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CRAIOVA

DOCTORAL SCHOOL

CLINICAL, MORPHOLOGICAL AND COLPOSCOPIC STUDY OF CERVICAL INTRAEPITHELIAL NEOPLASIA

DOCTORAL THESIS
ABSTRACT

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KEY WORDS: CERVIX, CITOLOGY, HPV, LSIL, HSIL, INTRAEPITHELIAL NEOPLASIA, COLPOSCOPY, SURGERY, MORPHOLOGY, IMMUNOHISTOCHEMISTRY.

GENERAL SECTION

1. ANATOMICAL AND HISTOLOGICAL ELEMENTS OF THE UTERINE CERVIX

The inferior female genital tract includes the cervix, the vagina and the vulva and it is unique in that these areas are easily accessible to both clinical and paraclinical assessment. A medical subspecialty – cervical/vaginal cytopathology, and an imaging exploration – colposcopy, are dedicated to the screening, diagnosis, and monitoring of premalignant lesions and intraepithelial cancers on this level.

When examined with the open speculum, the cervix appears as a circular structure. Consequently, topographical areas on the cervical surface are conventionally identified by analogy to a clock dial. The normal uterine cervix is approximately 3 cm long and approximately 2 cm in diameter, accounting for approximately 50% of the uterine volume in the nulliparous patients [1, 2].

Most of the external cervical region is covered by a stratified squamous non-keratinized epithelium. This region is also known as the ectocervix or exocervix.

Squamous cervical cells are arbitrarily divided into four distinct layers [3, 4]. The basal or germ cell layer is composed of one to two layers of small cuboid cells containing large round-oval dark nuclei. Mitoses are occasionally present at this level. The parabasal or spinocellular cell layer is composed of irregular polyhedral cells with large, dark, oval shaped nuclei. Nucleoli can be seen in most cases in these cells. The intermediate or navicular layer consists of flattened cells with a glycogen rich cytoplasm and comprises the majority of squamous cells. The nuclei are small, dark and round, and the nucleoli are not very visible. The superficial layer or the corneous layer consists of flat cells elongated with small pyknotic nuclei. Collagen is present in more superficial cells [3].

The samples of the Pap smear (Pap test) from the cervix involve scraping from the exocervical surface and a portion of the endocervical non-visualized surface using different sampling devices. As continuous maturation progresses to superficial layers, squamous cells lose functional desmosomes and split from each other. Therefore, the exocervical cells extracted for cytological examination are cells that have been exfoliated from the surface and appear under the microscope as individual cells in Papanicolaou samples. On the other hand, endocervical cells are not stratified and generally are not exfoliated. When scraped, endocervical cells are usually eliminated in agglomerations similar to microscopically examined cell clusters. Thus, harvested at the top of the estrogen screen, the cytological specimen will mostly contain superficial and intermediate cells [4].

2. KEY ELEMENTS IN COLPOSCOPY

The colposcope is the optical instrument that allows the illumination and the increased examination of the inferior genital tract. Intense light transilluminates the epithelium and magnification allows for careful examination of the surface epithelium and of the subepithelial blood vessels [5, 6].

In this chapter we have discussed current issues regarding the tools required for colposcopic examination (vaginal specimen, biopsy forceps, endocervical curettes or lateral vaginal wall retractors), the colposcopic examination technique, the acquisition and processing of data and images but also the particularities of colposcopic examination in pregnancy.

3. THE TRANSFORMATION ZONE OF THE CERVIX

Colposcopic examination is meant to detect the lesion when the cytological examination shows abnormal results. The most important objective of the colposcopic examination is to detect the abnormal transformation area [7, 8].

Within this chapter we have analyzed the following aspects: the normal transformation zone, the neoplastic change of the normal transformation zone and the abnormal transformation area.

Next, we have structured the current information regarding the aceto-white epithelium, the iodine-negative epithelium, the punctation, the mosaic, the atypical vessels, the leukoplakia, the erosion and ulceration and cervical flora.

4. PARTICULARITIES REGARDING THE IMPORTANCE AND BIOLOGY OF THE HPV VIRUS IN GENITAL INFECTIONS

The relationship between HPV and cervical cancer is now well established [9-15]. Theoretically, HPV infection causes almost all cases of CIN3 and cervical cancer and about 40-50% of vaginal and vulvar cancers, 50% of penile cancers, and 90% of anal cancers [15-19].

Different case-control studies have shown that the vast majority of women with cervical neoplasia have detectable levels of HPV DNA [37-48] and that the presence of high-risk HPV is predictive of an increased risk of high-grade CIN [20-23].

