University of Medicine and Pharmacy, Târgu Mures
School of Doctoral Studies

DETECTION AND TREATMENT OF CERVICAL INTRAEPITHELIAL LESIONS

Scientific Supervisor:
Prof. CONSTANTIN RĂDULESCU

PhD Candidate:
FLORIN COLŢESCU

Târgu-Mureş
2012

INTRODUCTION

Cervical cancer is the second leading cause of cancer-related deaths among Romanian women, following breast cancer and the first cause of cancer-related deaths in women belonging to the 25-44 year-old age group. This disease represents a major public health problem with significant social costs.

In 2008, the gross incidence rate in Romanian women was 29%ooo. The number of death cases caused by cervical cancer in Romania places our country on the first place in Europe, with a standardized mortality rate of 16.2%ooo women, with a total 3402 new registered cases and 2060 deaths.

It is very important to understand the mechanism of how HPV infection is involved in the pathogenesis of squamous intraepithelial lesions as well as their diagnostic methods, being aware that dysplasia is the precursor lesion for cancer.

This PhD thesis has been intended to perform a comprehensive evaluation of the complex diagnosis of cervical intraepithelial lesions, based on both traditional and modern methods of investigation such as immunohistochemistry.

The classical methods of evaluation included Pap smear test, colposcopy and histopathology examination, and in order to determine prognosis besides these HPV infection was determined and immunohistochemical examination of biopsy pieces performed.

THE OBJECTIVE OF THE STUDY:

- to identify the main risk factors involved in the pathogenesis of squamous intraepithelial lesions
- to identify the impact of HPV infection upon the cervical epithelium and the type of the produced lesions
- to establish the cytological, colposcopy and histopathology characters of the squamous intraepithelial lesions and their mutual correlation
- the analysis of the immunohistochemical markers p16 and Ki-67

This study comprises a general part and a special part.

The general part describes NIC risk factors, classification of precancerous lesions of the cervix, screening, diagnosis and evolution of NIC and treatment methods that can be applied.

The **special part** comprises the following chapters:

MATERIAL AND METHOD

1. The study comprises a number of 64 patients who underwent colposcopy, cytodiagnosis and biopsy examinations during January 2010 – December 2011 at the Saint Die Hospital (France). Testing for HR HPV was performed especially in case of ASCUS Pap smears.
2. Another aim of the study was to analyse the efficiency of LEEP and the incidence of complications in the pre- and post-operative period. The loop electrosurgical excision procedure (LEEP) is the most commonly used technique today for the practice of conisation, regardless of the size of the lesion or level of junction in the endocervix.
3. We performed a study in 119 patients who underwent cervical biopsy during 2008-2011 at the Saint Die Hospital, France (with 4 groups of results: normal and benign lesions, CIN 1, CIN 2 and CIN 3).

The objective of this study was to evaluate the utility of protein p16 ^{INK4A} overexpression as a specific marker of precancerous lesions of the uterine cervix.

RESULTS

The average age of patients was 33.5 years, ranging from 19 to 65 years. CUS results were mostly ASCUS (46.87%), followed by LSIL (32.81%), HSIL (12.5%). In 7.81% of the cases were benign alterations (inflammation).

An ASCUS result for CUS corresponded with a histological diagnosis of normal expression and benign lesions (60% of the cases), in 26.66% of the cases with low grade malignancy lesion and in 13.33% of the cases with high grade malignancy lesion.

In ASCUS patients who underwent HR HPV testing, the results were positive in case of all (100%) patients whose histological test showed high grade malignant lesions, in 66.66% of patients with low-grade malignant lesions and similarly 66.66% of the patients with normal histology and benign lesions.

Protein p16$^{INK4a\ tested}$ positive for 78.57% of CIN 1 patients and 100% for CIN 2 and CIN 3 patients. P16 expression was negative in 6 patients with CIN 1. (21.42%)

DISCUSSIONS

It is known that high-risk HPV are responsible for precancerous lesions and cervical cancers. HPV infection is common (about 7 out of 10 women are exposed to HPV infection during their

lifetime). The prevalence of the infection in subjects younger than 30 years old is estimated to be about 30%, 10% between the ages of 30 - 50 years old and 5% in subjects older than 50 years. The majority of HPV infected women successfully eliminate the virus by immune mechanisms within 12–18 months (in 60–80% of the cases).

HPV testing is more sensitive than CUS and allows patients to avoid colposcopy in about 50% of the cases, colposcopy being performed only in HR HPV positive patients. Colposcopy with biopsy is unnecessary and induces stress in ASCUS patients who tested negative for HR HPV. Colposcopic guided cervical biopsy allows the improvement of colposcopy results and provides histological results. Cervical biopsy results depend on the localization of the biopsy area

CONCLUSIONS

Immunohistochemical study of p16 protein is often useful as a complementary alternative to standard histology. The routine use of p16 in Romania can be an important tool for the diagnosis of CIN, especially in cases that are difficult to interpret (used to distinguish immature metaplasia of highly malignant lesions).

P16 protein expression reflects alterations in the HPV-induced cell cycle.

The importance of the use of p16 protein is to emphasise a persistent infection with oncogenic HPV, to reduce CIN 1 lesions presenting evolutionary risk and to differentiate immature metaplasia from HPV-induced lesions.

In our experience, LEEP could be considered the treatment of choice for cervical dysplasia when colposcopy is satisfactory, because it is effective, simple, fast, inexpensive, unaggressive, has a low morbidity and permits adequate pathological examination.

There is a direct correlation between the presence of HPV and grade of malignancy, thus all patients presenting high grade malignant lesions tested positive for HR HPV.

Negative HR HPV testing offers long term protection that cannot be provided only by sampling cervical-uterine smears. In our study neither of the patients who tested negative for HR HPV presented high grade malignancy lesions at the histology test.

Negative testing for HR HPV may allow the increase of screening interval (up to 5 years).

HPV testing should not be performed in patients with LSIL results when sampling cervical-uterine smears, because the HPV prevalence is highly increased and a positive HR HPV test result would only create panic in young Romanian patients.

Keywords: cervical intraepithelial neoplasia, infection, high-risk Papillomavirus, Pap smears, p16, loop electrosurgical excision procedure (LEEP)