Evaluation of HPV DNA HR assay in females as a marker of recurrent disease following treatment of cervical intraepithelial neoplasia

Ocena wartości testu DNA HPV HR u kobiet po leczeniu z powodu śródnabłonkowej neoplazji szyjki macicy jako markera nawrotu procesu chorobowego

Mikolajczyk Katarzyna[1], Kedzia Witold[2], Zaba Ryszard[1], Silny Wojciech[1]

[1] Chair and Department of Dermatology of the University of Medical Sciences in Poznan, Poland
[2] Department of Gynecological Oncology of the Chair of Gynecology and Obstetrics of the University of Medical Sciences in Poznan, Poland

Summary

Aim: *The basic assumption of the prevention of cervical cancer is to early detect and treat CIN (cervical intraepithelial neoplasia) as well as to prevent recurrence of neoplasia after therapy. This study involved comparison of the cytology test value and determination of HPV (human papilloma virus) DNA in women treated for CIN so as to find a sensitive and specific marker of disease recurrence.*

Methods: *A group of 107 females after CIN treatment underwent 14-month follow-up and regular cytological and molecular evaluations.*

Results: *Based on the follow-up data the recurrence of CIN was found in 9 females who despite effective therapy for the entire follow-up period were HPV positive. Evaluation of value of HR (high risk) - HPV DNA assay used to detect CIN showed its 100% sensitivity.*

Conclusion: *The HR-HPV DNA assay is likely to be a valuable diagnostic tool facilitating more precise detection of recurrent neoplasia risk than cytological test alone.*

Key words: **HPV** / **cervical neoplasia** / **recurrence of CIN** /

Author for correspondence:
Katarzyna Mikołajczyk
Chair and Department of Dermatology of the University of Medical Sciences in Poznan
Poland, 60-355 Poznan, Przybyszewskiego 49
phone: +48 61 869 12 85
fax: + 48 61 869 15 72
e-mail: katarzyna.mikolajczyk@op.pl

Otrzymano: **15.06.2011**
Zaakceptowano do druku: **16.08.2011**

Streszczenie

Cel pracy: *Podstawowym założeniem profilaktyki raka szyjki macicy, jest wczesne wykrycie i leczenie CIN a także zapobieganie nawrotom neoplazji po leczeniu. W pracy porównano wartość testu cytologicznego oraz oznaczania DNA HPV u kobiet leczonych z powodu CIN w celu poszukiwania czułego i swoistego markera nawrotu procesu chorobowego.*

Metoda: *14-miesięcznej obserwacji poddano 107 kobiet po leczeniu CIN, u których regularnie wykonywano ocenę cytologiczną i molekularną.*

Wyniki: *Nawrót CIN wykryto u 9 obserwowanych kobiet, które mimo skutecznej terapii przez cały okres obserwacji były HPV pozytywne. Ocena wartości testu na obecność DNA HPV HR, użytego do wykrycia CIN wykazała 100% czułość tej metody.*

Wnioski: *Test na obecność DNA HPV HR może być cennym narzędziem diagnostycznym, pozwalającym bardziej precyzyjnie niż badanie cytologiczne wykryć ryzyko nawrotu neoplazji.*

Słowa kluczowe: **HPV** / **neoplazja szyjki macicy** / **wznowa** / **CIN** /

Introduction

Malignant neoplasms are currently one of the most serious diseases affecting people, and the growing morbidity and mortality rates raise in consequence a lot of concern among medical professionals. The employment of more and more sophisticated diagnostic methods and effective therapy will not replace proper prophylaxis which – if used systematically and to an appropriate extent – undoubtedly contributes to the decreased incidence of malignant neoplasms. It is noticeable especially in case of prophylaxis of cervical cancer. Cervical cancer is the second most common malignancy in females worldwide [1]. According to the National Cancer Registry in Poland, cervical cancer makes up 5.2% of all cancers in women and morbidity rate is the third most common one after breast cancer (21.5%) and lung cancer (8.2%); at the same time is the most frequent cancer of the genital organs [1]. The reason for this unfavorable situation lies in low efficacy of programs against cancers of this organ and poor attendance rate in prophylactic medical examinations [2]. Documentation of Human Papilloma Virus (HPV) infection in etiology of this disease gives an outstanding chances to prevent and early detect cervical diseases and cervical cancer [3,4]. Recent studies showed that HPV DNA assay combined with a traditional cytology may lead to early detection of both primary cervical neoplasia as well as recurrence of neoplasia after therapy, decreasing the need for colposcopy and treatment [5, 6].