The issues addressed in this chapter relate specifically to HPV natural history and lifecycle, HPV genital infection mechanisms, HPV genital transmission features, co-factors of HPV infection due to cervical neoplasia and progression of HPV infection to intraepithelial neoplasia and cervical cancer.

5. MANAGEMENT OPTIONS AND SURGICAL TECHNIQUES ON THE ABNORMAL UTERINE CERVIX

In this final chapter of the general section, I have made reference to both management elements and to certain surgical techniques used in the treatment of intraepithelial cervical lesions. The management of cervical lesions has been addressed through the following aspects: the management of LSIL lesions in adolescents, in pregnancy and in postmenopausal women, the management of HSIL lesions in adolescents, young or pregnant women, the management of CIN2 and CIN3 lesions in adolescents and pregnancy and the management of the excisions with positive margins.

As far as the surgical techniques used are concerned, I have structured current information on loop electrosurgical excision (LEEP), electrosurgical conization and, last but not least, cold knife conization.

SPECIAL SECTION

1. INTRODUCTION

Cervical cytological screening is one of the major successes in the control and prevention of cervical cancer [24]. The Babes-Papanicolaou (Pap) test is based on the principle that epithelial squamous cells repeatedly exfoliate, so that normal or abnormal epithelial cells are desquamated at the cervical level and used for cytological examination [25].

Although the performance of exfoliative cytology has increased the ability to indirectly identify cervical neoplasia, it certainly has its limits, but the improvement of the conventional Papanicolaou smear, such as fluid sampling and electronic processing, has produced more effective results of this test [26].

It is widely accepted that HPV infection precedes the occurrence of the neoplastic disease within a variable timeframe, and HPV testing can detect 30 to 100% more preinvasive lesions compared to conventional cytology and 20-50% more precancerous lesions than liquid-based cytology [27-31].

1.1. THE OBJECTIVES OF THE STUDY

The objective of this doctoral study was to seek clinical correlations with influence on the cervical pathology in general and on cervical intraepithelial preinvasive lesions in particular.

In addition to this, the aim of this doctoral thesis was to analyze the cytological and colposcopic characteristics of the LSIL and HSIL cervical lesions and to draw histological and immunohistochemical correlations in these CIN1, CIN2 and CIN3 type lesions.

2. MATERIAL AND METHOD

The retrospective and prospective observational doctoral study was conducted between October 2016 and August 2018 on a group of 137 liquid-based cervical cytology patients, positive for LSIL or HSIL, or ASC-US, ASC-H, AGC and HR-HPV respectively.

The cases in the study groups were selected from the case studies of the Obstetrics and Gynecology Clinic II, The Emergency Clinical Hospital of Craiova, the General Surgery Clinic II and from private practice in an Obstetrics and Gynecology Medical Center in Craiova.

The average age of the patients in my series was 35 years (16-54 years).

2.1. CLINICAL STUDY

The following variables (**Table 1**): age, multiparity, pregnancy, previous use of an intrauterine device, the presence of the intrauterine device at the time of inclusion in the study, menopause, smoking, associated gynecological pathology, were considered in the perspective of the clinical study.

Table 1. Clinical features and the criteria for the inclusion in the study

The criterion for the inclusion in the study		N (%)	
PAP test detected cytological abnormality	ASC-US	20 (14.59)	
	HR-HPV	43 (31.38)	
	ASC-H	3 (2.18)	
	LSIL	28 (20.43)	
	HSIL	57 (41.6)	
	AGC	6 (4.37)	
Apparently inexplicable postcoital bleeding		78 (56.93)	
Associated vulvar or vaginal HPV lesion		19 (13.86)	
Cervical erosion at routine gynecological examination		21 (15.32)	
Uterine cervical ulceration at routine gynecological examination		16 (11.67)	
Cervical tumor at routine gynecological examination		5 (3.64)	
Partner diagnosed with lesion or condylomas in the inferior genital tract		13 (9.48)	
Cytology type across the study group N (%)		**LSIL** 28 (20.43)	**HSIL** 57 (41.6)
HPV Testing	HR-HPV N (%)	Different HPV * types N (%)	HR-HPV + Different HPV * types N (%)
	24 (17.51)	16 (11.67)	19 (13.86)
Clinical features			
Age (years) N (%)	16-21	32 (23.35)	
	21-30	40 (29.19)	
	30-40	33 (24.08)	
	40-50	23 (16.78)	
	50-60	9 (6.56)	
Multiparity		28 (20.43)	
Pregnancy		17 (12.4)	
The previous use of an intrauterine device		41 (29.92)	

