sciendo

ПРИЛОЗИ. Одд. за мед. науки, XL 1, 2019
CONTRIBUTIONS. Sec. of Med. Sci., XL 1, 2019

МАНУ
MASA

10.2478/prilozi-2019-0003

ISSN 1857-9345
UDC: 618.146-022.6:578.827]-076(497.7)"2016/2017

COMPARISON BETWEEN HPV DNA TESTING AND HPV E6/E7 MRNA TESTING IN WOMEN WITH SQUAMOUS CELL ABNORMALITIES OF THE UTERINE CERVIX

Drage Dabeski[1], Sotirija Duvlis[3], Neli Basheska[2], Vesna Antovska[1], Marjan Stojovski[1], Milka Trajanova[1], Goran Dimitrov[1], Ana Dabeski[1], Nadica Gureva-Gjorgievska[1]

[1] University Clinic of Gynecology and Obstetrics

[2] University Clinic of Radiotherapy and Oncology

[3] Institute of Public Health of Republic of Macedonia, University ″Ss. Cyril and Methodius″, Medical Faculty, Skopje, Republic of Macedonia

Corresponding author: Drage Dabeski, University Clinic of Gynecology and Obstetrics in Skopje, Republic of Macedonia, Majka Tereza 17, 1000 Skopje, Republic of Macedonia, tel.: 0038970577566; e-mail: drdabeski@yahoo.com

ABSTRACT

Introduction: The aim of the study was to compare the results of two human papillomavirus (HPV) diagnostic techniques: human papillomavirus deoxyribonucleic acid (HPV DNA) testing and human papillomavirus E6/E7 messenger ribonucleic acid (HPV E6/E7 mRNA) testing in women with squamous cell abnormalities of the uterine cervix.

Material and Methods: Comparative prospective study, conducted in the period from January 2016 to June 2017 of 128 sexually active women, age groups of 20 to 59 years (40.50 ± 10.85) with squamous cell abnormalities on the cervical cytology. All patients were subject to: HPV DNA testing, HPV E6/E7 mRNA testing and colposcopic cervical biopsy with endocervical curettage for histopathologycal analysis. HPV DNA testing was done using multiplex polymerase chain reaction (PCR) and reverse hybridization methods. HPV E6/E7 mRNA testing was done using real-time PCR method.

Results: Data analysis showed an association between the results of HPV DNA testing and HPV E6/E7 mRNA testing ($p<0.0001$). The concordance between the results of both tests was moderate (55.47%). The results show that HPV E6/E7 mRNA testing had a higer specificity 88.89% and positive predictive value (PPV) 93.59% for HSIL + invasive squamous cell carcinoma compared to HPV DNA testing that had specificity of 55.56% and PPV 84.61%, respectively.

Conclusion: The results of our study suggested that HPV E6/E7 mRNA testing is more specific and has a higher positive predictive value than HPV DNA testing and that viral oncoproteins E6 and E7 are superior biomarkers for the detection of high-risk HPV-associated squamous intraepithelial lesions of the uterine cervix**.**

Keywords: HPV, DNA, mRNA, E6/E7, uterine cervix

INTRODUCTION

Squamous cell carcinoma of the uterine cervix is the most common histological subtype of cervical cancer. About 90% of cases of cervical cancer are squamous carcinoma, 10% are adenocarcinomas and a small percentage of other subtypes [1]. The occurrence of cervical carcinoma is preceded by various forms of intraepithelial lesions involving a series of progressive morphological changes from productive human papillomavirus (HPV) infection-mild dysplasia to in situ carcinoma [2]. This phase is generally asymptomatic and occurs over a period of 10 to 20 years [3].

The six main clinical applications of human papillomavirus deoxyribonucleic acid (HPV DNA) testing are: triage of women with ASC-US (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesion); monitoring of women with abnormal screening results that are negative for colposcopy and/or biopsy; predicting a therapeutic outcome after treatment of an intraepithelial lesion; as a primary screening test; obtaining information on the existence of certain HPV genotypes and determining the prevalence of certain genotypes of HPV at regional, national or global levels [4,5].