Study objective

The aim of this study was to evaluate the value of HR-HPV DNA assay as a marker of recurrent disease in females after therapy for intraepithelial cervical neoplasia.

Study population and design

The studied material included pap smears and tissue specimens collected from the cervix in 107 females diagnosed at the Cervical Pathophysiology Lab (CPL) of the University Clinical Obstetrics and Gynecological Hospital in Poznan between June 2004 and October 2006. Females were referred to the CPL due to abnormal pap smears (ASCUS, LSIL, HSIL) [7].

Each subject had cervical biopsy under colposcopy. Histopathological examinations of the tissue specimens revealed various grade cervical intraepithelial neoplasia. Based on those results, 3 study groups were distinguished: 32 patients with CIN I, 43 patients with CIN II, and 32 patients with CIN III (mean age 31.7, 33.7, and 35.9 years, respectively). Prior to the onset of CIN treatment all patients had cytological samples collected from the cervical disk and canal in order to detect high oncogenic-risk HPV DNA. This examination was repeated in each woman at 2 to 4 months after the therapy. Control HPV DNA tests and cytological smears were also performed at 6-8 and 12-14 months after treatment. The patients with abnormal cytological smears (ASC-US, LSIL, HSIL) had another colposcopy performed and specimens collected for histopathological reanalysis.

The study participants declared complete sexual abstinence from treatment onset to the first control of HR-HPV DNA. During the study conduct, both females and their partners declared to avoid adulterous sexual intercourse outside marriage or partnership.

Human papilloma virus DNA was determined in the cellular material collected from the cervical disc and canal using Amplicor HPV equipment. Amplicor HPV quality test used amplification of DNA segment by polymerase chain reaction (PCR) and hybridization of nucleic acid to detect genotypes of high risk (high risk – HR) HPV DNA: 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59 and 68 in cervical canal cells drawn into liquid transport medium.

The Amplicor HPV test simultaneously performs amplification of PCR of the searched HPV DNA segment and B-globin DNA (cellular control). Amplification mixture (Master Mix) contains DNA starter pairs for 13 high risk HPV genotypes and B-globins. The detection of amplificated DNA fragments (amplicons) is carried out using oligonucleotide probe, which helps to independently identify HPV amplicons and B-globins amplicons. The use of AmpErase enzyme limits the risk of cross pollution, thus ensuring a selective amplification of the examined nucleic acid. This test provides repeatability of results and does not raise interpretative doubts, having high clinical sensitivity and specificity of 96 % [5].

Results

The group of 107 women with confirmed cervical intraepithelial neoplasia of various severity were followed-up: 32 patients with CIN I, 43 with CIN II and 32 with CIN III. Before the treatment, all subjects were HR-HPV positive. In 91 cases leep-loop conization was performed, 10 subjects underwent cryotherapy and in the remaining hysterectomy was carried out. At the first follow-up visit after end of treatment, 47 subjects (including 17 with CIN I, 19 with CIN II and 11 with CIN III) had persistent HR-HPV infection. Simultaneous cytological examination (ASCUS or LSIL) was abnormal in only 5 ones. Subsequent follow-up visits at 6-8 and 12-14 months proved persisting HR-HPV infections in 37 and 29 patients, respectively.

