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ОФИЦИАЛНО ИЗДАНИЕ НА БЪЛГАРСКОТО НАУЧНО ДРУЖЕСТВО
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HPV STATUS AFTER COLD KNIFE CONIZATION

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Abstract. Introduction. *The aim of this study was to determine whether HPV DNA test after cold knife conization is a predictive factor for CIN persistence or recurrence. The study also investigated whether HPV DNA test results should influence post cold knife excision surveillance.*

Materials and methods. *A retrospective observation study was performed on 738 patients who underwent cold knife conization for CIN or microinvasive cervical cancer at the University Clinic of Obstetrics & Gynecology, Medical Faculty, Ss. Cyril and Methodius University, Skopje from 1st June 2007 to 1st June 2009. A total of 217 patients met the inclusion criteria and were with complete data.The follow-up HPV DNA testing was performed at 8 months after cold knife conization, after which the patients were followed-up every 4 months till 24 months postoperatively.*

Results. *HPV DNA testing after 8 months after conization showed that 44 patients were HPV DNA positive and 199 were HPV DNA negative. Recurrent cytological abnormalities were found in 26 of the 44 HPV DNA positive patients, and in 12 of the 199 HPV DNA negative patients. Analysis showed that a positive HPV DNA result was a risk factor for recurrent/persistent cervical intraepithelial neoplasia.*

Conclusion. *HPV DNA testing 8 months after conization is important for predicting the risk of disease: persistence or recurrence. In addition, such testing can assist in designing patient management, since HPV DNA negative patients should undergo routine surveillance, while HPV DNA positive patients should undergo frequent and meticulous surveillance.*

INTRODUCTION

Ablative or excisional techniques as a conservative outpatient therapy for cervical intraepithelial neoplasia(CIN) is widely used all over the world. The prevention of invasive cervical cancer is the main objective of this treatment. [1] Conization of the uterine cervix with cold knife conization (CKC) is diagnostic and therapeutic procedure for cervical intraepithelial neoplasia (CIN).[2,3]

Conservative outpatient therapy in women with CIN reduces the risk of invasive cancer of the cervix by 95% during the first 8 years after treatment. However, even with careful, long-term follow-up, the risk of invasive cervical cancer among these women is about five times greater than that among the general population of women throughout that period. Careful follow-up is essential for at least 10 years after conservative treatment of CIN.[1] These findings indicate the importance of continuous and meticulous follow-up.

Factors reported to be associated with persistent or recurrent cervical neoplasms after conization include menopausal status, grade of dysplasia, follow- up cervical cytology, cone diagnosis of CIN 3, cone margin status, and positive endocervical curettage. [3,4,5] However, these factors are suboptimal predictors, and cannot be used to dictate the follow-up strategy after conization.

Frequent follow up with cytology and colposcopic evaluation of the cervix is the preferred strategy at present, on the other hand, if a cone biopsy has CIN-free section margins, the risk on recurrences is still in the range of 5–35% of women.[3,6,7]

High-risk human papillomavirus infection plays a predominant role in the pathogenesis of preinvasive and invasive cervical cancer and there is increasing evidence that testing for the presence of human papilloma virus DNA after cold knife conization may help predict the likelihood of persistent or recurrent disease.[8,9,10]

HPV DNA test after cold knife conization is a predictive factor for CIN persistence or recurrence. The study also investigated whether HPV DNA test results should influence post cold knife excision surveillance.

MATERIALS AND METHODS

Study design

A retrospective study was performed on 738 patients who underwent cold knife conization for CIN or microinvasive cervical cancer at the University Clinic of Obstetrics & Gynecology, Medical Faculty, Ss. Cyril and Methodius University, Skopje from 1st of June 2007 to 1st of June 2009. The routine diagnostic HPV tests and further HPV typing were done at the HPV Laboratory at the University Clinic of Obstetrics & Gynecology, Medical Faculty, Ss. Cyril and Methodius University, Skopje while the histopathological evaluation of tissue specimens removed at excision from the cervix were performed at the Department of Pathology, Medical Faculty, Ss. Cyril and Methodius University, Skopje. The protocol of the Clinic is that cytology check-ups are performed every 4 months in the following 2 years and HPV DNA testing from the second follow-up visit (8 months after the operation) and after that every 4 months (till the end of the second year, postoperatively).

