Cytology, colposcopy and immunohistochemistry study in preinvasive cervical lesions

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**Introduction**

Cervical carcinoma is the second malignancy as overall frequency in women after breast cancer. In developing countries, ranks first in the neoplasm in women.

The gravity is that cervical cancers survival rate is lower than in breast cancer and cervical cancer occurs in the last decades to an age increasingly younger.

Do not forget the fact that cervical neoplasm is one of the few sites of neoplastic diseases with long preclinical development. As cervical cancer is preceded by abnormal squamous cells of the malpighian epithelium or glandular cells of endocervical epithelium, covered under the generic name of preinvasive cervical lesions or cervical precancerous lesions.

These include a spectrum of changes that begins with minimum irregular progression to marked intraepithelial abnormalities and then to invasive carcinoma.

The present study deals with identifying the most effective conventional methods (cytology, colposcopy) and modern (cytology in the liquid medium, videocolposcopy, immunotyping HPV and immunohistochemistry) for early diagnosis of cervical preinvasive lesions of epithelium squamous.

**OBJECTIVES AND STUDY MATERIAL**

This study attempts to demonstrate the importance of early diagnosis of preinvasive cervical lesion, a reflection of the modern concept of approach to these injuries, according to the latest data epidemiologic, morphological and molecular biology.

The major objective pursued by me in this personal study is to determine the best combination of investigations that can diagnose early preinvasive lesions of the cervix, so they do not evolve to the stage of cervical uterus cancer. The study uses early diagnosis of preinvasive cervical lesions, both traditional methods such as cytology and colposcopy and modern diagnostic methods, namely videocolposcopy, cytology in liquid medium and immunohistoch.

Another objective was to analyze the specificity and sensitivity of videocolposcopy as predictor of preinvasive cervical lesions of its volume and its limits in these topographic analysis of immunohistochemistry lesions. The study of this doctoral thesis covers the proliferation cell markers Ki-67 and PCNA correlated with the severity or dysplastic lesions, and the study of B and T lymphocytes reaction of preinvasive cervical squamous lesions showed that their participation is limited, which allows progression of these lesions to invasive cancer.
Patients studied in this paper came from two sources and were divided into two groups:

Group A - the women aged between 16 and 78 years who participated in the action screening cervical cancer through Pap test conducted between 2007-2009 by the cabinet of Ambulatory Gynecology Clinic Specialty Hospital Emergency Craiova. This group included 3477 women, of which 957 were tested in 2007, 1293 in 2008 and 1227 in 2009. All these women had Pap test performed which consisted of the exo-endocervical brush cytology CervexBrush material from both the exocol and endocol.

In 2007, the cytological material collected was processed by two methods, both conventional and by the CYTOSCREEN method (cytology in liquid medium). In the other two years, 2008 and 2009 the cytotic test was performed only by the method.

Lot B - is formed by women who in their own initiative presented at the office of the gynecology GYN-MED Craiova in 2007-2009, in a total number of 1286 cases.

These women were not included in the criteria of an organized screening classic age, occupation, residence, territory but came to consult the gynecologist for prophylactic or diverse symptoms, such as being leucorrhoea, itching, postcoital bleeding, intermenstrual bleeding, pelvic pain.

Collection of study materials and investigations were made on steps that imposed the following algorithm:

- clinical examination, history, local examination speculum, feel of vaginal and digital rectal examination;
- cytology: conventional cytology, cytology in the liquid medium (in 2007);
- videocolposcopy classic colposcopic examination;
- histopathology;
- HPV typing and immunohistochemistry.

STUDY
Cytology

The study was retrospective on cytologic material (cervico-vaginal smears) obtained from women aged 18-78 years, women who participated in the act of screening for cervical cancer by Pap test performed within Emergency County Hospital Craiova from 2007 – 2009 and cytological material obtained from women who came on their own initiative to the gynecologic surgery GYN MED with years between 16-78 years,
from 2007 -2009. Thus, during this period included the screening action of 3477 women, of which 957 were tested in 2007, 1293 in 2008 and 1227 in 2009 (Table no. 1)- group A. Group B included 1286 women of which 388 were tested in 2007, 412 were tested in 2008 and 486 were tested in 2009.

All these women have Pap test was performed which consisted of harvesting the exo-endocervical brush cytology CervexBrush material from both the exocol and endocol level, material which was then processed by two methods.

Thus, in 2007, all patients received both cervico-vaginal cytology performed by the conventional method and the method CYTOSCREEN (cytology in liquid medium). In the other two years, 2008 and 2009, the cytostest was performed only by the conventional method, methods that will be presented below.

### Cytology TRIALS

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>Total ( nr/ % )</th>
</tr>
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<tbody>
<tr>
<td>ASCUS</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>17 / 36.95</td>
</tr>
<tr>
<td>LSIL</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>6 / 13.04</td>
</tr>
<tr>
<td>LSIL - ATYPICAL OF HPV INDUCED</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6 / 13.04</td>
</tr>
<tr>
<td>HSIL</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>15 / 34.78</td>
</tr>
<tr>
<td>Squamous carcinoma cell</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2 / 4.35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>18</strong></td>
<td><strong>14</strong></td>
<td><strong>14</strong></td>
<td><strong>46</strong></td>
</tr>
</tbody>
</table>

**Legend:**
- ASCUS - Atypical squamous cells of undetermined significance
- LSIL - Low grade squamous intraepithelial lesion
- HSIL - High grade squamous intraepithelial lesion

Thus, 15 of the 46 cases (23.91%) smears showed atypical squamous cells of undetermined significance (ASCUS).