Based on the analysis of cytology results, at follow-up II and III, persistent HPV infection was present in 9 and 18 patients, respectively. Based on the histological examinations over the entire follow-up period the recurrence of cervical intraepithelial neoplasia was found and confirmed in 9 treated subjects (2 of them were already present during the follow-up II visit). Among HPV negative patients, there was no recurrent CIN detected or diagnosed. On the other hand, despite effective elimination of HR-HPV during treatment, 9 women were reinfected with HPV by their sexual partner and it remained till the end of the follow-up period. The results of statistical analysis of all women, without considering the division with respect to neoplasia severity, demonstrated 100% sensitivity of both diagnostic tests for detecting recurrent CIN at follow-up visits II and III.

Table I. Statistical analysis of viral and cytological test results for detecting cervical intraepithelial neoplasia recurrence at follow-up visits II and III after treatment in all participating subjects.

TEST TYPE	FOLLOW-UP II		FOLLOW-UP III	
	HPV DNA	PAP	HPV DNA	PAP
SENSITIVITY	100%	100%	100%	100%
SPECIFICITY	66.7%	93.3%	78%	89%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH POSITIVE PREDICTION	5.41%	22.2%	24.2%	38.9%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH NEGATIVE PREDICTION	0%	0%	0%	0%

Table II. Statistical analysis of viral and cytological test results for detecting cervical intraepithelial neoplasia recurrence at the follow-up visits I, II and III after treatment in subjects with CIN.

TEST TYPE	I FOLLOW-UP		II FOLLOW-UP		III FOLLOW-UP	
	HPV DNA	PAP	HPV DNA	PAP	HPV DNA	PAP
SENSITIVITY	66.7%	16.7%	100%	66.7%	100%	100%
SPECIFICITY	59.5%	97.3%	70%	100%	83.8%	97.3%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH POSITIVE PREDICTION	21%	50%	35.3%	100%	50%	85.7%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH NEGATIVE PREDICTION	91%	87.8%	100%	94.8%	100%	100%

Table III. Statistical analysis of viral and cytological test results for detecting cervical intraepithelial neoplasia recurrence at follow-up I, II and III visits after treatment in subjects with CIN III.

TEST TYPE	I FOLLOW-UP		II FOLLOW-UP		III FOLLOW-UP	
	HPV DNA	PAP	HPV DNA	PAP	HPV DNA	PAP
SENSITIVITY	100%	66.7%	100%	66.7%	100%	100%
SPECIFICITY	72.4%	100%	75.8%	96.5%	82.7%	93.1%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH POSITIVE PREDICTION	27.3%	100%	30%	66.7%	37.5%	60%
PREDICTED DISEASE INCIDENCE IN PATIENTS WITH NEGATIVE PREDICTION	100%	96.7%	100%	96.5%	100%	100%

Specificity of viral test increases while specificity of cytology test decreases with the time lapse after treatment.

Statistical analysis of HPV DNA test in women with CIN II showed significantly higher sensitivity of this test at follow-up visit I and II as compared to the one of PAP test. As opposed to HPV DNA test specificity of cytological test for follow-ups is slightly higher.

Statistical analysis of HPV DNA test in women with CIN III showed 100% sensitivity both at I, II and III follow-up visits after treatment. Cytology test had 100% sensitivity only at the follow-up III visit after treatment. In turn, specificity of both tests was similar to the study group with CIN II.

