UNIVERSITY OF MEDICINE AND PHARMACY  
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DOCTORAL THESIS  
SUMMARY

CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS –  
EPIDEMIOLOGICAL AND ANATOMOCLINICAL  
CORRELATIONS

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cervical squamous intraepithelial lesions, risk factors, HPV infection, cytology, HPV genotyping, histopathology, statistical analysis

INTRODUCTION

Cervical squamous intraepithelial lesions (SILs) represent an important gynecological category of disease because of their potential to progress to invasive cervical cancer and involve the HPV (Human Papilloma Virus) as the major risk factor (5). SILs prevalence varies very widely, depending on socio-economic and geographic characteristics of the female population from 1.05% in studies on patients undergoing screening to 13.7% in women who have repeated sexually transmitted infections (127).

STUDY OBJECTIVES

The aim of study was to evaluate the impact of certain risk factors in pathogenesis of squamous intraepithelial lesions, the role of main diagnostic methods in identification of cytological, colposcopic and histopathological HPV-induced changes, and also the relation between the various diagnostic classes. The study objectives were:

• identifying of the main risk factors involved in the pathogenesis of squamous intraepithelial lesions
• evaluation of the impact of HPV infection on cervical epithelium and the types of lesions produced by HPV type
• establishing of the cytological, colposcopic and histopathological features of squamous intraepithelial lesions, their mutual correlation, and correlations with various risk factors

**HPV PATHOGENESIS IN SILs**

Recent progress in immunology, molecular biology and medical genetics, have demonstrated the central role of HPV infection in the etiology and pathogenesis of squamous cervical intraepithelial lesions (SILs) and cervical cancer (12, 169). There are more than 100 known HPV genotypes, grouped in cutaneous and mucosotropic and also known as low- and high-risk categories based on oncogenic properties. HPV6 and HPV11, classified as low-risk viruses are mainly responsible for genital warts, while the high-risk viruses (particularly HPV16, 18, 45 and 31) are found in varying proportions in HSILs (high-grade SIL), in which the annual rate of progression to invasive cervical cancer is less than 1%. HPV-dependent pathogenesis of SILs involves:

• squamous epithelial infection with HPV particularly high risk type followed by a HPV persistent infection particularly in squamous cells of the transformation zone

• HPV integration into host cell genome conferring neoplastic properties; SIL development and further progression to invasive cancer.

**MATERIAL AND METHODS**

**A. Investigated material.**

The study group included 556 female patients; the biological material investigated was represented by vaginal discharge (for microbiology and cytopathology) and fragments of cervical biopsy (histopathology and immunohistochemical); we also used clinical charts and registers with cytopathological, histopathological and immunohistochemical results.
B. Methods.

The analysis of clinical data was used to group the patients according to various risk factors. The cytopathological analysis was based on the microbiological examination of vaginal discharge, cytologic interpretation of PAP smear according to Bethesda system and HPV genotyping (PCR technique). The colposcopic evaluation with application of 5% acetic acid solution led to calculation of Reid index according to cervical lesion severity, while the histopathological study allowed getting the specific diagnostic and immunohistochemistry has evaluated certain indicators of adverse evolution. The statistical significance of relation between various clinical, cytological, colposcopic and histopathological factors was based on statistical analysis (Chi square test, Goodman and Kruskal's lambda (λ) test, Cramer test, the arithmetic mean and dispersion of individual values).

REZULTS

Clinical analysis. Addressability to gynecologic investigations of selected patients in our study was more pronounced in those of 31-34 group of age (24.10%), with the net predominance of women from urban environment.

Microbiological Study. Most patients had polymicrobial infections (44.24%), the most frequent involved germs being Candida albicans (34.89%) followed by Escherichia coli (15.11%). Clamydia trachomatis known to worsen the evolution of HPV infection was found in only 1.80% cases.

Cytopathological Study. According to Bethesda system used in PAP smear interpretation, the most patients were in LSIL class (55.40%) followed by the ASCUS with suspect colposcopy (23.02%); patients in HSIL class represented 10.67% cases; the rarest cytodiagnostic category was ASCH (3.24%).

HPV Genotyping. HPV genotyping was performed in 244 patients (43.88% cases), revealed infection with one or more of low-risk and/or high-
risk HPV types. Monotype HPV infection was found in 23.50% cases for the low-risk HPV category and in 8.99% cases for the high-risk category. In the low-risk HPV category, the most frequent were HPV type 61 (5.76% cases), followed by type 6 (4.68%) and 11 and 54 types (2.16% each). The high-risk HPV category included HPV 16 as the most frequent the type (8.99%); the second high-risk type as frequency was HPV 18 (5.04%), followed by HPV 33 and 52 types found in 3.96% cases each. Complex HPV infection with low and high-risk types was identified in 42 patients (7.55%).

