Morphoquantitative Analysis of the Intraepithelial Neoplastic Uterine Cells by Computer Image System

Análisis Morfoquantitativo de las Células Neoplásicas Intraepiteliales Uterinas por Sistema Computarizado

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SUMMARY: The purpose of the present research was to detect the DNA variations by Feulgen stained chromatin and the packing degree of the cervical chromatin in the clinical cases of human uterine carcinomas. Scanning microphotometry was carried out on slides prepared from block Feulgen-stained. Accurate control procedures were performed using normal human cell nuclei. The cervical cells was morphometric studied using the Image Lab System for citologic classification. The overall diagnostic accuracy for neoplasia type I (CIN I) and type II (CIN II), demonstrated by stain interpretation values of a sensitive detection of DNA neuroploidy and a significant difference between the two groups in scanning static cytophotometry.

KEY WORDS: Cervix; DNA; Cervical Cancer; Morphometry.

INTRODUCTION

Neoplasias of the uterine cervix in developed and under-developed countries are diagnosed in 171.640 women. In Brazil, the cancer of uterine cervix is believed to be the third most common amongst women, just behind skin cancer (not melanoma) and breast cancer. In the North-Eastern part of Brazil its occurrence is 30.2 per 100.00 inhabitants, 10% of which are malignant (Ministério da Saúde, 2005).

The disease starts at an early age and moves up gradually, thus allowing a better diagnostic according to Lima *et al.* (1999), Cramer & Cutler (1974) who came up with 15.6 years of time between both the initial injury and the phase clinically diagnosed. Several factors are responsible for the cancer of uterine cervix and, most of them are possibly from epithelial tissues lesions, Pappa *et al.* (2006); or now caused by papilloma virus (HPV) (Lopes *et al.*, 2002). Nowadays, various methods are used to have a better diagnosis of these injuries (Papanicolaou & Traut,

1943; Coplesson *et al.*, 1992) but, it was back in 1932 that Broders (1932) introduced the term carcinoma *in situ* used until now, and based upon architectural disturbances in both nuclear abnormalities and indifferenciation.

To show evidence of difficulties during diagnosis, Richard (1967) proposed that epithelial alterations of the uterine cervix, be divided in three levels used as intraepithelial neoplasias (CIN) I, II, and III for those more serious. Although it's known that the numerous diagnosed dysplasias by cytomorphology and histology are insufficient to detect the preventive malignancy even with a constant preoccupation of inner controls, Amaral *et al.* (2003), Pittoli *et al.* (2003) and Böcking *et al.* (1985) think DNA quantification can be used in different techniques such as Giemsa, Toluidine Blue, Gallocyanine (stain methods), and other coloration techniques. Böhm (1968) efforts have been made towards automatic diagnostic of tumors by flow cytometry to measure nuclear DNA.

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Morphologic techniques to classify malignant tumors are considered subjective and non reliable in their reproduction (Böcking & Auffermann, 1982). Objective techniques are necessary to accurately show a prognosis in case of cancer for the chosen therapy be both adequate and solely for that subject. DNA study, with no doubt, is the ideal parameter to be used as the condition for prognosis tests (Feulgen & Rossenbeck, 1924; Böcking & Auffermann).

Our purpose was to quantify possible DNA mutations through a computerized analysis of images along with Feulgen & Rossenbeck's histochemic reaction.

MATERIAL AND METHOD

A total of 103 uterine cervix biopsies from subjects between 15-66 years old (32 years on average) were used, with differences in cross-breeding as to active sexual life, not using hormonal adjustment, marital status as married, single and widows (facts from medical files), all this collected at the University Hospital and the Hospital of Sugar and Alcohol Agro-Industry in Alagoas, and the Hospital of Cancer in Pernambuco). These subjects were divided in two groups, following cervical intraepithelial neoplasias diagnostic of the cervix of uterus in level I (CIN I) and level II (CIN II), Tables I and II.