The inclusion criteria of this study were:

1. Patients whose HPV test results before the cold knife conization were available.
2. Negative margins on pathological examinations in patients after cold knife conization
3. Patients whose follow-up cytology results and HPV DNA test results after conization were available.

HPV detection and typing

First step in HPV testing was isolation of DNA from collected cells (exfoliated cervical cells in medium). The cervical cells were digested with an appropriate buffer containing Proteinase K. DNA Extraction employs glass fibers, fixed in column, that specifically binds DNA in the presence of a chaotropic salt. Genomic DNA was eluted by a low salt solution; a negative control was included for every DNA isolation (AccuPrep® Genomic DNA Extraction kit, Bioneer, Inc.)

HPV was detected by Multiplex PCR (polymerase chain reaction) with high sensitivity and specificity by applying DPO (Dual Priming Oligonucleotide) technology (Seeplex® HPV4A ACE Screening, Seegene, inc.). Positive and negative controls were included for every amplification. The internal control was DNA plasmid.[11,12]

HPV Screening can simultaneously genotype (HPV 16 and 18) and screen for 16 high-risk types ([illegible] 73, 82) and 2 low-risk types (HPV 6, 11). Aliquots of the PCR were run on a 1.5% agarose gel and analyzed under UV light following ethidium bromide staining.

The positive samples were genotyped with simultaneous amplification of specific E6 gene of several HPV types (6, 11, 16, 18, 31, 33, 45, 52, 58). Positive and negative controls were included in each of the tested series.[13,14]

The results were analyzed with agarose gel electrophoresis and visualized on UV transluminator.

Cytology

All of the cervical cytology tests in this study were liquid-based cytology (CYTOFAST by HOSPITEX DIAGNOSTICS, Sesto Fiorentino, Italy). All specimens were stained using the Papanicolaou method and were evaluated using the Bethesda III system (2001).

Histology

Serial section analysis of the tissue specimen excised was performed according to the standard protocol. Cone biopsies and LLETZ specimens are cut in parallel slices at 2,5 mm intervals, with each piece of tissue being embedded in an individual cassette. A six haematoxylin and eosin section is examined initially with a further six or more sections, being examined if the tissue is incomplete, if there is no evidence of cervical intraepithelial neoplasia, if there are features that are suspicious of invasive disease, or if margins are involved.

Statistics

SPSS software for Windows (version 9.0; SPSS inc., Chicago, IL) was used for analysis of data. Odds ratio and Z statistics was calculated expressing the statistical significance at 5% level.

RESULTS

From 738 patients treated with cold knife conization, a total of 217 patients met the inclusion criteria and were tested posi¬tive for the presence of the HPV. The median age of them was 39.3 years (range, 20 to 62 years), and 13 were postmenopausal.

Following conization, the diagnosis was CIN I in 25 patients, CIN II in 47 patients, and CIN III in 145 patients. The most frequent type before conization was HPV 16 (31%), followed by HPV 31 (14%), HPV 66(9%), HPV 6 (5%), HPV 58 (8%), HPV 33 (4%), HPV 18 (5%) and other types(14%). In 22.4% multiple infection was present.

HPV DNA testing **after 8-10 months** after conization showed that HPV DNA positive were 41 patients and 176 were HPV DNA negative.

Recurrent cytological abnormalities in 24 of the 41 HPV DNA positive patients were found, and in 9 of the 176 HPV DNA negative patients. Analysis showed that a risk factor for recurrent cytological abnormality was positive HPV DNA result (Odds ratio 26.1961 95% CI 10.4996 to 65.358 z statistic 7.001, Significance level $P < 0.0001$),

The types of recurrent cytological abnormalities were **ASCUS** in 12 patients, **LSIL** in 9 patients, and **HSIL** in 12 patients. Of these patients, regression to normal cytology in subsequent follow-up tests showed 7, and 26 underwent biopsies of the cervix. The biopsy results of those 26 patients showed that in 8 there was no dysplasia, while in 18 there was recurrent pathological abnormality.

