CYTO-HISTOPATHOLOGICAL CORRESPONDENCES IN INTRAEPITHELIAL LESIONS OF THE *CERVIX UTERI*

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Abstract. Our research focused on the evaluation of the correlation degree between the cytological and histopathological exam, with the aim to identify the advantages and limitations in the application of the two methods. The study group consisted of 28 patients diagnosed by conventional Papanicolaou cytology and routine histopathology exam. The results showed a correspondence between diagnoses with the association of benign cervical, uterine, and ovarian pathologies, sometimes with an upgrade of the intraepithelial lesions in histopathological exam. Conventional and liquid based cytology represent two accessible and well tolerated methods of diagnosis as well as of post-therapy monitorization. The histopathological examination is absolutely mandatory for confirmation of the diagnosis, for upgrade of intraepithelial high grade lesions to carcinoma, and for initiation of any therapeutic scheme.

INTRODUCTION

The investigations on the tumor pathology of the uterine cervix are rather easy and they provide complex research of cytology, colposcopy, biopsy, histology, and molecular biology type in order to demonstrate the mechanism of cervical carcinogenesis, in its sequences of evolution. Thus, the lesions are recorded in relation to the grade of the specific morphological anomalies to which the cytopathic effects of HPV infection are added, if noticed (Wright et al., 2002).
The development of screening strategies lead to a decrease of mortality by cervical cancer, due to a better understanding of the natural history of cervical neoplasia, the recognition of HPV role in carcinogenesis and of HPV infection latency, and allowed an early detection and initiation of therapy in the precursor stages.
Most cervical squamous cell carcinomas occur in the context of progressive intraepithelial lesions, their evaluation bearing fundamental importance in the prevention and prediction of cervical cancer development. The foundation of cervical carcinogenesis originates in cytological and histological results, with repercussions on therapy management (Wentzensen et al., 2009). On the basis of the data gathered until now, our research focused on the evaluation of the correlation degree between the cytological and histopathological exam, with the aim to identify the advantages and limitations in the application of the two methods.

MATERIALS AND METHODS

The study group consisted of 28 patients diagnosed, treated and monitored in the Obstetrics and Gynecology Clinic of the University Hospital „Elena Doamna” of Iasi between 2012 and 2014. The methods applied were conventional Papanicolaou cytology (Pap test) and routine pathology exam, on biopsy or hysterectomy specimens, processed by paraffin embedding and routine staining with Hematoxylin-eosin. The cytologic and histopathologic diagnostics were made on the biomedical routine microscope Nikon Eclipse 50i.
To assess the degree of slide representativity and the presence of the endocervical or metaplastic cells criteria of the Bethesda System 2001 have been used. If the slide contained two or more groups of endocervical/metaplastic cells with 5 or more cells each, or if the slide contained 10 or more glandular/metaplastic cells the criteria of endocervical cells presence was considered as achieved.
The cytologic diagnostics of LSIL (low grade squamous intraepithelial lesions) and ASC-US (atypical cells of undetermined significance) have imposed the recommendation for colposcopy with histopathological examination or Pap test reevaluation after 4 months. The patients diagnosed with HSIL (high grade squamous intraepithelial lesions) were subsequently investigated by colposcopy and histopathological examination.

RESULTS AND DISCUSSIONS

The results are presented comparatively for the two methods, cytology and pathology, respectively, in Table 1.