Discussion

Each time before qualification for treatment the test for HPV DNA oncogenic type was done. Regarding the study objective only women with cervical intraepithelial neoplasia and positive test result for HR-HPV DNA. Results regarding the first detection for virus genetic material after therapy pointed out that during 2 to 4 months after the end of therapy human papilloma virus persisted in 45/107 (42%) followed-up women. It seems to be of crucial importance that in 4 of 6 patients with suspected persistent neoplasia, in whom – according to histopathological report – it is beyond certainty as regard the complete the excision of neoplastic epithelium due to material fragmentation thus resulting in positive result of HPV DNA test at first follow-up visit after treatment. In spite of secondary exclusion of CIN treatment failure, viral infection persisted in these 4 patients on subsequent follow-ups. Similar relationship was described by Nagai in 5 patients with persistent cervical intraepithelial neoplasia selected out of 56 females treated for CIN [8]. Likewise, Nobbenhuis demonstrated high, though not full, correlation between persistent neoplasia and HR-HPV DNA identifying genetic material of oncogenic types of HPV in 27 of 29 inefficiently treated women [9]. Attention should be drawn to the fact that only 2 subjects with persistent cervical intraepithelial neoplasia after therapy had negative HR-HPV DNA molecular test. There are also some separate reports on much weaker relationship between persistent, ineffectively treated CIN and presence of HPV in the specimen collected from the cervical canal. Acladious et al. demonstrated presence of HR-HPV DNA only in 22 of 47 women ineffectively treated for CIN and diagnosed with persistent neoplasia [10]. Paraskevaidis et al. in a cross-sectional analysis of 11 multicenter studies addressing correlation between persistent neoplasia and chronic HPV infection maintained after ineffective treatment showed presence of HR-HPV DNA in 82.8% of women in whom CIN therapy failed [11, 12].

Our study involving 107 females treated for CIN and followed up from a period prior to the treatment and up to at least 12-14 months after its end – recurrent CIN was detected and diagnosed in 9 of 107 (8.4%) subjects. According to Angel Chao et al. the risk of recurrence of intraepithelial neoplasia in women treated previously for CIN of different severity, affects 10.3% of patients [13]. It was estimated by Flannely et al. that an average risk of having recurrent CIN refers to about 10% of women during 2 years after the end of therapy [14]. This author observed 765 females who underwent ablation of CIN and who afterwards were followed-up for 3 years [14]. Sarian et al. performed an analysis of 107 females treated for cervical intraepithelial neoplasia [15]. Twelve-month follow-up revealed recurrence of CIN in 10 (11.2%) of treated women. In our study, the recurring disease was accompanied to a great extent by persistent viral infection in patients in whom CIN treatment did not eliminate it. The high likelihood of such etiology of recurrent CIN was observed and confirmed using HR-HPV DNA assay (in cervical samples) in 7 subjects, who had the first check-up at months 2-4 after the treatment when they refrained from sexual intercourse. Only in 2 of 9 individuals, who had recurrent CIN, apart from the first check-up the viral infection was also detected during subsequent ones. In those patients the first HR-HPV DNA test scored negative. Also according to other authors all cases of recurrent neoplasia seem to concern women, who had HR-HPV DNA identified at follow-up after CIN therapy [14, 15].

No studies are available so far attempting to explain whether HR-HPV DNA presence results from persistent infection or is a secondarily acquired infection from a sexual partner during follow-up after therapy for CIN. These studies showed the development of recurring cervical intraepithelial neoplasia only in females treated for CIN II and CIN III. It is typical that in females treated for CIN II, recurrent disease was diagnosed in 6 patients all of which could be classified as CIN I typical changes. It is worth noting that all women with secondary infection confirmed during the follow-up had recurrent neoplasia, which was documented during the last third follow-up visit. Three cases of recurrent disease were detected in the female group treated for CIN III. Only one patient had low-grade neoplasia confirmed during the third follow-up visit. The remaining two patients were diagnosed with recurrent disease during the second and the third follow-up related to CIN II and preinvasive cervical carcinoma-like lesion, respectively. It should be highlighted that the cases of recurrent neoplsia in this group occurred only in women with HR-HPV infection persisted after treatment. The observations made could be of great practical importance with respect to planning disease monitoring after CIN treatment and determining the schedule for follow-up HR-HPV DNA test as a relapse marker. Taking into account the fact that in followed-up women referred with CIN it is likely that the source of positive HPV test will be persistent or acquired infection. We have to remember, that any of sexually transmitted disease may be a risk factor of acquire HPV infection [16, 17, 18].

