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RISK FACTORS ASSOCIATED WITH PERSISTENCE, RECURRENCE AND PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) AFTER COLD KNIFE CONIZATION

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ABSTRACT

Objective: The aim of this study was to evaluate the significant risk factors that influence on persistence, recurrence and progression of CIN after cold-knife conization (CKC)

Design: Prospective follow-up study.

Methods: We retrospectively analyzed the likelihood of recurrent/residual disease in 143 pa-tients (mean age 39, 3 years, range 22 to 58 years) who underwent cold knife conization for CIN at the General Hospital Remedika, Department for Gynecology and Obstetrics, Skopje, Mace-donia in a period of 5 years.

Results: Women with HR- HPV DNA after cold knife conization had a 17,5 % risk of reccur-ence, whereas 0,8% of women with absent HR – HPV DNA were with recurrent/persistent CIN.

Conclusion: Persistence of HR-HPV DNA was only significant risk factor for recurrence and progression of cervical intraepithelial neoplasia (CIN) after Cold Knife Conization. Women with post-treatment HR-HPV types should be carefully followed-up every 4 months in a period of 2 years and all cytology abnormalities, followed by histological verification should be appropriately treated. Long-term follow-up strategy is obligatory because HR-HPV infection plays a predominant role in the pathogenesis of pre-invasive and invasive cervical cancer.

Key Words: Cold Knife Conization; CIN, HR- HPV Infection; Persistence; Recurrence.

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INRODUCTION

The third most common cancer in female population is the cervical cancer with an estimated 530,000 new cases in 2008 from GLOBOCAN (IARC) data. More than 85% of the cases are reported in developing countries, where it accounts for 13% of all female cancers. According to the WHO (World Health Organization) the incidence of cervical cancer in Europe region is 3.8% (age-standardized rate (ASR) is 10.1%), the mortality rate is 3.4% (ASR is 3.9%) and the 5-year prevalence is 4.6%. In our country the ASR incidence of cervical cancer 22% and ASR mortality rate is 9.9% [1, 2].

The human papillomaviruses (HPVs) are a family of more than 100 serotypes divided into low risk and high risk types. In the pathogenesis of pre-invasive and invasive cervical cancer the persistent infection with high-risk human papillomavirus serotypes (including types 16, 18, 31, and 45) plays a predominant role [3]. Fortunately, not all HPV infections lead to cervical cancer for the reason that human immune system is very powerful in highly effective clearance of most recent infections. However, if the infection is persistent for a long time, generally over 10-12 years, the transformation of the normal cervical cells might progress to cervical cancer. Basically, every tumor cell of the cervical cancer comprises sequences of high risk HPV types, therefore tests for DNA HPV typing, are one of the key tools for diagnosis and post treatment surveliance. Since nowadays it has been proved that cervical intraepithelial neoplasia (CIN,) when untreated, leads to invasive cervical cancer, early diagnosis and appropriate treatment is essential. Cervical conization including all its modalities with well-defined benefits and inconveniences (cold-knife conization, laser, or electrosurgical loop), is a cone-shaped excision from the cervix uteri including the transformation zone and section of the endocervical canal which plays a crucial role in diagnosis and treatment of cervical intraepithelial neoplasia (CIN). Nevertheless, cold-knife conization maintains the cleanest histological specimen proving to be still the best tool in the management (both diagnosis and treatment) of cervical intraepithelial neoplasia [4, 5].

The aim of this study was to evaluate the significant risk factors that influence on persistence, recurrence and progression of CIN after cold-knife conization. Presence of high risk-human papilloma virus (HR-HPV), age, education, menopausal status, smoking habits, parity, cytology grade, punch biopsy histological evaluation and margin involvement have been observed as risk factors associated with residual/recurrent disease in CIN treatment. HR- HPV infection plays a predominant role in the pathogenesis of preinvasive and invasive cervical cancer [6].

Frequent follow-up with cytology and colposcopy of the cervix is preferred strategy at present. Management of cervical pre-neoplasia starts with an abnormal Pap smear result. Patients with HSIL should be referred promptly for colposcopic–directed biopsy assessment, followed by one of the excision procedures (Cold Knife Conization or LLETZ).

