

АКУШЕРСТВО И ГИНЕКОЛОГИЯ

OBSTETRICS AND GYNECOLOGY

The official journal of the Bulgarian Scientific Society
of Obstetrics and Gynecology



Volume 52
ISSN 0324-0959

2'2013

ОРИГИНАЛНИ СТАТИИ

Димитров Р. - Биометрия и оценка на леваторния хиатус с триизмерна ехография на тазовото дъно 3

Корновски Я., Е. Исмаил, С. Славчев - Лимфни метастази и свободна от заболяване преживяемост при пациенти с рак на маточната шийка 9

ОБЗОРИ

Лазаров Н., Л. Лазаров, С. Лазаров - Оперативното лечение - прогностичен фактор за пациентки с рак на яйчника I-II ст. 13

Ковачев С. - Социални и сексуални рискови фактори за вагинална дисбактериоза 17

Слънчева Б., Хр. Мумджиев - Деца, родени малки за гестационната възраст - дефиниция, етиология и неонатално поведение 25

Слънчева Б., С. Хитрова, Д. Марков, Л. Вакрилова, Т. Праматарова, Н. Яръкова, О. Бранков - Вродени белодробни лезии при новородени - преглед на литературата с описание на три случая 33

Мумджиев Хр., Б. Слънчева - Интраутеринната хипотрофия и програмиране на здравето. Късни проблеми при новородените деца с интраутеринна хипотрофия 40

КЛИНИЧНИ СЛУЧАИ

Цанкова М., Б. Маринов, Д. Божилов, Е. Пирнарева - Placenta accreta - пренатална диагноза, поведение 48

Божинова С., К. Порожанова - Простагландин F2 α (Prostin 15 M) за прекъсване на бременност във II-ри триместър 53

Михайлова И., В. Първанова, П. Костова - Дисгерминома на яйчника. Представяне на клиничен случай 56

ЗА ПРАКТИКАТА

PrenaTest[®] е вече на пазара в над 30 страни от Европа, Азия и Близкия Изток 60

Велев Р., М. Мирчева - Приложение на Gynofit в акушерската практика 61

НАШИ ЧУЖДЕСТРАННИ ГОСТИ

Dimitrov G., B. Talevska, S. Nikolovski, M. Micevska, S. Panov, Gl. Dimitrov - HPV status after cold knife conization... 65

ORIGINAL ARTICLES

Dimitrov R. - Biometry and assessment of the levator hiatus by three-dimensional pelvic floor ultrasound 3

Kornovski Y., E. Ismail, S. Slavchev - Lymph node metastases and disease-free survival in cervical cancer patients 9

REVIEW

Lazarov N., L. Lazarov, S. Lazarov - Operative treatment - prognostic factor in patients with ovarian cancer stage I-II 13

Kovachev S. - Vaginal disbacteriosis - social and sexual risk factors 17

Slancheva B., Hr. Mumdzhev - Small for gestational age newborns - definition, etiology and neonatal treatment 25

Slancheva B., S. Hitrova, D. Markov, L. Vakrilova, T. Pramatarova, N. Yarukova O. Brankov - Congenital cystic lung lesions - review of the literature with three clinical cases 33

Mumdzhev Hr., B. Slancheva - Intrauterine hypotrophy and programming the health status. Late problems in newborns with intrauterine hypotrophy 40

CLINICAL CASES

Tsankova M., B. Marinov, D. Bozhilov, E. Pimareva - Placenta accreta - prenatal diagnosis, treatment 48

Bojinova S., K. Porozhanova - The use of Prostaglandin F2 α (Prostin 15M) for termination of second trimester pregnancy 53

Mihaylova I., V. Parvanova, P. Kostova - Dysgerminoma of the ovary. Presentation of case report 56

FOR THE PRACTICE

PrenaTest[®] 60

Velev R., M. Mircheva - Application of Gynofit In Obsetrics 61

OUR FOREIGN GUESTS

Dimitrov G., B. Talevska, S. Nikolovski, M. Micevska, S. Panov, Gl. Dimitrov - HPV status after cold knife conization... 65

АКУШЕРСТВО И ГИНЕКОЛОГИЯ

ОФИЦИАЛНО ИЗДАНИЕ НА БЪЛГАРСКОТО НАУЧНО ДРУЖЕСТВО
ПО АКУШЕРСТВО И ГИНЕКОЛОГИЯ

Volume 52

ISSN 0324-0959



"АКУШЕРСТВО И ГИНЕКОЛОГИЯ"
се индексира в MEDLINE

"AKUSHERSTVO I GINECOLOGIA"
Is indexed in MEDLINE

РЕДАКЦИОННА КОЛЕГИЯ

Ст. ИВАНОВ - главен редактор
Г. ГОРЧЕВ - зам. гл. редактор
М. СИРАКОВ - секретар

Членове:

Ил. КАРАГЪЗОВ, А. ДИМИТРОВ,
Т. ЧЕРНЕВ, И. КОЗОВСКИ, В. ЗЛАТКОВ
Н. ВАСИЛЕВ, Н. ДОГАНОВ, А. НИКОЛОВ

EDITORS

St. IVANOV - Editor-in-chief
G. GORCHEV - Managing editor
M. SIRAKOV - Scientific secretary

