

CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN). COMPARISON OF HISTOLOGICAL AND PAP RESULTS

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ABSTRACT

Objectives: The aim of this study is to investigate the compatibility of pathohistological and Pap findings on material obtained by cervical biopsy and excoaltation, and to investigate the compatibility of qualitative histological pattern and diagnosis

Material and Methods: The study included 152 patients with premalignant lesions of the cervix who underwent Pap testing and targeted cervical biopsy. Archival data on Pap test and histologic diagnosis were collected from available medical records. Information on age and advisable clinical diagnoses was obtained from referrals for pathohistologic examination of tissue. The age of the subjects was determined at the time the tissue samples were collected for analysis. Data on the quality of the material were obtained from microscopic evaluation of the collected material

Results: The largest number of patients was diagnosed with cervical intraepithelial neoplasia type 3 (CIN 3) cytologically (57.2%) and pathohistologically (55.2%). The largest percentage of treated material was rated as very good (46.1%). The majority of respondents (92.4%) had no difference in diagnosis based on Pap test and pathohistological analysis. There was no statistically significant difference in the diagnosis made on the basis of the Pap test and pathohistologic analysis depending on the quality of the material

Conclusion: There was a strong positive correlation between the grade established by cytology (Pap test) and the pathohistological diagnosis (PHD). There was no statistically significant difference in the diagnosis made on the basis of the Pap test and pathohistological analysis depending on the quality of the material.

Keywords: cervical intraepithelial neoplasia; Pap test; histopathology; punch biopsy

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INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is a premalignant disease of the cervix that regresses spontaneously in most cases [1]. CIN lesions can develop into invasive cancer over time [2,3]. CIN is primarily a disease of sexually active women, and given the risk factors, it is considered a sexually transmitted disease

Histologically, atypical cells are found within the epithelium with nuclear hyperchromasia and mitosis in the surface layers of the epithelium, but without evidence of stromal invasion. The basic criteria for the diagnosis of intraepithelial neoplasia are: Inequality in shape and size of cells and nuclei, maturation disorder, disruption of the relationship between cytoplasm and nucleus, and the presence of mitosis. Taking into account the thickness of the involvement of the multilayer squamous epithelium, we distinguish

three stages of CIN [4]. CIN 1 is the mildest form of cervical intraepithelial neoplasia. Measured at the basal membrane, the cellular changes do not affect more than 1/3 of the thickness of the epithelium. A small number of mitoses are found in the basal layer, and atypical cells with atypical nuclei are rare. CIN 2 is a moderately severe form in which the cell changes are present in the lower 2/3 of the mucosal epithelium as measured from the basal membrane. Cell arrangement and maturation are also disturbed, and immature cells with increased chromatin content are visible. The basal cell layer is enlarged and contains a greater number of mitoses and a greater number of atypical-looking cells. CIN 3 is the most severe form of dysplasia and carcinoma in situ (CIS). At this stage, dysplastic cells are located in more than 2/3 of the epithelial thickness or the entire epithelial thickness is affected. Numerous mitoses and numerous cells with

marked nuclear changes are present. Signs of epithelial maturation and differentiation are found only in the upper third of the epithelium. Numerous studies have confirmed that CIN is a precancerous condition that transforms into invasive cancer in a certain percentage of women. The main difference between carcinoma in situ (CIS) and invasive cancer is that in the former the thin basal membrane separating the epithelium from the subepithelial connective tissue containing blood and lymphatic vessels is still intact, whereas in invasive cancer malignant cells penetrate the basal membrane [5,6].

The three main methods for detecting intraepithelial neoplasia are the PAP test, colposcopy with targeted biopsy, and pathohistological analysis of biopsies [2]. The Pap test is a morphologic screening test that detects pre- and early-stage cervical cancer. It is used to identify women with CIN 2 and more severe lesions (CIN 2 plus). Today, the PAP test is still considered the best screening method for cervical epithelial atypia [6,7]. It is recommended that every sexually active woman should have a PAP test at least once every three years. A cytologic smear of the cervix should be performed 5 to 7 days after sexual abstinence or menstruation, and vaginal lavage is prohibited before collection of the smear to obtain a sufficient number of cells for analysis [8,9].

The changes detected on squamous cells are divided into three groups: atypical squamous cells (ASC), squamous intraepithelial lesion (SIL), and squamous cell carcinoma [1]. ASCs are divided into atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells in which HSIL (ASC-H) cannot be ruled out, and atypical squamous cells in which invasion cannot be ruled out. SIL is divided into low-grade SIL (Levis dysplasia or CIN I) and high-grade SIL (media and gravis dysplasia, carcinoma in situ or CIN 2 and 3). Squamous cell carcinoma is a definite finding of malignant squamous cells [10,11].