HPV DNA tests only detect HPV infection. Based on these tests, it can not be concluded whether it is a transient (80%) or a long-term/persistent (20%) HPV infection [6]. Human papillomavirus E6/E7 messenger ribonucleic acid (HPV E6/E7 mRNA) tests determine the oncogenic activity of the virus and represent a good clinical biomarker for the prediction and detection of the direct risk of developing cervical cancer [7].

The five most important advantages of HPV E6/E7 mRNA tests over HPV DNA tests are: they are more specific, non-invasive tests; have a greater medical predictive value (greater prognostic significance) in relation to HPV DNA detection tests [8]; correlate better with cytology and histology [9]; directly detect the expression level of oncogen HPV E6 and E7 (mRNA) and at the same time detect, differentiate and type high-risk HPV-genotypes -16, -18, -31, -33 and -45 [10].

The aim of the study is to compare the HPV DNA and HPV E6 E7 mRNA test results in women with squamous cell abnormalities of the uterine cervix, to prove that HPV E6/ E7 mRNA testing has greater specificity and greater positive predictive value than HPV DNA testing, to prove that viral oncoproteins E6 and E7 are predictive biomarkers for the detection of high-risk HPV-associated squamous intraepithelial cervical lesions.

MATERIAL AND METHODS

Comparative prospective study, conducted in the period from January 2016 to June 2017 at the University Clinic of Gynecology and Obstetrics and Radiotherapy and Oncology in Skopje and the Institute of Public Health of the Republic of Macedonia in Skopje of 128 sexually active women, age groups of 20 to 59 years (40.50 ± 10.85) with squamous cell abnormalities on the cervical cytology, who came to their annual gynaecological exam at University Clinic for Gynaecology and Obstetrics in Skopje. The study did not include: pregnant women, women with previous cervical surgery (conization, carbon dioxide laser vaporization and total hysterectomy) and women with previous abnormal cytological and histopathological findings of the uterine cervix.

In all patients was done: HPV DNA testing, HPV E6/E7 mRNA testing and colposcopic cervical biopsy with endocervical curettage for histopathological analysis.

CYTOPATHOLOGICAL ANALYSIS. All samples for cytology were taken using the Thin Prep PAP smear cytology and were analyzed in the Laboratory of the University Clinic of Gynecology and Obstetrics in Skopje by a doctor-cytopathologist. Cytological results were classified according to the revised Bethesda classification [11,12], such as: ASC-US, Atypical Squamous Cells of Undetermined Significance; ASC-H, Atypical Squamous Cells cannot exclude a high-grade squamous intraepithelial lesion; LSIL, Low-grade Squamous Intraepithelial Lesion (CIN 1, Cervical Intraepithelial Neoplasia grade 1); HSIL, High-grade Squamous Intraepithelial Lesion (CIN 2, Cervical Intraepithelial Lesion grade 2; CIN 3, Cervical Intraepithelial Neoplasia grade 3; CIS, Carcinoma In Situ) and invasive squamous cell carcinoma.

HISTOPATHOLOGICAL ANALYSIS. Samples for histopathological analysis were taken to the University Clinic of Gynecology and Obstetrics in Skopje and were analyzed at the University Clinic of Oncology and Radiotherapy in Skopje, Department of Histopathology and Clinical Cytology, by an experienced expert in pathohistology. According to the morphology of the bioptic samples, the cervical findings were characterized as: normal finding (nonspecific cervicitis); LSIL (mild dysplasia, flat condyloma, cervicitis chronica virosa); HSIL (moderate and severe dysplasia, in situ squamous cell carcinoma) and invasive squamous cell carcinoma [13].