Colposcopic evaluation. Colposcopic examination with 5% acetic acid application was performed in 430 patients (77.34%) particularly in ASCUS (usually over age 40 years) and ASCH (regardless of patient age) class and also in cases with LSIL (particularly associated with high-risk HPV) and in HSIL patients (to view the transformation zone for targeted biopsy). Cases with index Reid 3-4 (probably CIN1 or CIN2 lesion) were most frequent category, (32.37%); followed by index Reid 0-2 category, suggesting CIN1 lesion (25.18%) and Reid index 5-8 (probably CIN2 or CIN3 lesion) in 10.43% cases.

Histopathological study. On microscopic examination, the most frequent diagnostic class was CIN1 (42.81% cases) followed by the condylomas (plate and acuminated lesions) (19.78%). Among high-grade squamous intraepithelial lesions, CIN 2 was found in 11.51% cases and CIN3 represented 6.12% cases. Reactive changes associated with inflammation were present in significant number of cases (15.11%).

Statistical Analysis evaluated relation between various risk factors, colposcopic index Reid and cytological and histopathological diagnosis classes. Statistical correlation was highly significant between low risk HPV infection (documented by cytology and HPV testing) and presence of warts (95.83%) and CIN 1 (26.27%). High-risk HPV infection was strongly related with cervical intraepithelial neoplasia (86.86% of CIN1 and 87.50% of CIN 2 had oncogenetic viral infection). There was a strong association between cytologic
LSIL class and Reid colposcopic score 3-4 and also between ASC-US class and Reid score 0-2. The Reid index 3-4 was correlated with cytodiagnostic classes of LSIL and ASCUS (partly) and also with histopathological diagnosis of condylomas and CIN1. The statistical correlation was strong between 5-8 Reid index and presence of moderate or severe dysplasia on histopathology and also with HSIL and ASCH cytodiagnostic classes.

Analyzing the distribution of lesions of the cervix in relation to age group, the LSIL and ASCUS (most cases) categories, corresponding to condylomas and CIN1 lesions were found mostly between patients in 30-39 group of age (45.45% of condylomas and 47.90% of CIN1). The CIN2 and CIN3 lesions, overlapping ASCH and HSIL cytodiagnostic classes, involved predominantly patients in 30-39 group of age, for CIN2 (53.13%) and one decade later, for CIN 3 (52.94% cases). A relatively high number of CIN2 lesions were found in 40-49 group of age patients (21.88%), while CIN3 was also frequent found in patients over 50 years of age (41.18% cases).

**CONCLUSION**

- In our study, the occurrence and progression of cervical squamous intraepithelial lesion appearance and progression appear to be influenced by various risk factors (age, environment (urban), oral contraceptives).
- Cervicitis and cervical hypertrophy often represented the clinical expressions of coinfection in HPV-induced squamous intraepithelial lesion
- The microbiological study of vaginal discharge has demonstrated an increased incidence of polymicrobial infections; the most frequently involved germs were Candida albicans and Escherichia coli; we also found positive cases for Chlamydia trachomatis involved in progression of HPV infection.
- In the cytological study based on 2001 Bethesda system for cytologic interpretation, LSIL class comprised the majority of cases, followed by ASCUS with suspect colposcopy.
• HPV genotyping study identified HPV 61 and 6 as the most frequent low-risk HPV types, while in high-risk category prevailed HPV types 16 and 18, followed by HPV 33 and 52; infection with multiple HPV types (low-risk and/or high-risk) was relatively frequent.

• Colposcopic study revealed, Reid score 3-4 (probably CIN1 or CIN2 lesions) in most cases; the rarest were cases with Reid index of 5-8 suggesting high-grade lesions (CIN2-CIN3).

• Histopathological study revealed CIN1 as the most frequent lesion followed in order of frequency by condylomas and CIN 2 and CIN 3 lesions.

• The statistic analysis established a high significant concordance between variable as cytopathological SILs category (Bethesda interpretation system), colposcopic Reid index and histopathological diagnosis.

• The statistically high significant concordance between the cytodiagnostic classes (Bethesda system), the lesion value of Reid index (on colposcopy) and histopathological diagnostic revealed the importance of cervicovaginal cytology screening in detecting of HPV infection and subsequent cervical squamous intraepithelial lesions.
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Paper works presented at Congresses and Conferences