Since the occurrence of cervical cancer may be extended in a period over 20 years, long term follow-up after conization is prescribed. Therefore, early detection of treatment failure is important.

PATIENTS AND METHODS

Study Population

A retrospective analysis was used to examine 143 women who underwent cold knife conization (CKC) for CIN, ina 5 year period, at the General Hospital Remedika, Department for Gynecology and Obstetrics, Skopje, Republic of Macedonia.

This study includes 143 patients. Their inclusion characteristics are shown in Table 1. The inclusion population of this study were patients who underwent CKC because of HSIL with or without presence of HR-HPV (patients with mixed HPV types, low-risk HPV and high risk HPV also were included in HPV high risk group) and followed-up with cytology/colposcopy and HPV tipization every 4 months in a period of 2 years, but at the first follow-up visit, 4 months after conization, only cytology and colposcopy were performed. After that period, twice a year cytology/colposcopy and HPV tipization has been made in next 2 years. A logistic regression analysis was performed to determine the risk factors for persistence, recurrence and progression of cervical intraepithelial neoplasia (CIN) after cold knife conization with independent variables such as HR-HPV presence, age, menopausal status, smoking habits, parity, cytology grade, punch biopsy histological evaluation and margin involvement. All p-values were considered as significant when $p \leq 0, 05$.

Table 1—Characteristics of 143 Patients

Characteristics	No (%)
Age (mean)	39,3
Menopausal status	
No	130 (91,6)
Yes	13 (8,4)
Parity	
Nullipara	99 (69,2)
1-3	25 (17,5)
≥3	19 (13,3)
Smoking habits	
Smoker	82 (57,3)
No smoker	61 (42,7)
Education	
Elementary school	34 (23,8)
Secondary school	64 (44,8)
High school	45 (31,4)

Methods

HPV was detected by Polymerase Chain Reaction (PCR) method in the Laboratory for Molecular Biology, Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, R. Macedonia.

The material for analysis (exfoliated cells in medium) was analyzed 24-48 hours after sample collection. The cervical cells were collected and digested with an appropriate buffer containing Proteinase K and 0,5% SDS. The total DNA was isolated with NaCl/chloroform extraction and ethanol precipitation. The PCR amplification was performed with 3 pairs of consensus primers (MY09/11, GP5+/6+, HPVpU 1M/2R) specific for L1 and E6/E7 regions of the HPV genome (thermocycler Perkin Elmer Geneamp PCR System 2400). Positive and negative controls were included in each of the tested series. The positive primers were genotyped and digested with 7 restrictional endonucleases (AfaI, HaeIII, PstI, AccI, AvaII, BglII, AvaI) specific for "low-risk" HPV types (6, 11, 40, 42, 43, 44, 54, 55, 61, 70, 72, 81, MM8, CP6108) and "high-risk" HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, MM4, MM7, MM9). The results were analyzed with agarose gel electrophoresis and visualized on UV transluminator. The viral genotype was determined through the length of restrictional fragments of the electrophoresis gel [7].

Table 2—Preoperative and Postoperative Findings of 143 Patients

Findings	No(%)
HPV- high risk before CKC	
presence	137(95,8)
absence	6 (4,2)
Cytology	
ASCUS	45 (31,5)
ASC-H	18 (12,6)
LSIL	27 (18,9)
HSIL	53 (37,0)
Biopsy histology	
CIN I	12 (8,5)
CIN II	39 (27,2)
CIN III	92 (64,3)
Resection margin after CKC	
Negative	113 (79)
Positive	30 (21)
HSIL involving margins	5 (16,7)
LSIL involving margins	25 (83,3)

ASCUS: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells – high grade, LSIL: low-grade squamous intraepithelial lesion, HSIL: high- grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia

Conventional PAP was used and the results were interpreted according to the Bethesda System classification 2001 [8].