Table 1. Comparative results of cytological and histopathological (HP) exam

CASE	CYTOLOGIC DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	OBSERVATIONS
1.	HSIL*	Cervical intraepithelial neoplasia. Severe cervical dysplasia (CIN 3*)	Correspondence between diagnoses with the association of benign uterine and ovarian pathologies
2.	LSIL*. HPV*	CIN2*. CIN3. HPV* infection.	Upgrade of the intraepithelial lesions in HP exam
3.	Atrophy	Chronic cervicitis	Correspondence between diagnoses; surgical intervention required for uterine pathology
4.	Inflammatory hemorrhagic smear	Cervical polyp	Relative correspondence between diagnoses, in the context of benign cervical pathology
5.	Inflammatory smear	Benign endometrial pathology	Relative correspondence between diagnoses, in the context of benign endometrial pathology
6.	HSIL. HPV	CIN1*. CIN2. HPV infection	Correspondence between diagnoses with benign uterine pathology
7.	LSIL. HPV	CIN1. CIN2. HPV infection.	Upgrade of the intraepithelial lesion in HP exam
8.	Inflammatory preatrophic smear	CIN1. HPV infection. Endocervical polyp	HP exam discovered an incipient intraepithelial lesion and the presence of HPV
9.	Inflammatory smear	CIN1. HPV infection	HP exam discovered an early intraepithelial lesion and the presence of HPV, associated with benign uterine and ovarian pathology
10.	ASC-US*	CIN1. HPV infection	HP exam determined the relative diagnosis categorization into an early intraepithelial lesion and the presence of HPV
11.	LSIL. HPV	CIN1	Correspondence between diagnoses associated with ovarian benign pathology
12.	ASC-H*	CIN1. CIN2. HPV infection	HP exam determined the relative diagnosis classification into a severe intraepithelial lesion, and the presence of HPV
13.	HSIL	CIN2. CIN1. HPV infection	Correspondence between diagnoses
14.	LSIL. HPV	CIN1. HPV infection	Correspondence between diagnoses
15.	LSIL. HPV	CIN1. HPV infection	Correspondence between diagnoses
16.	HSIL	Cervical squamous cell carcinoma *in situ* (CIS)	Diagnosis upgrade from severe lesion to carcinoma *in situ*
17.	LSIL. HPV	CIN1. HPV infection	Correspondence between diagnoses
18.	ASC-US	Mild epithelial dysplasia of the cervix (CIN1). HPV infection	HP exam determined the relative diagnosis classification into a severe intraepithelial lesion, and the presence of HPV
19.	LSIL	Minor dysplasia (CIN1). HPV infection	Correspondence between diagnoses, with evidence of the cytopathic HPV effect in HP exam
20.	Inflammatory smear	CIN1. HPV infection	HP exam discovered an incipient intraepithelial lesion and the presence of HPV
21.	LSIL. HPV	CIN1. HPV infection	Correspondence between diagnoses

CASE	CYTOLOGIC DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	OBSERVATIONS
22.	LSIL. AGC- NOS*	CIN1	Correspondence between exocervical diagnoses
23.	ASC-US*	CIN1. CIN2. HPV infection	HP exam determined the relative diagnosis classification into a severe intraepithelial lesion and the presence of HPV
24.	LSIL. HPV	CIN 1. HPV infection	Correspondence between diagnoses
25.	LSIL. HPV	CIN 1. HPV infection	Correspondence between diagnoses
26.	HSIL	Cervical squamous cell carcinoma *in situ* (CIS)	Diagnosis upgrade from severe lesion to carcinoma *in situ*, in association with benign uterine and ovarian pathology
27.	ASC-H*	CIN 2. HPV infection	HP exam determined the relative diagnosis classification into a severe intraepithelial lesion and presence of HPV
28.	HSIL*	Cervical squamous non-keratinized invasive carcinoma pT1bNx	Diagnosis upgrade from severe lesion to invasive carcinoma, associated with benign pathologies of the uterus and appendages

**CIN1- mild cervical intraepithelial neoplasia; CIN2 – moderate cervical intraepithelial neoplasia; CIN3 – severe cervical intraepithelial neoplasia; HPV – human papilloma virus; ASC-US – atypical squamous cells of undetermined significance; ASC-H – atypical squamous cells-cannot be excluded high-grade squamous intraepithelial lesion; LSIL- low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesion; AGC-NOS – atypical glandular cells-not otherwise specified.*

In the histopathological investigation performed on our study group, according to WHO guidelines for cervical benign, precursor, and tumoral lesions (Kurman et al., 2014), the diagnoses have been the following: squamous cell carcinoma non-keratinized 8072/3: 1 case (3.44%), CIN1: 14 cases (48.27%), CIN1-2: 4 cases (13.79%), CIN2-3: 1 case (3.44%), squamous cell carcinoma *in situ* 8070/2: 2 cases (6.89%), and endocervical polyp 8015/3: 2 cases (6.89%).

Considering the utility of cytological follow-up for the cases that underwent more conservative but also radical surgical procedures, we selected 9 patients from our cases which were monitored in the same medical facility. They had hysterectomies in 6 cases and excisional biopsies in 3 cases.

The cytological diagnosis has the advantage to expand the limits and the possibilities of histopathological evaluation due to the variety of cellular elements it can identify, especially when biopsy cannot be performed, providing the identification of lesions that escape from the clinical examination (Apgar, Brotzman, 2004). The cytological method has a high sensitivity, with fewer than 10% false negative results, recorded due to the lack of shedding of the malignant cells or to collection errors.

Furthermore, the method has a high efficiency, with a low percentage of false positive results (Asotic et al., 2014). In our study group, we have recorded diagnosis consistency in 14 cases (50%), 9 cases (32%) having a cytological and histopathological correspondence of LSIL (Fig. 1) and CIN1 with HPV infection, 3 cases yielding false negative results (10.71%), and no false positive results, thus demonstrating the value of this method as well in our research.