HPV DNA TESTING. The analysis of the samples from the cervical swabs, detection and HPV typing were made at the University Clinic of Gynaecology and Obstetrics in Skopje, in the Laboratory for HPV typing. The first step in HPV testing was the isolation of DNA from the collected cells of the cervical swabs. For isolation of DNA series of three paraffin cuts were prepared. Cuts were incubated in 1 ml of xylene, 5 minutes at 55°C, and centrifuged at 10 000 G for five minutes at room temperature. The same procedure was repeated two more times.

After careful removal of the remains of xylene, the samples were briefly incubated twice in 1 ml of 100% ethanol, and centrifuged for 5 minutes at room temperature. After the removal of ethanol, short drying followed in air and incubation overnight in buffer with freshly added proteinase K at 55°C. The second step was the detection of DNA in HPV by using polymerase chain reaction (PCR). To verify the quality and integrity of the isolated DNA, actually of a present inhibitor, for each sample a reaction of multiplication of the specific primers for beta globin PC04 and GH20 was first made. Three pairs of primers were used, common to a larger number of HPV types: degenerate primers My09/My11 and CPI/CPII G and Gp5/6+. The samples were carried through all reactions with primers specific to high-risk and low-risk HPV genotypes. The third step was genotyping by using reverse hybridization. It is a method that is based on the hybridization of specific DNA probes that are immobilized on nitrocellulose or nylon tapes. It is a set of primers (SPF 10) with aim-propagation of the L1 gene on the viral DNA. The product of amplification with SPF primers is the size of 65 bp, and allows detection of 25 new genotypes. Denatured biotinylated PCR products are hybridized with specific oligonucleotide probes that are immobilized as parallel lines on membrane strips. After hybridization and washing with streptavidin, alkaline phosphatase is added, which binds to the biotinylated hybrids formed previously. Incubation with BCIP (5-bromo-4-chloro-3-indolyl-phosphate)/ NBT (nitro blue tetrazolium) chromogen gives purple precipitate and the results are interpreted visually.

HPV E6/E7 mRNA TESTING. All samples for HPV E6/E7 mRNA testing were taken from the cervix, using a "blue" cytobrush at the University Clinic of Gynaecology and Obstetrics in Skopje and were analyzed in the Laboratory of the Institute of Public Health of the Republic of Macedonia in Skopje by a doctor-virologist. We used tests that use the Real-Time PCR method. The results were analyzed and shown qualitatively, whether there is the presence or absence of HPV E6/E7 oncoproteins [14]. HPV E6/E7 mRNA testing was done in three steps. The first step was the isolation of viral RNA using the commercial test of the Nuclisense nucleid acid isolation test (Biomerieux) by following the manufacturer's recommendations. The socond step was the detection of viral RNA. The detection method is based on the application of NASBA (Nucleic Acid Sequence Based Assay) technology and the Easy Q Nuclisens platform was used. Detection was performed using the PreTect HPV Proofer test, Nor Chip AS, Norway. The test enables qualitative molecular detection of mRNA from oncogens E6 and E7 to the 5 most common HPV genotypes: -16, -18, -31, -33, -45. The third step of HPV E6/E7 mRNA was software processing and displaying the results.

STATISTICAL ANALYSIS. Data were analysed by a specific software for data-bases (Excel). Statistical analysis of the established statistical series was made with the statistical program SPSS (Statistical Package for Social Sciences), version 23.0. The structure of numerical signs was analysed by determining the measures of central tendency (arithmetical mean) and measures of dispersion (standard deviation). Analysis of the relationship (the existence of association) between two sets of attribute variables was performed using the Chi-square test. Analysis of the relationships (the existence of correlation) between two sets of numerical variables was performed using the regression analysis and coefficient of linear correlation. The concordance among the DNA and RNA tests results was evaluated by using Cohen's kappa statistic: k value of <0.20 indicated poor concordance, k value from 0.21 to 0.40 indicated sufficient concordance, k value from 0.41 to 0.60 indicated moderate concordance, k value from 0.61 to 0.80 indicated good concordance and k value >0.80 indicated very good concordance. The sensitivities, specificities and PPVs for the HPV DNA and RNA assays were estimated by comparison with the cytological and histological findings. Statistical significance was defined as a p value <0.05.