The presence of the intrauterine device at the time of inclusion in the study	6 (4.37)
Menopause	8 (5.83)
Smoking	54 (39.41)
Associated gynecological pathology	32 (23.35)
N – case number; ASC-US - Atypical squamous cells of undetermined significance; HR-HPV - High risk HPV; ASC-H - Atypical squamous cells - cannot exclude HSIL; LSIL - Low-grade squamous intraepithelial lesion: HSIL - High-grade squamous intraepithelial lesion: AGC - Atypical glandular cells.	
Types - 31, 33, 35, 39, 45, 51, 52, 56, 58, 66.	

2.2. COLPOSCOPIC STUDY

The colposcopic evaluation was performed using a video-colposcopic imaging equipment to capture and store images to configure the study database.

The colposcopic evaluation was performed by initially applying the normal saline solution (0.9% sodium chloride solution). The next step in assessing cervical lesions was the application of 3-5% acetic acid, maintaining tissue contact for 2 minutes. The final stage of the colposcopic examination was the administration of diluted Lugol iodine solution (half-diluted or one-quarters diluted iodine solution), previously checking whether the patient is allergic to iodine (**Table 2**).

Table 2. Colposcopic evaluation

The purpose of the colposcopic examination[*1]	Identification/ Differentiation	The solutions used	Magnification
➢ The visualization of the uterine cervix and the storage of images; ➢ Detailed description of the squamous-columnar junction: • Identification of the transformation area; • The size, shape, contour, location and extent of lesions; • Correlations with the Pap test;	▪ Leukoplakia; ▪ Abnormal vascularization. .	○ Saline solution (0.9% Sodium Chloride) [*2]	Low (3.75x)
			Medium (7.5x)
			High (15x)
	• Differentiation between normal and abnormal epithelium; • Aceto-white tissue intensity.	○ Acetic acid (3-5%)	Low (3.75x)
			Medium (7.5x)
			High (15x)
	▪ Iodine solution capture; ▪ Lesion margins;	Lugol solution (half or quarter-diluted	Low (3.75x)
			Medium (7.5x)
			High (15x)

	iodine solution)	
• Augmentation of therapeutic management .	▪ The characteristics of the lesion margins; ▪ Lesion extension.	

[1] The colposcopy has been performed only by specialists trained and certified according to the legislation; [2] Native and green filter rating.

2.3. THE STUDY ON THE SURGERY OF THE CERVIX

Excisional biopsy and ablative surgery procedures were performed in this study. In my group, I had patients diagnosed with LSIL and HSIL (CIN1, 2 and 3), and therefore the techniques used for ablative surgery were LEEP (loop electrosurgical excision procedure), electrical scalpel excision and electrosurgical conization or the *top hat* loop electrosurgical excision procedure (**Table 3**).

Surgical procedures were performed in 98 of the cases in my study group, while conservative treatment, with or without excisional biopsy, was adopted in 39 of the cases (**Table 4**).

Table 3. Surgical strategy

Surgical technique	Colposcopic Control	
LEEP/ loop electrosurgical excision procedure	Single section (one stroke of the loop)	▪ Lugol solution appliance to determine the lesion margins; ▪ Marking the starting and ending (start / stop) of the loop stroke (hour 3 and hour 9 respectively); ▪ Different loop sizes -adapted to the lesion extension; ▪ Section depth: 5-8 mm; ▪ Safety margin of 4-6 mm outside of the lesion; ▪ Power output : 30-50 Watt; ▪ Power output prior to tissue contact; ▪ Slow and continuous loop stroke; ▪ Hemostasis control; ▪ Suction.
	Multiple sections (several strokes of the loop)	▪ Similar to single section; ▪ Marking of the initial and final (start/stop) loop stroke (6 o'clock and 12 o'clock respectively); ▪ One or two loop sizes; ▪ Two or more tissue specimens.
Electrical scalpel excision	▪ Various blade sizes for the electrical scalpel; ▪ Single or multiple sections (one or more strokes of the	

	excision blade);
Top hat loop electrosurgical excision procedure	▪ Section depth: 15 mm; ▪ Circular incision of approximately 8 mm in the cervix; ▪ Power output: 40-50 Watt;
Electrosurgical conization	○ Continuous movement; ○ Hemostasis control; ○ Suction.