Table I. Benign cervical intraepithelial neoplasias diagnostic (CIN I) subjects in North-Eastern Brazil.

Neoplasia	Marital Status		Race
Level I (CIN I)	Single	08	White
		00	Black
	Married	37	White
		00	Black
	Widows	02	White
		00	Black
	Unknow	09	
Total		56	

Table II. Moderate cervical intraepithelial neoplasias diagnostic (CIN II) subjects in North-Eastern Brazil.

Neoplasia	Marital Status		Race
Level II (CIN II)	Single	08	White
		03	Black
	Married	32	White
		02	Black
	Widows	01	White
		01	Black
	Unknow	00	
Total		47	

All diagnoses were confirmed, those with CIN III, incompatible diagnosis and incomplete or unknown facts being ruled out.

The material of blocks handed over were slice on 5µm of width (Spencer microtome), colored with hematoxiline – eosine for histology analysis and Feulgen & Rossenbeck histochemical reaction while being bleached with Fast-Green to quantify possible DNA alterations. Typical cells, mainly lymphocytes, were used as control colored with hematoxiline-eosine.

After Feulgen & Rossenbeck histochemic reaction, eight to fifteen areas were analyzed on slide, reaching a total of one hundred cells, then captured by the Image-Lab software connected to microscope (Leica DMLS), video camera (Samsung 410-SHC-NAD), computer (Pentium III) for analysis and morphometric quantification. The equipment was balanced and the sequenced readings using both Haroske *et al.* (1994) and Giroud & Montmassou (1989) security measure. The facts obtained were submitted to statistics analysis.

RESULTS

Several slides and blocks (61. 17%) were disregarded due to a failure to marital status identification, race, age and sexual orientation; with tissues incorrectly wrapped and misplaced; with incompatible diagnosis, such as slides with low optic resolution. Thus a total of forty subjects in group 1 and 2 remained, representing (38.83%) of the sample (Tables III and IV).

Table III. Benign cervical intraepithelial neoplasias (CIN I) diagnostic subjects in North-Eastern Brazil.

Neoplasia	Marital Status		Race
Level I (CIN I)	Single	00	White
		04	Black
	Married	00	White
		14	Black
	Widows	00	White
		02	Black
	Unknow	00	
Total		20	

It was impossible to establish any fact as to racial aspects because de casuistic has incomplete information, such as neoplasias occurrence in ages. For neoplasias disparity levels (light and moderate) using lymphocyte (Fig. 1), we

can observe in the level light, CIN I (Fig. II) a higher intensity between channels 2.3 to 6.8. In Fig. III, as to neoplasias intensity in moderate level (CIN II) there was a higher prevalent in channels 1 to 5, having a modest superposition in some cases.

Table IV. Moderate cervical intraepithelial neoplasias diagnostic (CIN II) subjects in North-Eastern Brazil.

Neoplasia	Marital Status	Race
Level II (CIN II)	Single	00 White
		02 Black
	Married	02 White
		16 Black
	Widows	00 White
		00 Black
	Unknow	00
		20
Total		

Fig. 1. Lymphocytes intensity distribution in several channels in female subjects with cervix of uterus neoplasias diagnostic.

Fig. 2. Benign level of Intensity Prevalence in subjects with cervical intraepithelial neoplasia (CIN I) diagnostic.

Fig. 3. Moderate level of intensity prevalence in subjects with cervical intraepithelial neoplasia (CIN II) diagnostic.