Recurrent pathological abnormalities in 13 of the 41 **HPV DNA positive** patients were found, and in 5 of the 176 **HPV DNA negative** patients. Analysis showed that a positive HPV DNA test result was a risk factor for recurrent pathological abnormality (Odds ratio 15.8786 95% CI 5.2533 to 47.9945 z statistic 4.899, Significance level $P < 0.0001$).

The types of recurrent pathological abnormalities were **CIN I** in 7 patients, **CIN II** in 5 patients and **CIN III** in 6 patients. In 12 patients laser vaporization – ectocervical lesion was done and 6 had hysterectomies.

The types of HPV that occurred in recurrent CIN were HPV 16(71%) and other HPV types (29%). The multiple infection was present in 8 patients with recurrent pathological abnormalities.

DISCUSSION

The relationship between a persistent HR-HPV infection and CIN2+ is well established.[15,16,17]

We studied the value of the presence of HPV DNA 8, 16 and 24 months after conisation in predicting recurrences. Our results show that at 6 months after cold knife conization a positive HPV DNA test is more predictive for posttreatment than abnormal cervical cytology. The negative predictive value of a HPV DNA test in negative cytomorphologically normal cervical smear is very high and the presence of high-risk HPV 24 months after treatment is a risk-factor for new post-treatment CIN 2/3. [18] Therefore, we consider HPV DNA testing valuable in the early detection or prediction of post-treatment CIN.

There is increasing evidence that HPV DNA testing after cold knife conization is important for detecting persistent or recurrent disease.[19] The accuracy in predicting recurrence in cases of abnormal cytology is higher if HR-HPV DNA is present (50 versus 0%). Bar-Am et al.[20] demonstrates that adding HPV testing to cytology serves as an extra safety measure in predicting recurrences and helps to select women for colposcopy. Paraskevaidis et al. [21] demonstrated that HPV DNA testing predicts treatment failure more accurately than either the first post-treatment Pap smear or positive cone margins. The 2001 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines state that HPV DNA testing is acceptable for post treatment surveillance. HPV DNA testing post-cold knife conization is useful for detecting not only persistent disease but also recurrent disease. [22] The sensitivity, specificity, positive and negative predictive values of HPV DNA testing for detecting persistent or recurrent disease after cold knife conization have been reported in several studies. In particular, the negative predictive value was found to be very high in all studies. [20,21,23]

We conclude that women with positive HR-HPV DNA after conization need intense follow up since they are at increased risk for recurrent or residual/ recurrent CIN. This may be even more important for older women. If HR-HPV DNA is absent, then risk of recurrence is low; Nevertheless, in other studies, some rare recurrent cases that were HPV negative could be detected by cytology only.[23]

The 2001 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend that HPV DNA testing be performed at least 6 months after treatment to provide sufficient time for clearance of the HPV infection, and that it can be performed at 12 months after treatment unless a patient has risk factors for persistent/recurrent CIN, such as a large lesion or endocervical extension.[22]

We advocate to monitor women 8 months after initial treatment both by HPV DNA testing and cervical cytology. In case of a positive cytology test, biopsies are indicated. Retesting by both tests should be considered at 16 and 24 months after initial treatment to avoid missing cervical carcinomas because of detection problems. Moreover, it is known that acquisition of HPV is increased in women with a history of CIN lesions.

CONCLUSION

HPV DNA testing 8 months after conization is important for predicting the risk of disease persistence or recurrence. In addition, such testing can assist in designing patient management, since HPV DNA negative patients should undergo routine surveillance, while HPV DNA positive patients should undergo frequent and meticulous surveillance.

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