RESULTS

Of the 128 examinate patients aged from 20 to 59 years (40.50 ± 10.85): 28 (21.87%) were aged 20-29; 38 (29.69%) 30-39 years; 30 (23.44%) 40-49 years and 32 (25.00%) were aged 50-59 years.

Cytopathologically, there were: 13 (10.16%) ASC-US cases, 7 (5.47%) ASC-H cases, 31 (24.22%) LSIL cases, 56 (43.75%) HSIL cases and 21 (16.40%) invasive squamous cell carcinomas. Histopathologically, there were: 9 (7.03%) non-neoplastic lesions, 41 (32.03%) LSIL cases, 54 (42.19%) HSIL cases and 24 (18.75%) invasive squamous cell carcinomas.

HPV DNA infection was detected in 75.00% (96/128) of the patients studied. Data analysis showed an increase in HPV infection, along with an increase in the histopathological grade of cervical lesion. The lowest percentage was observed in LSIL 63.41% (26/41), with an increase up to 83.33% (45/54) in HSIL and 87.50% (21/24) in invasive

squamous cell carcinoma (chi-square test=7.0506, p=0.029443, p<0.05) (Table 1). The data analysis showed an association between the presence of HPV DNA infection and the occurrence of squamous cell abnormalities of the uterine cervix (chi-square test=4.8204, p=0.028125, p <0.05).

HPV E6/E7 mRNA infection was detected in 60.94% (78/128) of examined patients. Data analysis showed an increase in HPV E6/E7 mRNA infection, along with an increase in the histopathological grade of cervical lesion. The lowest percentage was found in LSIL 9.76% (4/41), followed by 92.59% (50/54) in HSIL and 95.83% (23/24) in invasive squamous cell carcinoma of the uterine cervix (chi-square test=82.7796, p<0.00001, p<0.05) (Table 1). Data analysis showed an association between the presence of viral oncoproteins E6 and E7 and the incidence of squamous cell abnormalities of the uterine cervix (chi square test=10.0967, p=0.001485, p<0.05).

Table 1. *Comparison between HPV DNA testing and HPV E6/E7 mRNA testing in relation to histopathological diagnosis*

	Hystopathological diagnosis	HPV DNA testing				HPV E6/E7 mRNA testing				concordance		p	Cohen kappa (k)	95% CI
		HPV DNA positive		HPV DNA negative		HPV E6/ E7 mRNA positive		HPV E6/ E7 mRNA negative						
		n	(%)	n	(%)	n	(%)	n	(%)	n	(%)			
	Normal finding (n=9)	4	(44.44)	5	(55.56)	1	(11.11)	8	(88.89)	1	(11.11)	0.444	0.270	-0.191 – 0.731
LSIL (n=41)	Cervicitis chronica virosa (n=20)	13	(65.00)	7	(35.00)	1	(5.00)	19	(95.00)	1	(5.00)	1.000	0.055	-0.056 – 0.166
	Flat condyloma (n=2)	2	(100)	0	(0)	0	(0)	2	(100)	0	(0)	0.333	0.000	0.000 – 0.000
	Mild dysplasia (n=19)	11	(57.89)	8	(42.11)	3	(15.79)	16	(84.21)	3	(15.79)	0.228	0.240	-0.020 – 0.500
HSIL (n=54)	Moderate dysplasia (n=15)	12	(80.00)	3	(20.00)	14	(93.33)	1	(6.67)	12	(80.00)	0.200	0.444	-0.152 – 1.000
	Severe dysplasia n=(23)	19	(82.61)	4	(17.39)	21	(91.30)	2	(8.7)	19	(82.61)	0.0237	0.623	0.160 – 1.000
	In situ squamous cell carcinoma (n=16)	14	(87.50)	2	(12.50)	15	(93.75)	1	(6.25)	14	(87.50)	0.125	0.623	-0.006 – 1.000
	Invasive squamous cell carcinoma(n=24)	21	(87.50	3	(12.50)	23	(95.83)	1	(4.17)	21	(87.50)	0.125	0.467	-0.132 – 1.000
	Total (n=128)	96	(75.00)	32	(25.00)	78	(60.94)	50	(39.06)	71	(55.47)	<0.001	0.439	0.282 – 0.596