Moreover, the histopathological examination has been superior to cytology because it determined an upgrade of lesions in 4 cases (14.28%), and the corresponding categorization of cytologically suspicious lesions, classified as ASC-US and ASC-H, in other 4 cases (14.28%), their histopathological examination revealing the presence of intraepithelial lesions associated to HPV

infection (Figs. 3, 4). However, although the cytological exam provided the orientation in these cases, we believe that only the histopathological examination can exactly establish the type of lesion.

In 3 cases (10.71%), the cytological exam recorded a high grade intraepithelial lesion (Fig. 2), which has been upgraded to carcinoma *in situ* (Cis) (2 cases) (Fig. 5) and to invasive carcinoma (1 case) (Fig. 6), the relative error being the result of the difficulty of a clear differentiation between severe lesions and carcinoma in conventional cytology.

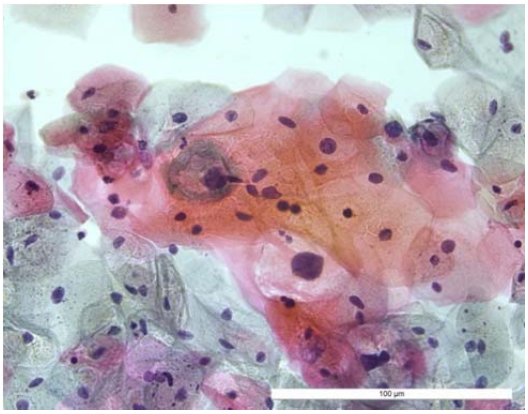

Fig. 1. LSIL, koilocytes, conventional Pap smear, x40

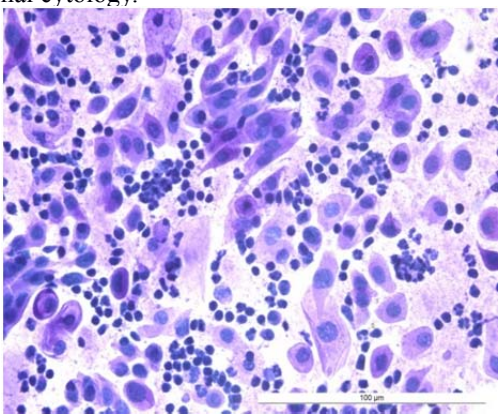

Fig. 2. HSIL, conventional Pap smear, x20

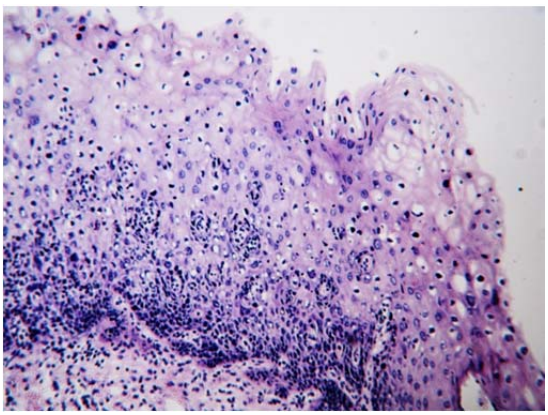

Fig. 3. CIN1, HPV infection, HE x20

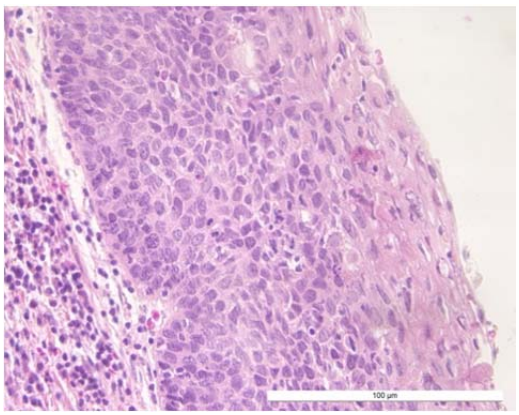

Fig. 4. CIN2, HE x20

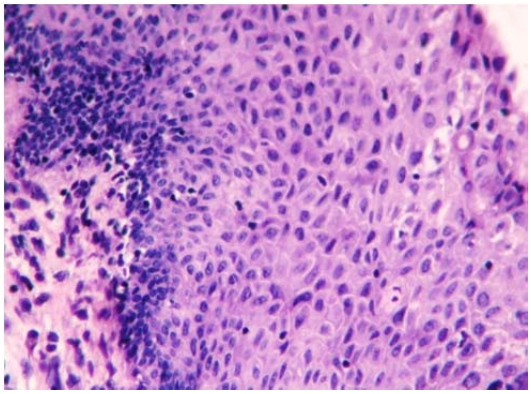

Fig. 5. Carcinoma *in situ*, with numerous mitoses, HE, x20

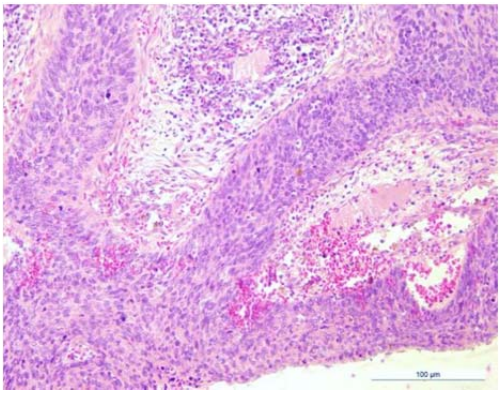

Fig. 6. Invasive squamous cell carcinoma, non-keratinized subtype, HE, x10

The degree of accuracy of the cytological exam depends on the collection procedure of the biological product and the processing method, liquid based cytology providing the best technique for the removal of artefacts caused by fixation and staining (Moosa et al., 2014). Thus, we believe that in our study group, classic cytology yielded falsely negative results due to these error causing factors.

The introduction of colposcopy in the investigation of the lesional cervix allowed the uncovering of subclinical lesions, which, later on, were designated as dysplasias and which, in theory, led to the hypothesis of lesional continuity, with the emergence of malignant lesion within the benign precursor (Milenkovic et al., 2012).

Besides the cytological data, the histopathological data significantly contributed to our knowledge on cervical carcinogenesis, by their integration into a unified hypothesis. This founded the role of the cytological exam in large screening activities, the identification of cell atypia triggering the histopathological examination, even when clinical and colposcopy data recorded no abnormalities (Saha, Thapa, 2005).

Bethesda system acquired the value of medical expertise, clearly drafted and providing an accurate and uniform terminology for diagnosis (Solomon et al., 2002), and for the inter-disciplinary communication. Thus, the three categories of CIN have been replaced by low grade and high grade intraepithelial lesions. We have noted that classic cytology showed high accuracy in the detection of high grade lesions (HSIL), with the observation that in 2 cases, the diagnosis was upgraded to Cis by the histopathological exam.