Biopsy, in contrast to the PAP test, is an invasive method indicated for suspicious or pathologic clinical, cytologic, or colposcopic findings, or for discrepancies between colposcopic and cytologic findings, and for equivocal colposcopic findings that cannot be explained by cytologic findings [12]. Today, many gynecologists prefer conization if the change is more extensive or if it spreads to the cervical canal. In addition to its diagnostic value, it also has therapeutic value, as the change can be completely removed in this way [9]. After the biopsy, the tissue samples are sent in separate vials in 10% formaldehyde for histopathological analysis, which is considered the "gold standard" in the diagnosis of preinvasive cervical changes [2].

The aim of this study is to investigate the concordance of histopathological and PAP findings and the dependence of the quality of the pathohistological specimen and diagnosis on the material obtained by targeted cervical biopsy and endocervical curettage.

SUBJECTS AND METHODS

Study design and participants

This study was designed as a cross-sectional study [10]. Cervical smears (Papanicolau test) and biopsy material from 152 women were analyzed over a one-year period. Patients were divided into groups according to age, PAP and PH findings. Pathohistologic material was grouped according to the quality of the material and designated as very good, good, or poor material [13].

Materials and methods

The material was obtained by targeted biopsy and excochleation from patients treated at the Department of Gynecology and Obstetrics, Osijek College Hospital. The material was pathohistologically processed in the Department of Pathology and Forensic Medicine, Osijek College Hospital, in a period of one year (from January 1 to December 31, 2012). The material was processed retrospectively. The material was treated with standard histochemical technique, embedded in paraffin blocks and stained with standard histochemical stain of HE (Hemalaun Eosin). Part of the material was processed with immunohistochemical methods CINtec (p16) and Ki67.

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Statistical methods

Categorical data are presented in absolute and relative frequencies. Normality of the distribution of numerical variables was tested using the Kolmogorov-Smirnov test. Numerical data are described by the median and the limits of the interquartile range. The χ^2 test was used to determine the differences in proportions between the independent samples. To determine the differences in scalar variables between groups, the Kruskal-Wallis test was used. To determine the correlation between ordinal and scalar variables, Spearman correlation was used. The selected significance level is $\alpha = 0.05$. Statistical analysis was performed with the SPSS statistical program (version 16.0, SPSS Inc., Chicago, IL, USA) [13,14].

RESULTS

The study was conducted on 152 patients who underwent PAP testing and PH analysis of material obtained by targeted biopsy or excochleation over a one-year period. The mean age of the subjects was 32.5 years (interquartile range: 27 to 41). There was no statistically significant difference in the age of the subjects depending on the grade determined by the analysis of the sample obtained by the PAP -test (Kruskal-Wallis test, $p = 0.116$) or PH -analysis (Kruskal-Wallis test, $p = 0.165$).

Cervical biopsy was performed in most subjects (86.8%) (Table 1).

Table 1. Percentage of subjects who underwent biopsy or excochleation

Materials	Number of subjects (%)
Biopsy	132 (86.8)
Excochleate	20 (13.2)
Total	152 (100)

Of the total 152 subjects, 57.2% had a cytologic diagnosis of CIN 3 (Table 2).

Table 2. Percentage of subjects depending on the cytological diagnosis made on the PAP test

Cytological diagnosis	Number of subjects (%)
Cervical intraepithelial neoplasia grade 1 (CIN 1)	24 (15.8)
Cervical intraepithelial neoplasia grade 2 (CIN 2)	30 (19.7)
Cervical intraepithelial neoplasia grade 3 (CIN 3)	87 (57.2)
Atypical squamous cells of undetermined significance (ASC-US)	4 (2.6)
Atypical squamous cells in which HSIL cannot be ruled out (ASC-H)	4 (2.6)
Squamous cell cancer	2 (1.3)
Atypical glandular cells of undetermined significance (AGC-US)	1 (0.7)
Total	152 (100)

The highest percentage of subjects had a pathohistologic diagnosis of CIN 3 (55.2%) (Table 3).

Table 3. Percentage of subjects depending on the pathohistological diagnosis made on pathohistological (PH) analysis of the material

Cytological diagnosis	Number of subjects (%)
Cervical intraepithelial neoplasia grade 1 (CIN 1)	30 (22.4)
Cervical intraepithelial neoplasia grade 2 (CIN 2)	26 (19.4)
Cervical intraepithelial neoplasia grade 3 (CIN 3)	74 (55.7)
Granulations	1 (0.7)
Inflammatory polip	1 (0.7)
Squamous cell cancer	2 (1.5)
Total	134 (100)

The highest percentage of respondents had very good quality materials (46.1%) (Table 4).