Legend: n, number; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CI, confidence interval

Out of a total of 128 patients, 71 (55.47%) were HPV positive for both tests. Data analysis showed an association between the results of HPV DNA testing and HPV E6/E7 mRNA testing (chi-square test=27.3504, p<0.0001, p<0.05). The concordance between the results of both tests was moderate (55.47%; kappa=0.439; 95%CI: 0.282-0.596). HPV DNA testing was superior to the detection of LSIL (63.41%; 26/41 versus 9.76%; 4/41), while HPV E6/E7 mRNA testing was superior to the detection of HSIL (92.60%; 50/54 versus 83.33%; 45/54) and invasive squamous cell carcinoma of the uterine cervix (95.83%, 23/24 versus 87.50%; 21/24) (Table 1).

Data analysis from both tests showed: good concordance between the results of patients with severe dysplasia (82.61%; kappa=0.623) and in situ squamous cell carcinoma (87.50%; 0.636), moderate concordance between those with mod-

erate dysplasia (80.00%; kappa=0.467), sufficient concordance between those with mild dysplasia (15.79%; kappa=0.240) and normal histopathological findings on the uterine cervix (11.11%; kappa=0.444) and invasive squamous cell carcinoma of the uterine cervix (87.50%; kappa=0.270) and poor concordance between the results of patients with cervicitis chronica virosa (5.00%; kappa=0.055) and flat condyloma (0%; kappa=0.000) (Table 1).

Table 2. *Sensitivity and specificity of HPV DNA and HPV E6 / E7 mRNA tests in correlation to histopathological diagnosis*

Testing	Sensitivity			Specificity		
	Estimated value	95%CI	(%)	Estimated value	95%CI	(%)
HPV DNA	0.7731	0.6854-0.8427	77.31	0.5555	0.2265-0.8466	55.56
HPV E6/E7 mRNA	0.6471	0.5535-0.7309	64.71	0.8888	0.5067-0.9942	88.89

Legend: HPV, human papillomavirus; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; CI, confidence interval

Table 3. *Positive predictive value of HPV DNA and HPV E6/E7 mRNA tests in correlation to histopathological diagnosis*

Hystopathological diagnosis	Positive predictive value (%)	
	HPV DNA testing	HPV E6/E7 mRNA testing
LSIL	63.41	9.76
HSIL	83.33	92.59
Invasive squamous cell carcinoma	87.50	95.83

Legend: HPV, human papillomavirus; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion

The accuracy of HPVDNA testing was 75.78%, while the accuracy of HPV E6/E7 mRNA testing was 66.41%. The results show that HPV E6/E7 mRNA testing had a higer specificity 88.89% and positive predictive value (PPV) 93.59% for HSIL + invasive squamous cell carcinoma compared to HPV DNA testing that had specificity of 55.56% and PPV 84.61%, respectively (Tables 2 and 3).