The follow-up of post-therapeutic alterations by exfoliative cytology raises certain difficulties, due to alteration of both normal and malignant cells. The alterations in normal cells are manifested by changes in cell volume, which may reaches up to x3-6 the normal size in all epithelial levels, and in the basic components of the cell. Thus, in the nucleus, karyolysis, pyknosis, karyorrhexis may be observed, associated to cytoplasmic vacuolizations of various degrees and occurrence of multinucleated cells (Koss, Melamed, 2006).

In our study group, we monitored 9 cases (32.14%) after surgical therapy. The records showed that the lesions went into remission after therapy, without noticing any evidence of HPV persistence of, except for 2 cases with HSIL (7.14%), where excisional biopsy and adjuvant therapy have not been efficient. Later, in these cases, local (conization) or extensive (hysterectomy) surgery have been necessary, following the general assessment of context by the

multi-disciplinary team.

The investigation performed support the screening value, by the comparative analysis with the histopathological exam, only 3 cases (10.71%) being false negative. This is in accordance with the literature data, that emphasize the importance of national screening programs of the cervical carcinoma, which are organized in order to detect the precursor and invasive cervical lesions, but in incipient stages, when an efficient treatment can be applied. The Papanicolaou smear is the only screening test which has determined the decreasing of the incidence and mortality rate of a cancer (Rodriguez et al., 2012). However, we believe that the accurate diagnosis still remained the advantage of histopathology, as demonstrated in 8 cases (28.57%) from the study group.

CONCLUSIONS

Conventional and liquid based cytology represent two accessible and well tolerated methods of diagnosis as well as of post-therapy monitorization, which lead to a relatively similar rate of detection of atypia in squamous epithelium, liquid based cytology providing superior detection of endocervical and high grade squamous lesions. The accuracy of the diagnosis is higher in the case of simultaneous use of the two methods, the conventional cytological exam and the monolayer cytology, the latter preserving the biological material for diagnosis and supplementary tests, if necessary. Nevertheless, the histopathological examination is absolutely mandatory for confirmation of diagnosis, for upgrade of intraepithelial high grade lesions to carcinoma, and for initiation of any therapeutic scheme.

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