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**THE PREDICTIVE VALUE OF PRE-CONIZATION HPV LOAD  
IN PROGRESSION AND RECURRENCE OF CERVICAL  
INTRAEPITHELIAL NEOPLASIA**

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# BACKGROUND

- Persistent infection with carcinogenic HPV types plays a major role in the development of cervical intraepithelial neoplasia and it may particularly be affected with high HPV load as a result of viral replication (Costa et al., 2002).
- Systematic reviews indicate overall pooled failure rates of 5-15% for the different modalities with no significant difference between them (Kyrgiou et al. 2006).
- Most failure occur within 2 years after treatment (Alonso et al., 2006).
- Several studies have suggested that the amount of HPV should be an important factor for progression from HPV infection to cervical cancer (Paraskevaïdis E et al., 2004; Zielinski GB et al., 2004).
- A nested case control-study of the relation between the amount of HPV DNA and development of carcinoma in situ by analysis of archived cervical smear samples was performed by *Josefsson AM et al in 2000*. High amounts of HPV 16 DNA estimated by PCR that used 5' exonuclease (TaqMan) method, were found to be a major risk factor for development of cervical carcinoma in situ.

*Alonso et al., 2006* used high HPV load cutoff of 1000 RLU/PC and reported that pre-conization HR-HPV load as determined using Hybrid Capture II was associated with higher risk of persistent/recurrent disease according to univariate analysis.

*Song et al., 2006* reported that HPV infection after conization with negative margins was persistent in 9.8% of the patients with a low viral (<500 RLU/PC) and 43.8% of the patients with high viral load ( $\geq 500$  RLU/PC). Their multivariate analysis showed that high viral load was the only independent predictor of HPV persistence.

*Park et al., 2008* performed a retrospective study using lower cutoff (100 RLU/PC) and reported that after adjusting for other risk factors, the pre-conization HR-HPV load was an independent risk factor for residual or recurrent disease according to multivariate analysis.

- According to our knowledge there is only one prospective study assessing the predictive value of pretreatment HPV load on progression and recurrence of the cervical intraepithelial disease.

# OBJECTIVES

## General objectives

1. To contribute in terms of improving the management and patient counseling.

## Specific objectives

1. To find the correlation between the pre-conization HPV load and progression/recurrence of cervical intraepithelial neoplasia.
2. To contribute in prediction the time of progression/recurrence of cervical intraepithelial neoplasia.
3. To find if there is any association between other risk factors (age, parity, menopause, marginal status, glandular extension, type of CIN of the cervical cone, smoking after conization and the number of sexual partners after conization) with the progression/recurrence of intraepithelial neoplasia.

## Patients

Women undergoing cold-knife conization at the University Clinic of Obstetrics and Gynecology in Skopje, Republic of Macedonia because of biopsy confirmed cervical CIN 2,3 and Ca in situ.

The recruited population will be adjusted for:

- Age
- Parity
- Menopause
- Glandular extension
- Margin status
- Type of cervical cone CIN
- Number of sexual partners after conization
- Smoking after the conization

## **Inclusion criteria**

1. Histologically confirmed CIN<sub>2</sub>, CIN<sub>3</sub> and Ca in situ in the conization specimen (cold-knife conization).

## **Exclusion criteria**

1. Patients who will undergo hysterectomy after conization.
2. Patient with current referral for hysterectomy because of other uterine pathology (e.g., fibroids)
3. Pregnant patients or patients who want to become pregnant.
4. Patients with previous surgical treatment for cervical disease.

# METHODS

## Study design

Prospective cohort study.

The recruited patients will be divided into two groups according to the pre-conization HPV load estimated at least 3 weeks before conization.

- I. Low HPV load (1-100 RLU/PC)
- II. High HPV load (>100 RLU/PC)



# DATA COLLECTION

## Detection of HPV DNA

We will use the Hybrid Capture II system (HC II) for detection of HPV. Light measurements will be quantified using a luminometer and will be expressed by comparing the relative light units (RLU) of clinical samples with a positive control (PC).

RLU/PC ratios will be calculated as the ratio of the specimen luminescence to the luminescence of the 1.0 pg/ml HPV 16 cutoff standard (i.e. 100 000 HPV16 genomes/ml) which is known to represent a semi-quantitative value for the cumulative viral burden of one or more of the 13 oncogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68).

A RLU/PC ratio of 1 or higher will be considered as a positive result, as proposed by the manufacturer (equivalent to 1 pg/ml of HPV DNA).

*Capture is reported to have 90-95% sensitivity and negative predictive value above 90-95%.*



## **Cytology**

Conventional cytology will be used (Bethesda 2001).

## **Colposcopy**

Colposcopy will be performed using Zeiss OPM1F (Karl Zeiss, Germany).

For the histopathological grading of the lesions, CIN nomenclature will be used. Directed punch biopsies will be taken from all colposcopic abnormalities, according to routine procedures. In patients with unsatisfactory colposcopy, the endocervical extension of the squamocellular junction will be explored using a Kogan forceps and an endocervical curettage will be performed.

All patients will undergo cytology, HR-HPV testing and colposcopy at the 3<sup>rd</sup> and 6<sup>th</sup> month after conization, and subsequent follow-ups of 3 to 6 months will be individualized according to the obtained results of the last visit and the severity of the dysplasia.

## **Questionnaires**

Parity, smoking habits, number of sexual partners.

# DEFINITIONS

*Residual or recurrent disease* is reported as a histological diagnosis of cervical intraepithelial neoplasia of any grade during the 24 months of post-treatment follow up [Alonso et al., 2006].

*Menopause* is defined as the time of the final menstrual period followed by 12 months of amenorrhea.

*Cone margins* will be reported as involved if the distance between cervical intraepithelial neoplasia and the resection surface is  $<1$  mm [Costa et al., 2000].

# STATISTICAL ANALYSIS

- Data will be analyzed using SPSS software for Windows.
- Comparisons of frequency distributions will be analyzed using Chi-squared test and Fisher's Exact test and comparisons of mean and median values between groups will be performed using Student's T-test and Mann-Whitney U test.



## ➤ **ETHICAL CONSIDERATION**

This project will be submitted to the Ethical Committee of the Ministry of Health of R. Macedonia. An informed consent will be obtained prior to patient enrollment in the study.

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THANK YOU!!!