Table 4. *Distribution of HPV genotypes detected with HPV DNA and HPV E6/E7 mRNA tests in 128 patients*

HPV genotype	HPV E6/E7 mRNA testing				HPV DNA testing				Detected genotype with both tests		p	Cohen kappa (κ)	95%CI
	Presence		Absence		Presence		Absence						
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)			
16	45	(35.16)	83	(64.84)	42	(32.81)	86	(67.19)	40	(31.25)	<0.0001	0.878	0.791-0.966
18	18	(14.06)	110	(85.94)	10	(7.81)	118	(92.19)	9	(7.03)	<0.0001	0.603	0.384-0.822
31	15	(11.72)	113	(88.28)	15	(11.72)	113	(88.28)	14	(10.94)	<0.0001	0.924	0.821-1.000
33	8	(6.25)	120	(93.75)	7	(5.47)	121	(94.53)	6	(4.69)	<0.0001	0.788	0.555-1.000
45	13	(10.16)	115	(89.84)	9	(7.03)	119	(92.97)	9	(7.03)	<0.0001	0.802	0.614-0.989
16/18	13	(10.16)	115	(89.84)	7	(5.47)	121	(94.53)	7	(5.47)	<0.0001	0.677	0.438-0.916

Legend: HPV, human papillomavirus; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; CI, confidence interval

Data analysis from both tests showed: very good concordance between the results of patients with HPV-16 and HPV-31, good concordance between those with HPV-33, HPV-45 and HPV-16/18 and a moderate concordance between the results of patients with HPV-18 (Table 4).

DISCUSSION

Persistent high-risk HPV infections are the most common risk factors for the occurrence of squamous cell cervical abnormalities. 75% of the sexually active population, in the course of their lives, was in contact with one or more HPV genotypes [15]. In this study, HPV DNA infection was detected in 75% of patients. This relatively high percentage of HPV DNA infection in patients with squamous cell abnormalities of the uterine cervix corresponds to some previously published studies [16,17]. According to the known prevalence of HPV genotypes in invasive cervical cancer, more than 80% of potential cases can be detected by the PreTect HPV-Proofer assay. According to the International Agency for Research on Cancer analysis of 3 085 invasive cervical carcinoma, the five most common HPV

genotypes were with decreasing frequency, HPV-16, -18, -45, -31, and -33. These genotypes were found in 82.9% of the cases [18].

In our study, high-risk HPV E6/E7 mRNA infection was detected in 60.94% of patients. This relatively high percentage of high-risk HPV E6/E7 mRNA infection in patients with squamous cell cervical abnormalities corresponds to some previously published studies; The study of Fontecha et al., compiled in the period from 2007 to 2014, showed HPV E6/E7 mRNA positivity in 68.29% of women, who for the first time were HPV E6/E7 mRNA tested [19], the study by Oliveira et al. from 2013, worked on 554 women, detected the expression of viral oncoproteins E6 and E7 in 55.10% of women surveyed [20], in a study by Cattani et al. from 2009, worked on 400 HPV DNA positive women, 61.20% of women were HPV E6/E7 mRNA positive [21], study by Tuney et al. from 2017, showed an expression of viral oncoproteins E6 and E7 in 55.60% of women with abnormal cervical cytological findings [22].

Data analysis from our study showed an association between the results of HPV DNA testing and HPV E6/E7 mRNA testing ($p<0.0001$). An association between the results of HPV DNA testing and HPV E6/E7 mRNA testing was also detected in the study of Doganov A. et al. from 2012 [23]. The concordance between the results of both tests was moderate (55.47%). The study of Trope et al. from 2009, worked on 643 women with HSIL-finding on the PAP test, showed concordance of 63.80% between the results of HPV DNA and HPV E6/E7 mRNA tests [24]. In the study of Spathis et al. from 2012, worked on 1 173 women at the University Clinic in Ioannina, a concordance of 71.60% was found between the HPV DNA and HPV E6/E7 mRNA tests [25]. The study of Salimovic-Besic from 2013, conducted on 105 women with abnormal cervical cytological findings, showed a concordance of 56.00% between the HPV DNA test and HPV E6/E7 mRNA test, with low HPV E6/E7 mRNA positivity in women with cytological findings of ASC-US on the PAP test [26].