Results

Of total 143 patients who underwent cold knife conization (CKC) with the mean age 39,3 years (range 22 to 58 years), 82 patients (57,3%) were smokers and only 13 patients (8,4 %) were postmenopausal. 99 patients (69,2%) were nullipara and 44 patients (30,8%) had children. 24 patients were excluded of this study because of inadequate follow-up. 5 patients had HSIL involving margins after conization and underwent hysterectomy. For the remaining 114 patients who were obtained in this study, CIN I involving margins was diagnosed in 25 patients and 89 were with negative resection margins.

114 patients were followed-up with cytology/colposcopy and HPV tipisation every 4 months in a period of 2 years, but at the first follow-up visit, 4 months after conization only cytology and colposcopy was performed. After 4th follow- up visit, 12 months after cold knife conization, results represented detection of

Table 3—Follow-up of the 114 Patients with HPV Presence/Absence, 12 Months After Conization

		Neg. No (%)	Pos. No (%)	p-value
Age	≥45	91	10	0.1734
	<45	10	3	
Cytology	LSIL	34	4	0.4292
	HSIL	50	12	
Biopsy histology	Low	38	4	0.7901
	High	64	8	
HPV	Low	42	10	0.0198
	High	38	25	

HR-HPV DNA in 25 patients. Cytological abnormality was detected in 16 patients, who underwent colposcopic–directed biopsy of the cervix. 8 of them had HR-HPV type, and 1 had mixed HPV types and 1 one was without HPV. The biopsy results of those 16 patients showed that 4 had no dysplasia, 4 had CIN I while 8 had recurrent high pathological abnormality and underwent repeated conization, laser CO2 vaporization or hysterectomy (depended of the grade of CIN, age and parity). After 24 months, only 4 of remaining untreated patients showed HR-HPV with cytological abnormality and after biopsy of the cervix, 2 had no dysplasia, and 2 had CIN I ectocervicaly. Those two patients underwent laser CO2 vaporization. Only 20 patients of all 114 developed residual/recurrent disease during the follow-up period.

Analysis showed that presence of HR-HPV DNA was the only significant risk factor for persistence, recurrence and progression of cervical intraepithelial neoplasia (CIN) after Cold Knife Conization. (p-value 0,0198). Age, education, menopausal status, smoking habits, parity, cytology grade, punch biopsy histological evaluation and margin involvement were not significantly associated with persistence/ recurrence of cervical intraepithelial neoplasia (CIN).

Discussion

Several publications describe significant relationship between HR-HPV persistence and residual/ recurrent cervical intraepithelial neoplasia describing an excellent sensitivity and negative predictive value of HPV DNA testing after conization for predicting recurrence [9, 10]. They conclude that persistence or clearance of HR-HPV DNA is an early valid prognostic marker of treatment failure for CIN2+ and is more accurate than cytology or section margin status at the time of conization. In the meta-analysis of the 11 studies, the negative predictive value (NPV) for recurrent/residual disease of HR- HPV testing was 98% (95% CI 97-99%), that of resection margins 91% (95% CI 87-94%), and that of cervical cytology 93% (95% CI 90-95%) [11]. Results demonstrate that the presence of HR-HPV, as well as multiple HPV types pre-conization, is associated with higher rates of residual/recurrent disease after conization [12].

Our findings suggested that women with HR-HPV DNA after cold knife conization had a 17,5 % risk of recurrence, while only 0,8 % of women with absent HR – HPV had a risk to develop recurrent disease. Margin status was not significantly associated with human papillomavirus status. Age, education, menopausal status, smoking habits, parity, cytology grade, punch biopsy histological evaluation also are not significant risk factors for persistence/ recurrence of cervical intraepithelial neoplasia (CIN).

Conclusion

Persistence of HR-HPV DNA was only significant risk factor for recurrence and progression of cervical intraepithelial neoplasia (CIN) after Cold Knife Conization. Women with post-treatment HR-HPV types should be carefully followed-up every 4 months in a period of 2 years and all cytology abnormalities, followed by histological verification should be appropriately treated. Long-term follow-up strategy is obligatory because HR-HPV infection plays a predominant role in the pathogenesis of preinvasive and invasive cervical cancer.

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