As a result, a test should be ordered to identify virus genetic material during the first follow-up visit after treatment and at least 12 months after treatment completion [19]. Provided that the first follow-up visit takes place in a patient who did not have sexual contact, a positive result of HR-HPV DNA test identifies subjects with persistent infection. The second test performed after at least 12 months confirms persistent infection in HR-HPV DNA positive women in the first examination or identifies secondarily infection in women negative at the first follow-up after treatment. In our studies it was proved that both sources of HR-HPV infection may contribute to CIN recurrence. Similarly, the studies of Kreimer and Sarian showed that none of the followed-up HR-HPV DNA negative females after CIN treatment had recurrent disease. Sarian et al. revealed that 11 of 107 subjects had recurrent cervical intraepithelial neoplasia due to CIN II or CIN III [15]. Most recurrent diseases involved high-grade neoplasia confirmed in 9 women. The remaining two patients had medium-grade neoplasia. Molecular tests for HR-HPV DNA were positive in

all women who experienced recurrent neoplasia following CIN treatment [15]. Kreimer et al. came to similar conclusions. They presented 34 recurrences of CIN during 24-month follow-up in 607 treated subjects. Again, all recurrences were documented in HR-HPV DNA positive women in cervical material [20]. What raises a great interest is the comparison of the effectiveness of HR-HPV DNA test between our studies and those of Kreimer carried out in the comparable study groups.

The studies in previously treated CIN patients performed in order to detect and diagnose another recurring neoplasia, the sensitivity was 100% both with respect to viral and cytological diagnostics of diagnostics used successively at the follow-up visits II and III. These results were also confirmed by Kreimer et al. who demonstrated 100% sensitivity for both tests during the first follow-up visit after treatment and its values for cytodiagnostics and molecular test detecting HR-HPV DNA which remained unchanged until the end of 12-month follow-up [20]. Number of investigators determined the sensitivity of HR-HPV DNA test as 100% in identifying development of CIN in women after neoplasia treatment [8,21]. Other studies with analogous examinations results showed slightly lower sensitivity of HR-HPV DNA test as opposed to 100% presented in this study. On the basis of data gathered during the follow-up of the larger group of females Nobbenhuis and Paraskevaidis showed 93% sensitivity of HR-HPV DNA test in detecting recurrence of CIN [9, 11, 12].

In our studies 66.7% and 78% specificity was obtained at follow up visit II and III, respectively, in molecular diagnostics of recurrent CIN in females previously treated for neoplasia. It should be added that the percentage of negative tests results for HR-HPV DNA in treated healthy subjects increases along with follow-up duration. In the available literature, individual investigators present different specificity of the HR-HPV DNA test as a method for diagnosing development of CIN recurrence in treated women. Lin et al. monitored 75 subjects after treatment of CIN and as a result gained 48% specificity for viral diagnostics in detection of recurrent disease [21]. Specificity of HR-HPV DNA tests for CIN detection given by other authors are as follows: 88%-Nagai, 86%-Nobbenhuis, and 84%-Paraskevaidis [8, 9, 11, 12].

In this study, an increase of specificity with respect to viral diagnostics is parallel with gradual decrease in specificity for cytodiagnostics – from 93.3% at the first follow-up visit to 89% at the second one. Based on these observations there is the need for oncologic follow-up in subjects treated for CIN for more than only 12 months. Diagnostic value of HR-HPV DNA molecular test, as a method of detection of recurrent CIN in previously treated women, increases along with time and seems to be of great significance during baseline follow-up and in the second year after treatment.

An attention should be drawn to significance of initial viral test for prediction of cervical intraepithelial neoplasia in females receiving effective treatment of CIN, who however were not cured of viral infection or acquired secondary infection, as all women who experienced recurrent CIN were HPV DNA positive at the second follow-up after treatment. A significant fact is that 7 of 9 subjects with CIN recurrence had HR-HPV DNA detected at the first follow-up visit after treatment for neoplasia.

Conclusion

The HR-HPV DNA assay is likely to be a valuable diagnostic tool facilitating more precise detection of recurrent neoplasia risk than cytological test alone.