Table 4. Case management in the group

Surgical strategy N = 98 (71,53%)	**_LEEP_** **N (%)**	45 (45.91)
	Electrosurgical conization **N (%)**	39 (39.79)
	Top hat loop electrosurgical excision procedure **N (%)**	14 (14.28)
Conservative strategy N = 39 (28,46%)	**_Excisional biopsy_** **N (%)**	14 (35.89)
	Conservative medical treatment (± colposcopic reassessment*) **N (%)**	25 (64.1)
N – case number; LEEP - loop electrosurgical excision procedure; * 6-8 months or 8-12 months.		

2.4. MICROSCOPIC MORPHOLOGICAL STUDY

In the microscopic morphological study, tissue specimens from both excisional biopsies and ablative surgical procedures were analyzed.

All tissue specimens were initially fixed in a 10% neutral formaldehyde solution at ambient temperature and subsequently included in paraffin after the histopathological protocol.

Specimens were cut using an HM350 microtome equipped with a water section transfer system (STS, microM).

For the histological study, sections with a thickness of 5 microns were made, subsequently revealed by classical histological colouring.

2.5. IMUNOHISTOCHEMICAL STUDY

For immunohistochemical staining, the lamellae were dewaxed and hydrated similarly to the Hematoxylin-Eosin technique. After hydration, the antigen was

exposed by heat with microwaves, and the pH of the solutions, their temperature and duration of treatment were the most important factors (**Table 5**).

Table 5. The antibodies used in the immunohistochemical technique

Primary antibody	Producer	Clone	Antigenic exposure solution	Dilution primary antibody	Secondary antibody
Anti Ki67	Dako	Clone MIB-1	EDTA pH 9	1:50	Monoclonal Mouse Anti-Human Ki67
Anti-p53	Dako	DO-7	EDTA, pH 9	1:50	Monoclonal Mouse Anti-Human p53 Protein
Anti-HPV	Dako	Clone K1H8	Citrate pH 6	1:50	Monoclonal Mouse Anti-Human Papilloma Virus (HPV)
Anti-p63	Dako	4A4	Citrate pH=6	1:50	Monoclonal Mouse Anti-Human p63 protein
Anti-BCL-2	Dako	Clone 124	EDTA pH 9	1:50	Monoclonal Mouse Anti-Human BCL-2 Oncoprotein
Anti CD20	Dako	Clone L26	Citrate pH 6	1:50	Monoclonal Mouse Anti-Human CD20cy
Anti CK7	Dako	Clone OV-TL 12/30	Citrate pH 6	1:50	Monoclonal Mouse Anti-Human Cytokeratin 7

3. RESULTS

When age range is considered, clinical features revealed an increased incidence of cervical abnormalities in the age range of 21-40 years: 40 (29.19%) cases in the age group of 21-30 years respectively, and 33 (24.08%) cases in the age group of 30-40 years, followed by the 16-21 years age group (23.35%), the 40-50 years age group (16.78%) and 6.56% in the age group of 50-60 years.

The data analysis of the cytological abnormalities identified by the Babeş-Papanicolaou test revealed the presence of HSIL in 57 (41.6%) cases, HR-HPV in 43 (31.38%), LSIL in 28 (20.43%), ASC-US in 20 (14.59%) cases, and AGC and

ASC-H in 6 (4.37%) and 3 (2.18%) respectively, of the cases in the group analyzed in this study.

As far as HPV infection is concerned in the study group, and following HPV genotyping, we diagnosed 24 (17.51%) cases of high-risk HPV type infections (types 16, 18), 16 (11.67%) cases of other types of HPV infections (31, 33, 35, 39, 45, 51, 52, 56, 58, 66), while 19 (13.86%) of the cases had mixed infections (HR-HPV + different HPV types). Thus, the overall incidence of HPV infection in the entire study was 43% (59 cases).

Also, considering the inclusion criteria in the study on the one hand and the recommendation for colposcopy, on the other hand, we have found that the apparently unexplained postcoital bleeding occurred in over half of the cases studied (78 / 56.93%). HPV or associated vulvar or vaginal lesions were diagnosed with an incidence of 13.86% (19 cases), while cervical erosion in routine gynecological examination was identified in 21 (15.32%) of the cases studied.

Similarly, cervical ulceration in routine gynecological examination was diagnosed in 16 (11.67%) cases, the presence of a tumor / pseudotumoral formation in routine gynecological examination was found in 5 (3.64%) cases, while 13 (9.48%) of the patients had a partner diagnosed with a lesion or condyloma in the inferior genital tract.