Thus, 9 patients cytological diagnosis was ASC-US, letting the clinician such cellular changes that could have been reactive. Another group of patients in the study, was represented by those that Cytodiagnosis was low graded squamous intraepithelial lesion (LSIL), consisting of 12 cases.

Changes in the cellular smears of these patients consisted of moderate hypertrophy of the nucleus, cytoplasmic nucleotide ratio increased, the most obvious change in shape
and size of kernels from one cell to another. Very important in these cases were citodiagnostic cromatiniens changes and nuclear contour. Thus, condensed chromatin was fine, evenly distributed, and nuclear contour was smooth, such aspects are seen on smears of six of the patients.

In this study, an important group of patients were represented by those whose smears were classified in group of high-grade lesions. Thus, 15 cases (34.78%) cytopatologic diagnosis was high grade squamous intraepithelial lesion (HSIL).

In patients with HSIL smears were found posters of cells in deep layers of heavy hypertrophy kernels with a significant increase in nucleotide-cytoplasmic ratio and moderate hipercromazy. Condensed chromatin is finely granular or older, but evenly distributed. The kernels showed thickened nuclear membrane and irregular nuclear contours.

In another case, the smears presented very frequently plaques of squamous cell and small-moderate size, with diskariotics kernels. Therefore recommended that in the cytopatologic bulletin, the patient to be subjected to further exploration by colposcopy and biopsy, stating that there is a high probability that the lesion is carcinoma in situ. Histopathology confirmed this.

**Colposcopy STUDY**

During the years of study, we identified major and minor colposcopic changes to 514 women included in groups A and B.

Of these 312 women had colposcopy and 202 minor changes women have experienced major changes.

Minor colposcopic changes were most common aceto-white fine epithelium and fine mosaics.

In our study, 312 women were diagnosed with minor injuries colposcopy, 204 women had minor injuries outside the transformation zone and 108 women had minor lesions located within the area of transformation. Histological diagnosis by colposcopic biopsy targeted to the 204 women with minor injuries outside the transformation zone showed that 68% of them were benign metaplasia and regenerative processes in progress, 17% were CIN I, CIN II and 13% only 3% of CIN III.

**Figure 1**

Mosaic histological correlations of outside the transformation zone
For the 108 women who had minor injuries located within the area of the histological processing, revealed CIN III lesions in 66 women, CIN II lesions in 15 women, CIN I lesions in three women and the 16 women were diagnosed with ongoing metaplasia lesions.

Figure No.2
Mosaic histological correlations within the transformation zone
Major colposcopic changes

Major colposcopic changes are represented by the aceto-white dense epithelium, coarse mosaic, atypical vessels, erosion and iodine negative areas.

In the study of the 202 major colposcopy with colposcopic changes were made to 190 targeted colposcopic biopsy, and among them a number of 157 biopsies showed lesions CIN II or CIN III, a total of 12 biopsies showed lesions type CIN I, and 21 biopsies showed regenerative changes, metaplasia current koilocites acanthosis and presence.

Fig No 3
Distribution of histopathological lesions in 190 biopsies performed for major colposcopy changes

Histological studies and immunohistochemistry

Morphological diagnostic methods have been imposed in the detection and diagnosis of female genital cancer due to their ability to signal the early presence of neoplasia, the preinvasive state, even in the absence of conclusive clinical data. In recent years, better characterization and differentiation of Scum intraepithelial lesion types we use, besides classical microscopic studies, and various immunohistochemical techniques that increase accuracy of diagnosis by histopathology.

For assessment of proliferation activity made several immunohistochemical examinations. Thus we used Ki-67 imunomarkers, imunomarker PCNA, P53 protein and the immune response of B and T perilezional lymphocytes.

Histological and immunohistochemical study is essential in determining the type of the pre-invasive lesion, its enlargement and conduct therapeutic formulation.
CIN I lesions were characterized by the presence of cellular abnormalities in the third deep epithelium that disorganized in its architecture.

Cell proliferation markers PCNA and Ki-67 had an intense reaction, but only one third lower in the epithelium, while the P53 marker had a reduced reactivity.

CIN II lesions were characterized by atypical cells present in two thirds below the epithelium associated with significant changes in the connective tissue subepithelium. Ki-67 proliferation factors and PCNA were highly positive in two thirds below the epithelium, marking kernels cell division, while the P53 marker had a low reactivity.

CIN III lesions were fully characterized by disruption of cervical epithelium architecture by atypical cells with proliferative character. Ki-67 proliferation factors and PCNA were highly positive, positivity that manifested to the surface of the epithelium. And in this type of injury marker P53 had a weak reactivity, which indicates that there are serious preinvasive squamous lesions in the TP53 gene encoding P53 protein is not altered.

Local immunological reaction in preinvasive squamous lesions was poor, both B cells and T lymphocytes are poorly represented at the level of injury or periligional, which makes us believe that the immune response is inhibited early in premalignant and malignant lesions of the cervix by various molecules cell signaling.

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