Table 4. Percentage of respondents regarding material quality

Material quality	Number of subjects (%)
Very good	70 (46.1)
Good	58 (38.2)
Poor	24 (15.8)
Total	152 (100)

In the majority of respondents (92.4%), there was no difference in the diagnosis made by PAP test and PH analysis (Table 5).

Table 5. Difference between cytological and pathohistological diagnosis

Difference	Number of subjects (%)
Cytological diagnosis is more difficult than PHD	8 (6.1)
No difference	122 (92.4)
PHD is more difficult than cytological diagnosis	2 (1.5)
Total	132 (100)

There is no statistically significant difference in the diagnosis made with the PAP test and pathohistological analysis depending on the quality of the material (χ^2 test, $p = 0.618$) (Table 6).

Table 6. Difference in diagnosis between cytology and PH analysis depending on the quality of the material

Difference	Material quality			Total	p
	Very good	Good	Poor		
Cytological diagnosis more difficult than PHD	5	3	0	8	=0.618
No difference	52	47	23	122	
PHD more difficult than cytological diagnosis	1	1	0	2	
Total	58	51	23	132	

There is a strong positive correlation between the grade determined by cytology (PAP test) and the pathohistological diagnosis (PH) (Spearman's $\rho = 0.961$, $p < 0.001$). Part of the material used in this study was processed by CIN-tec (p16) and Ki67 immunohistochemical methods (Figures 1 and 2).

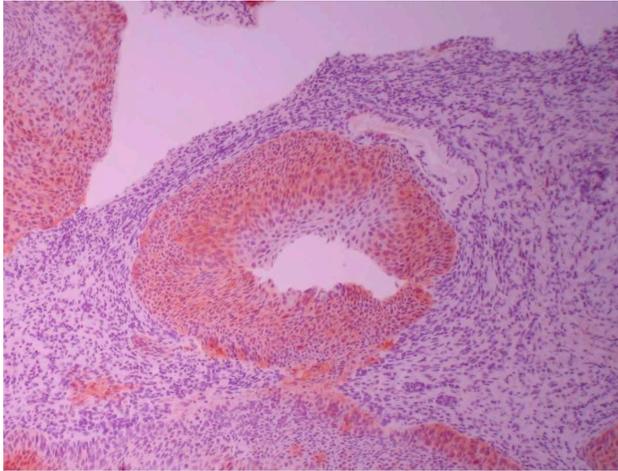


Figure 1. Cervical intraepithelial neoplasia grade 3 (CIN 3). IHC p16 (CIN-tec, DAKO) 200x.

Source: professor Milanka Mrčela, PhD, Clinical Department of Pathology and Forensic Medicine, University Hospital Center Osijek, material of the respondents from the conducted research

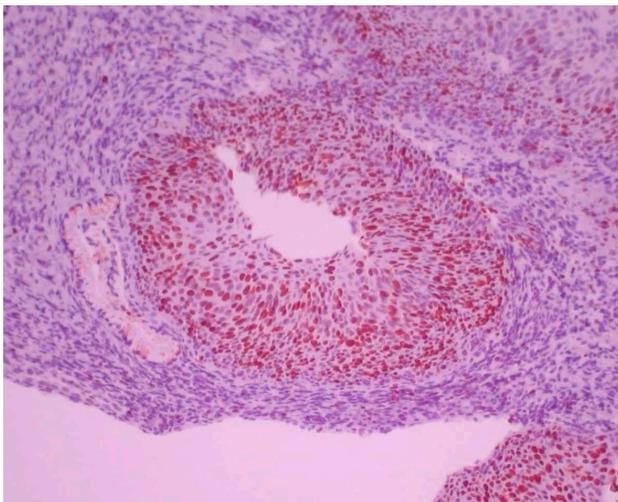


Figure 2. Cervical intraepithelial neoplasia grade 3 (CIN 3). IHC Ki-67 DAKO, 200x.

Source: professor Milanka Mrčela, PhD, Clinical Department of Pathology and Forensic Medicine, University Hospital Center Osijek, material of the respondents from the conducted research

DISCUSSION

This study examined data from patients with preinvasive cervical changes over a one-year period, from January 1, 2012, to December 31, 2012. We analyzed material obtained by the PAP test and targeted biopsy and excochleation of the cervix for pathohistological analysis (PH). In the literature, we found that the average age of patients with preinvasive cervical changes was 32 years,

which is consistent with the average age of patients in this study [11].

Preinvasive changes are usually thought to occur in women in their early thirties, although recently they have been more common in the early twenties or even earlier, as was also observed in this study [15,16].