From the perspective of clinical data, we also identified 17 (12.4%) pregnant patients, all cases being unique pregnancies. Multiparity was found in 28 (20.43%) cases, while a previous use of an intrauterine device was recorded in 41 (29.92%) cases and the presence of the intrauterine device at the time of inclusion in the study was recorded in 6 (4.37%) of the cases included in the study group.

Last but not least, smoking was identified in the anamnesis of 54 (39.41%) cases, the presence of menopause in 8 (5.83%) cases and associated gynecological pathology was diagnosed in 32 (23.35%) of the studied cases.

The cytological and colposcopic findings characteristic of LSIL and HSIL, CIN1 and CIN2,3 respectively, were consistent in 113 (82.48%) of the 137 cases in the study and discordant in 24 (17.51%) of these. There were 13 (54.16%) HR-HPV and other HPV positive types discordant cases and 11 (45.83%) positive HPV-HPV respectively (**Table 6**).

Table 6. Colposcopic findings

LSIL - N (%)		HSIL - N (%)	
Absent vascularization	9 (32.14)	Coarse vascular changes	40 (70.17)
Fine mosaic	15 (53.57)	Coarse mosaic	18 (31.57)
Fine punctation	13 (46.42)	Coarse punctation	24 (42.1)
Flat delineation	22 (78.57)	Flat surface	30 (52.63)
Increased and irregular condyloma	2 (7.14)	Iodine-negativity	57 (100)
Pale and translucent aceto-white epithelium	26 (92.85)	Thick aceto-white epithelium	50 (87.71)
Mat white epithelium	8 (28.57)	Yellow appearance	39 (68.42)

		(Lugol) in a previously dense aceto-white region	
Diffuse margins	7 (25)	Obvious and sharp external contour	54 (94.73)
Geographic display margins	20 (71.42)	Lesion in the proximity of SCJ	13 (22.8)
N – case number; SCJ – squamocolumnar junction.			

The ablative surgical procedure in the study group was represented by LEEP in most cases (45.91%), followed by electrosurgical conization in 39 (39.79%) of cases and *top hat* loop electrosurgical excision in 14 (14.28%) of these.

Post-surgical colposcopic reassessment was performed at 6 months and 1 year respectively after surgery. 6 months post-procedure colposcopy was available in 94 (95.91%) of cases, while colposcopic examination at 1 year was performed in 63 (64.28%) of the cases in the ablative surgical group.

 The cytological and colposcopic reassessment identified the presence of residual disease in 7 cases (7.14%), while most cases (92.85%) benefited from surgical healing without residual disease.

As a result of the histological and immunohistochemical study, for CIN1 lesions, the defining cellular element for low risk dysplastic lesions was represented by the koilocytes. These structures are located in the two superior thirds of the squamous epithelium, characterized by the presence of superficial or intermediate type cells with nuclei that are up to three times the size of a normal nucleus of the intermediate cell.

CIN2 lesions or moderate dysplasia are diagnosed when proliferation in basal or parabasal cells and abnormal cells reach two-thirds of the thickness of the epithelial surface. Dysplastic cells show abnormal polarity, hyperchroma, and mitotic abnormalities are identified beyond the lower third of the epithelium.

Severe cervical dysplasia or CIN3 lesions were diagnosed by the presence of the immature cellular proliferation process throughout the thickness of the epithelium. Cellular mitosis is present in this case very close to or even at the epithelial surface. Differentiation of CIN3 by in situ carcinoma is due to the presence of one or more residual mature cell surfaces in the upper levels.

4. DISCUSSIONS

We have included CIN1, CIN2 and CIN3 intraepithelial cervical pathology in this study, analyzing cytological, colposcopic and morphological abnormalities in the cervix, also incorporating doctoral research into clinical characteristics of patients as well as surgical pathology data.

I consider this study to be important, giving the advantage of a complex analysis of cervical lesions on the one hand and on the other hand, with the correlations it reveals in the context of cervical intraepithelial neoplasia in liquid-based cytology, colposcopy, histology and immunohistochemistry.

It is widely acknowledged that HPV infections play an extremely important role in the occurrence of cervical lesions and their progression to invasive lesions. HPV infection is the most common sexually transmitted disease. Various studies on the incidence of HPV have shown rates ranging from 12-56% in women under 21 years of age, decreasing to about 2-7% for women aged over 35 [32-35]. Moreover, about 75% of *de novo* HPV infections occur between the ages of 15-24 [35].