The results of our study showed that HPV E6/E7 mRNA testing was more specific (88.89%) and higher positive predictive value (93.59%) of HPV DNA testing. A greater specificity (50.00%) and greater positive predictive value (62.00%) of HPV E6/E7 mRNA testing was shown by the study of Duvlis S. et al. from 2015 conducted on a screening group of 413 Macedonian women [27], as well as some other previously published studies; the study of Cattani et al. since 2009, it has been developed for 180 women, showed more specificity (72.70%) of HPV E6/E7 mRNA testing than HPV DNA testing (56.20%) [21]. In the study of Munkhdelger et al. from 2014, conducted on 188 women with squamous intraepithelial cervical lesion, HPV E6/E7 mRNA testing was more specific than HPV DNA testing (85.00% versus 40.83%) [28].

CONCLUSION

The results of our study have suggested that HPV E6/E7 mRNA testing is more specific and has a higher positive predictive value than HPV DNA testing and that viral oncoproteins E6 and E7 are superior biomarkers for the detection of high-risk HPV-associated squamous intraepithelial lesions of the uterine cervix.

REFERENCES

1. Scott M. The pathology of cervical cancer. In: Ayhan A, Reed N, Gulterin M, Dursun P, editors. Textbook of Gynaecological Oncology. 3rd ed. Ankara, Turkey: Gunes Publishing; 2016. pp. 376-380.
2. American College of Obstetricians and Gynaecologists. ACOG Practice Bulletin No99: Management of abnormal cervical cytology and histology. Obstet Gynecol 2008; 112(6); 1419-1444. Reaffirmed 2010.
3. Martin CM, O'Leary JJ. Histology of cervical intraepithelial neoplasia and the role of biomarkers. In: Arul Kumaran S, editor. Best Practice & Research: Clinical Obstetrics & Gynaecology. 25th ed. Philadelphia, USA: Elsevier; 2011. pp. 605-615.
4. Abreu ALP, Souza RP, Gimenes F, et al. A review of methods for detect human papillomavirus infection. Virology Journal 2012; 9:262.
5. Cuzick J, Arbyn M, Sankaranarayanan R, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. 2008; 26:29-41.
6. Rosenblatt S, Wroclawski ER, Lucon AM, Pereyra EAG, editors. HPV in Clinical Practice. Sao Paulo: Atheneu; 2005.