DISCUSSION

Existing difficulties in having a correct diagnostic, mainly the malignity initial phase, as for morphology aspects in relation with DNA nucleus, was a first found. In fact, they made us disqualify several subjects' material (61.1%) for having a totally irregular distribution of chromogen results in diverse nucleus cells and a major intensity in a specific place, perhaps, due to the different fixing agents during impregnation method (Hanselaar *et al.*, 1992). The usage of different fixing agents with different fixation methods shows some varieties about chromogen intensity in the ionic hydrogen concentration and the hydrolise time in Feulgen's reaction (Böhm; Böhm *et al.*, 1968) that could interfere in the condensation of chromatin's spatial distribution and, apart from tracking the DNA, does the same with proteins (Stedman & Stedman, 1950), Umayahara *et al.* (2002). Meanwhile, uncharacteristic DNA distributions, for some authors, in comparison with results from the epithelial of the uterine cervix cytometry and its DNA, aren't an early malignity diagnostic method, Haroske *et al.* (2001), Ishikawa *et al.* (2002). Most of them consider aneuploidia as a malignity factor (Bollmann, 2003).

Level I (CIN I) neoplasias, we believe, can be subjected to different interpretations, based upon difficulties for, most dysplasia and malignity levels are detected through morphologic monitoring. Normally, degeneratives are followed by uncertainties due to a lack of confirmation in cells differentiations outset. Benign dysplasia lesions (CIN I) aren't easily identified, mainly because the change to malignant lacks observation (Böcking & Hilgarth, 1983; Zhang *et al.*, 2005). To detect cancerous cells the usage of histochemic technique linked with cytometry is undoubtedly

valid (Fu *et al.*, 1981; Böcking & Hilgarth; Wied *et al.*, 1996). The advantage to link these methodologies is the confirmation of the beginning of lesions, even though the subject doesn't show dysplasia during observation routine, given aneuploid cells synonymous with malignity (Auffermann & Böcking, 1983, Böcking *et al.*, 1984; Sági *et al.*, 2005).

Our studies reveal a different concentrations in DNA cells distribution, as to moderate dysplasia (CIN II) indication and morphologic diagnostic, in opposition with those with dysplasia in benign level (CIN I), leading to a possible observation of the beginning of a lesion as observed by Böcking *et al.* (1984, 1985). According to Saccomano (1982), lesions considered moderate dysplasias (CIN II) change to carcinoma *in situ* within eight years. Although morphologic evidences of malignity are obvious, when the carcinoma *in situ* stage occurs, aneuploid cells presence in benign dysplasia (CIN I), possibly is an indication of pre-cancerous cells. Böcking *et al.* (1985) and Méhes *et al.* (2004) believe demonstration of DNA ploidy can be used as a quality control in cytology and a morphologic observation in histology.

Histochemic techniques is common in the initial diagnostic of the uterine cervix pathologies and neoplasias (Böcking & Chatelain, 1992; Pinho & Mattos, 2002). Meanwhile, a good technique usage in DNA proof needs knowledge, standardization from the beginning of the material acquisition and its fixation, to the histochemic marker and reading from computerized equipments, to get precise diagnostics (Zuna *et al.*, 2002). When we compared our findings with Arcuri *et al.* (2002) and Schoolland *et al.* (2002), we found a huge percentage of unused material, for failure to reach a proof of basic conditions, or simply having mistaken diagnostics. We know, public hospitals have different financing cycles in a year, thus, usually allowing a usage of different fixing agents, difficulties in storage and handling so, standardizing the beginning of the process is essential independently from a technique to be used.

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RESUMEN: La investigación tuvo como objetivo detectar las posibles variaciones del DNA en el núcleo de las células marcadas por la reacción histoquímica de Feulgen en individuos con diagnóstico de neoplasias intraepiteliales de grados I y II del cuello uterino. La microcitometría fue realizada en láminas provenientes de hospitales universitarios y estatales, sometidas a reacción de Feulgen y analizadas en el sistema de imagen (Image Lab) para cuantificación. Los resultados encontrados entre las células neoplásicas de grado I (NIC I) y del grado II (NIC II) demostraron que el método es sensible y eficiente en la cuantificación entre las diferentes clasificaciones, ayudando así en los diagnósticos de las lesiones del cuello uterino en su fase inicial.

PALABRAS CLAVE: Cuello Uterino; DNA; Cáncer Cervical; Morfometría.

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