In terms of my study data, I have identified the presence of different types of HPV, including especially those at high risk in over 43% of the analyzed cases.

Liquid-based cytology has some advantages over conventional Papanicolaou smear, including more complete removal of exfoliated cells on the examination blades, random and probably more representative cell transfer on blades, and improved microscopic view [36].

In my group, all cases were examined by liquid-based cytology, giving greater precision to the cytological examination.

Furthermore, published data show that mono-layered cytological blades obtained in the liquid medium are more reliable as compared to the results of the conventional screening method [37].

Given the clinical data from this study, it should be noted the significant number of 16-21 year old cases (32 / 23.35%) who met one or more of the inclusion criteria. These data are integrated with HPV infection, much more common in adolescent patients. Thus, 12 (37.5%) of these patients had HPV infection. As compared to Sanjose et al. [38] study results, this is a rather high prevalence, compared to the 23% presented by these authors. Otherwise, it should be emphasized that, on the one hand, adolescents have high rates of infection and, on the other hand, compensatory and increased rates of viral clearance [34, 39, 40].

HPV infection is mainly a young woman's pathology, and the physiological changes that occur during adolescence make them even more vulnerable to this virus [41].

The usefulness of colposcopy in its early detection capability of cervical dysplasia has increased over time, due to the implementation of the risk score, which allows integration of the colposcopic diagnosis with the corresponding histopathological diagnosis [37].

The data from the study indicate that the sensitivity and specificity of the combined methods (cytology - HPV genotyping - colposcopy), along with histological and immunohistochemical confirmation in selected cases, provide better surgical management and improved prognosis of these cases.

Bornstein et al. consider that the colposcopic results can be evaluated in the context of a colposcopic diagnosis, their precision being proportionate to the examiner's experience, ranging from minor findings to invasive cancer or other conditions such as erosion, condyloma, polyps, cysts, endometriosis, inflammation, vaginal stenosis or anomalies of the transformation area [42].

Colposcopic examination during the gestation period is primarily intended to exclude the invasive cancer diagnosis and secondly to provide further details on the CIN conservative management [43, 44].

In pregnant patients in this study, lesion regression was observed in 3 (17.64%) cases, their persistence in 9 (52.94%) cases, while lesion progression was noted in 5 (29.41%) cases.

The immunohistochemistry uses a step-by-step approach of a set of generic markers that have the advantage of high sensitivity and specificity and classical correlation with morphological parameters, thus completing histopathological examination [45].

The morphological examination of tissue specimens certainly provides important elements for the diagnosis, but IHC techniques provide additional prognostic information or can be retrospectively adapted and reviewed to examine tissues, thus allowing a more accurate diagnosis [45, 46].

5. CONCLUSIONS

Liquid-based cytology, along with DNA-HPV genotyping, provides an increased rate of detection of intraepithelial cervical lesions.

The sensitivity and specificity of the combined methods - cytology, HPV genotyping and colposcopy - together with histological and immunohistochemical confirmation in selected cases, ensure more effective surgical management and improved prognosis of these cases.

Cervical surgery under colposcopic control, observing current protocols, is important for achieving safety limits in cervical intraepithelial lesions.

The significant correlation between cytology, colposcopy, histology, and IHC in this study supports the idea that the association of fluid-based cytology with correct sampling to ensure good quality specimens, together with a rigorous colposcopic examination are reliable solutions for both the exact diagnosis of CIN, and for selective or appropriate surgical management.

The data from this study suggest that LEEP and electrosurgical conization are safe surgical techniques in the treatment of CIN.

Cervical surgery should be practiced with caution, especially in nulliparous women, given the risk of premature birth or premature rupture of the membranes, associated with cervical surgery, irrespective of its extent.

Cervical surgery procedure in adolescent patients is an exception in the vast majority of cases.

Colposcopy in pregnancy seeks to exclude the invasive cancer diagnosis and brings details of CIN's conservative management.

The objective of cervical assessment in CIN associated with pregnancy is to continue it and exclude invasive disease. The treatment of cervical pathology in pregnancy should be postponed, with the exception of invasive disease.

The classical histological staining of Hematoxylin-Eosin revealed changes in the CIN cell nucleus-cytoplasmic ratio.

IHC has shown that abnormal cell proliferation is increased and correlated with the intensity of BCL-2 and HPV oncogenes.

The inflammatory response is strongly positive in the perilesional area and CK7 is expressed in normal and negative glandular cells in cells with dysplastic changes, this being a prognostic marker in CIN.

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