Editors

I. KARAGIOZOV, A. DIMITROV,
T. CHERNEV, I. KOZOVSKI, V. ZLATKOV
N. VASSILEV, N. DOGANOV, A. NIKOLOV

РЕДАКЦИОНЕН СЪВЕТ

Б. Слънчева, Б. Налбански, Ж. Карагъзова,
Б. Стамболов, М. Янков, С. Божинова,
Й. Попов, Е. Рачев, Б. Маринов,
М. Попова, С. Иванов
К. Пуле (Италия), Г. Креацас (Гърция),
Уайнбаум (САЩ), Х. Хошиай (Япония),
А. Е. Шиндлер (Германия),
В. Кесич (Сърбия и Черна гора), Н. Хакер (Австралия)

Технически редактор Е. Павлова

EDITORIAL BOARD

B. Slancheva, B. Nalbansky, Z. Karagiozova,
B. Stambolov, M. Yankov, S. Bojinova,
Y. Popov, E. Rachev, B. Marinov,
M. Popova, S. Ivanov,
C. Pulle (Italy), G. Creatsas (Greece),
Weinbaum (USA), H. Hoshiai (Japan),
A. E. Schindler (Germany),
V. Kesic (Serbia and Montenegro), N. Hacker (Australia)

Technical editor E. Pavlova

Адрес на редакцията:
ул. Здраве 2, София 1431
Тел. 02 / 91-72-353
Факс 02 / 851-72-71
e-mail: bsobgyn@abv.bg
Моб. 0888 92 56 26

HPV STATUS AFTER COLD KNIFE CONIZATION

Goran DIMITROV¹, Biljana TALEVSKA², Sotir NIKOLOVSKI³, Megi MICEVSKA⁴, Saso PANOVA⁵, Gligor DIMITROV⁶

1,2,4 - University Clinic of Obstetrics & Gynecology, Medical Faculty, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia

3 - Clinical Hospital Dr. Trifun Panovski, Bitola, Republic of Macedonia

5 - Faculty for Natural and Mathematical Sciences, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia

6 - Private General Hospital Remedika, Skopje, Republic of Macedonia

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 (

16, 18, 31, 33, 35, 39, 41, 42, 43, 44, 45, 49, 51, 52, 53, 55, 56, 59, 66, 68, 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 transilluminator.

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 positive 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 conization 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 cytologically 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.

- Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia. *Lancet*. 1997 Apr 5;349(9057):978-80.
2. Kucera E, Sliutz G, Czerwenka K, Breitenecker G, Leodolter S, Reinthaller A. Is high-risk human papillomavirus infection associated with cervical intraepithelial neoplasia eliminated after conization by large-loop excision of the transformation zone? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2001 Dec 10;100(1):72-6.
 3. Nagai Y, Maehama T, Asato T, Kanazawa K. Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence? *Gynecol. Oncol.* 2000 Nov;79(2):294-9.
 4. Chen Y, Lu H, Wan X, Lv W, Xie X. Factors associated with positive margins in patients with cervical intraepithelial neoplasia grade 3 and postconization management. *Int J Gynaecol Obstet.* 2009 Nov;107(2):107-10.
 5. Ørbo A, Arnesen T, Arnes M, Straume B. Resection margins in conization as prognostic marker for relapse in high-grade dysplasia of the uterine cervix in northern Norway: a retrospective long-term follow-up material. *Gynecol. Oncol.* 2004 May;93(2):479-83.
 6. Gardeil F, Barry-Walsh C, Prendiville W, Clinch J, Turner MJ. Persistent intraepithelial neoplasia after excision for cervical intraepithelial neoplasia grade III. *Obstet Gynecol.* 1997 Mar;89(3):419-22.
 7. Narducci F, Occelli B, Boman F, Vinatier D, Leroy JL. Positive margins after conization and risk of persistent lesion. *Gynecol. Oncol.* 2000 Mar;76(3):311-4.
 - Res. 2002 Dec;22(6B):3733-6.
 9. Paraskevaidis E, Arbyn M, Sotiriadis A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G, et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat. Rev.* 2004 Apr;30(2):205-11.
 10. Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *Lancet*. 1999 Jul 3;354(9172):20-5.
 11. Chun JY, Kim KJ, Hwang IT, Kim YJ, Lee IK, Kim JK. Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. *Nucleic Acids Res.* 35(6):40 (2007)
 12. Jin Han. Highly Specific Dual Priming Oligonucleotide and Its Application to Multiplex PCR. 11th AsianPacific Congress of Clinical Biochemistry (October 14th-19th 2007, Beijing, China).
 13. Hong JH, Lee JK, Lee NW, and Lee KW. Comparison of the Seeplex HPV4 ACE Screening and Hybridcapture 2 Assay for Detection of High-risk HPV and HPV-16, -18. Eurogin 2008, Joining forces for cervical cancer prevention. (Nice, Acropolis, France. November 12-15,2008).
 14. Shim HS, Noh SM, Park AR, Lee YN, Kim JK, Chung HJ, Kang KS, Cho NH. Detection of sexually transmitted infection and human papillomavirus in negative cytology by multiplex PCR. *BMC Infect Dis.* 2010;10(284):1-8