This may be related to the fact that women have sexual intercourse earlier and the most important cause of sexually transmitted cervical intraepithelial neoplasia is human papillomavirus (HPV), as confirmed by numerous studies [17,18]. In addition to the diagnoses of cervical intraepithelial neoplasia, several patients with a cytologic diagnosis of atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells in which a high-grade squamous lesion (ASC-H) cannot be excluded, and several patients with squamous cell carcinoma were included in this study. In the literature found, the most common abnormal cytologic finding is an atypical squamous cell of undetermined significance (41.8%), while in this study, the most common abnormal cytologic finding is a grade 3 cervical intraepithelial neoplasia (CIN 3) [19].

In contrast to cytologic findings, the most common finding in pathohistologic findings in the literature is cervical intraepithelial neoplasia grade 1 (50.2%), which is not the case in this study. The most common diagnosis in cytological findings is cervical intraepithelial neoplasia grade [19]. A smaller percentage were patients with cytological and pathohistological diagnosis of cervical intraepithelial neoplasia grade 2 (CIN 2) and cervical intraepithelial neoplasia grade 1 (CIN 1). In both the literature and this study, the lowest percentage (0.5%) consists of patients diagnosed with invasive cervical cancer [19].

Cervical cancer most commonly affects women between the ages of 45 and 55 years, which is not the case in this study, in which the age of patients with cervical cancer was higher [3,19]. In this study, all patients underwent PAP testing followed by targeted cervical biopsy. Few patients underwent excision based on pathohistological diagnosis, while in the literature all patients underwent PAP test, after which targeted biopsy was performed only in the patients with suspected premalignant or malignant changes [3].

In this study, there is a significant positive correlation between histological and cytological findings. The increase in severity of histologic lesions was associated with more severe cytologic findings ($p < 0.001$). These results are in agreement with the findings of other authors [3,19]. Cytologic and pathohistologic diagnoses did not agree in a certain number of patients in this study. In most cases, cytologic diagnosis was more difficult than pathohistologic diagnosis. In a very small number of patients in this study, the pathohistologic diagnosis was more difficult than the cytologic diagnosis. Cytologic diagnosis depends on the quality of collection and processing and the subjective judgement of the cytologist, but even with a cervical biopsy, the change can be completely removed, so a repeat PAP test may be normal

The patients' pathohistologic material used in this study was grouped according to the quality of the material and labelled as very good, good, and poor. The highest percentage of material used was classified as very good. From the statistical analysis, it can be concluded that there was no significant discrepancy in the diagnosis based on the PAP test and the pathohistological analysis depending on the quality of the material ($p = 0.618$).

However, in a certain number of patients, it was not possible to determine the pathohistological diagnosis in those patients in whom the material was considered poor, leading to the conclusion that the quality of the material influences the diagnosis. The literature shows that 5 to 22% of patients with a cytological diagnosis of atypical squamous cells of undetermined significance (ASC-US) have CIN₂ plus, which was the case in this study, where CIN₂ or even CIN₃ was found in the pathohistological findings in patients with a cytological diagnosis ASC-US [20]. In a number of patients with a cytologic diagnosis of ASC-US, a pathohistologic diagnosis could not be made due to poor material quality.

To reduce these potential errors and increase the overall sensitivity of the test, several new techniques have been developed in recent years and have been proposed as complementary replacement methods for the Papanicolaou test. Therefore, immunohistochemical detection of HPV is now increasingly used. The most commonly used biomarker has been the progression marker p16 and the proliferation biomarker 17 Ki-67. In oncogenic transformation of cervical cells due to persistent high-risk HPV infection, there is a disruption in the regulation of the cell control cycle, leading to accelerated proliferation detected by overexpression of Ki-67 as well as overexpression of p16, resulting in loss of regulatory control over the cell cycle [21-23]. Some of the material used in this study was processed by CINtec (p16) and Ki-67 immunohistochemical methods. Data analysis showed that in the majority of patients who underwent immunohistochemical staining, the diagnosis was confirmed by the PAP test, which is in agreement with the results of other authors [24].

CONCLUSION

Based on the conducted research and the results obtained, it can be concluded that there is a significant positive correlation between cytological and pathohistological findings in the detection of premalignant and malignant changes of the cervix ($p < 0.001$). The PAP test is reliable in diagnosing cervical lesions, especially more severe cervical lesions such as CIN₂, CIN₃ and squamous cell carcinoma. The specificity of the Papanicolaou test is satisfactory and does not depend on the severity of the cervical lesion, and the sensitivity of the test increases with the severity of the cervical lesion. It can also be concluded that there is no statistically significant discrepancy between the diagnosis by the PAP test and the pathohistological analysis depending on the quality of the material.

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