References

1. National Cancer Registry In Poland. Warsaw, M.C. Sklodowska Oncology Center, 2006.
2. Franco E, Duarte-franco E, Ferenczy A. Prospects for controlling cervical cancer at the turn of the century. *Salud Publica Mex.* 2003, 45, 367-375.
3. Kailash U, Hedau S, Gopalkrishna V, [et al.]. A simple 'paper smear' method for dry collection transport and storage of cervical cytological specimens for rapid screening of HPV infection by PCR. *J Med Microbiol.* 2002, 51, 606-610.
4. Munoz N, Castellsaque X, de Gonzalez A, Giessmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine.* 2006, 24, 1-10.
5. Molijn A, Kleter B, Quint W, van Doorn L. Molecular diagnosis of human papillomavirus (HPV) infections. *J Clin Virol.* 2005, 32, 43-51.
6. Kędzia W, Józefiak A, Pruski D, [et al.]. Human papilloma virus genotyping in women with CIN1. *Ginekol Pol.* 2010, 81, 664-667.
7. Spaczyński M, Karowicz-Bilińska A, Rokita W, [et al.]. Attendance rate in the Polish Cervical Cancer Screening Program in the years 2007-2009. *Ginekol Pol.* 2010, 81, 655-663.
8. 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, 79, 294-299.
9. Nobbenhuis M, Meijer C, van der Brule A, [et al.]. Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. *Br J Cancer.* 2001, 84, 796-801.
10. Acladious N, Sutton C, Mandal D, [et al.]. Persistent human papillomavirus infection and smoking increase risk of failure of treatment of CIN. *Int J Cancer.* 2002, 98, 435-439.
11. Paraskevaidis E, Arbyn M, Sotiriadis A, [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, 30, 205-211.
12. Paraskevaidis E, Lolis D, Koliopoulos G, [et al.]. Cervical intraepithelial neoplasia outcomes after large loop excision with clear margins. *Obstet Gynecol.* 2000, 95, 828-831.
13. Chao A, Lin C, Hsueh S, [et al.]. Usefulness of human papillmavirus testing in the follow-up of patients with high-grade cervical intraepithelial neoplasia after conization. *Am J Obstet Gynecol.* 2004, 190, 1046-1051.
14. Flannelly G, Bolger B, Fawzi H, [et al.]. Follow up after LLETZ: could schedules be modified according to risk of recurrence? *BJOG.* 2001, 108, 1025-1030.
15. Sarian L, Derchain D, Pitta D, [et al.]: Factors associated with HPV persistence after treatment for high-grade cervical intraepithelial neoplasia with large loop excision of the transformation zone (LLETZ). *J Clin Virol.* 2004, 31, 270-274.
16. Duś M, Łuczkowska M, Żaba R. Genital ulcer diseases as an entry for HIV infection. *Post Dermatol Alergol.* 2009, 26, 206-211.
17. Jakubowicz O, Żaba R, Czarnecka-Operacz M. Treatmet of infections caused by Herpes simplex virus type 1 and Vericella-zoster virus. *Post Dermatol Alergol.* 2010, 27, 303-307.
18. Jakubowicz O, Żaba R, Czarnecka-Operacz M. Serological tests for syphilis performed in the Sexually Transmitted Diseases Diagnostic Laboratory in Poznan during 2005-2009. *Post Dermatol Alergol.* 2010, 27, 275-281.
19. Kędzia W, Pruski D, Józefiak A. Genotyping of oncogenic human papilloma viruses in women with HG SIL diagnosis. *Ginekol Pol.* 2010, 81, 740-744.
20. Kreimer A, Guido R, Solomon D, [et al.]. Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease. *Cancer Epidemiol Biomarkers Prev.* 2006, 15, 908-914.
21. Lin C, Tseng C, Lai C, [et al.]. Value of human papillomavirus deoxyribonucleic acid testing after conization in the prediction of residual disease in the subsequent hysterectomy specimen. *Am J Obstet Gynecol.* 2001, 184, 940-945.