7. Hwang SJ, Shroyer KR. Biomarkers of Cervical Dysplasia and Carcinoma. Journal of Oncology 2012; 2012:9.
8. Coquillard G, Palao B, Patterson BK. Quantification of intracellular HPV E6/E7 mRNA expression increases the specificity and positive predictive value of cervical cancer screening compared to HPV DNA. Gynecol Oncol 2011; 120(1):89-93.
9. Sorbye SW, Arbyn M, Fismen S, et al. HPV E6/E7 mRNA Testing Is More Specific than Cytology in Post-Colposcopy Follow-Up of Women with Negative Cervical Biopsy. PLoS ONE 2011; 6(10):e26022.
10. Kraus I, Molden T, Holm R, et al. Presence of E6 and E7 mRNA from Human Papillomavirus Types 16, 18, 31, 33 and 45 in the Majority of Cervical Carcinomas. J Clin Microbiol 2006; 44:1310–1317.
11. Zerat L. La nouvelle terminologie de Bethesda: quellschangements? Rev Prat Gynecol Obstet. Numero Special 2002; 3-10.
12. Solomon D, Davey D, Kurman R. The 2001 Bethesda System. Terminology for reporting results of cervical cytology. JAMA 2002; 287:2114-19.
13. Kurman RJ, Carcangin ML, Herrington CS, Young RH, editors. World Health Organization Classification of Tumors of Female Reproductive Organs. 4th ed. Lyon, France: IARC Press; 2014.
14. Castle PE, Dockter J, Giachetti G, et al. A cross-sectional study of a prototype carcinogenic human papillomavirus E6/E7 messenger RNA assay for detection of cervical precancer and cancer. AACR 2007; 13:2599-2605.
15. Vieira L, Almeida A. The cytology and DNA detection by the Papillo Check test in the diagnosis of human papillomavirus infection. Eur J Microbiol Immunol 2013; 3(1):61-7.
16. Mazarico E, Gonzalez-Bosquet E. Prevalence of infection by different genotypes of human papillomavirus in women with cervical pathology. Gynecol Oncol 2012; 125(1):181-5.
17. Pista A, de Oliveira CF, Lopes C, et al; CLEOPATRE Portugal Study Group. Human papillomavirus type distribution in cervical intraepithelial neoplasia grade 2/3 and cervical cancer in Portugal: a CLEOPATRE II Study. Int J Gynecol Cancer 2013; 23(3):500-6.
18. Bzhalava D, Eklund C, Dillner J. International standardization and classification of human papillomavirus types. Virology. 2015; 476:341-4.
19. Fontecha N, Basaras M, Hernaez S, et al. Assessment of human papillomavirus E6/E7 oncogene expression as cervical disease biomarker. BMC Cancer 2016; 16:852.
20. Oliveira A, Verdasca N, Pista A. Use of the Nucli SENS Easy Q HPV assay in the management of cervical intraepithelial neoplasia. J Med Virol 2013; 85(7):1235-1241.
21. Cattani P, Siddu A, DÓnghia S, et al. RNA (E6 and E7) assay versus DNA (E6 and E7) assay for risk evaluation for women infected with human papillomavirus. J Clin Microbiol 2009; 2136-2141.
22. Tuney I, Altay A, Ergunay K, et al. HPV types and E6/E7 mRNA expression in cervical samples from Turkish women with abnormal cytology in Ankara, Turkey. Turk J Med Sci 2017; 1508-155.
23. Doganov A, Kostova P, Boyanova M, et al. Application of the RNA-based HPV test in the diagnostics of cervical epithelial changes. Akush Ginekol 2012; 51(3):22-4.
24. Trope A, Sjoborg K, Eskild A, et al. Performance of human papillomavirus DNA and mRNA testing strategies for women with and without cervical neoplasia. J Clin Microbiol 2009; 47(8):2458-2464.
25. Spathis A, Kottaridi C, Chranioti A, et al. mRNA and DNA detection of human papillomaviruses in women of all ages attending two colposcopy clinics. PLoS One 2012; 7(11):e49205.
26. Salimovic-Besic I, Tomic-Cica A, Smailjic A, et al. Comparison of the detection of HPV-16, 18, 31, 33 and 45 by type-specific DNA- and E6/E7 mRNA-based assays of HPV DNA positive women with abnormal Pap smears. J virol Methods 2013; 194(1-2):222-228.
27. Duvlis S, Popovska-Jankovic K, Sarafimovska-Arsova Z, et al. HPV E6/E7 mRNA versus HPV DNA biomarker in cervical cancer screening of a group of Macedonian women. J Med Virol 2015; 87(9):1578-1586.
28. Munkhdelger J, Choi Y, Lee D, et al. Comparison of the performance of the Nucli SENS Easy Q HPV E6/E7 mRNA assay and HPV DNA chip for testing squamous cell lesions of the uterine cervix. Diagnos Microbiol Inf Dis 2014; 422-427.

Резиме

СПОРЕДБА МЕЃУ ХПВ ДНК-ТЕСТИРАЊЕТО И ХПВ Е6/Е7 ИРНК-ТЕСТИРАЊЕТО КАЈ ЖЕНИ СО СКВАМОЗНИ КЛЕТОЧНИ АБНОРМАЛНОСТИ НА ГРЛОТО НА МАТКАТА

Драге Дабески[1], Сотирија Дувлис[3], Нели Башеска[2], Весна Антовска[1], Марјан Стојовски[1], Милка Трајанова[1], Горан Димитров[1], Ана Дабески[1], Надица Гурева-Ѓоргиевска[1]

[1] Универзитетска клиника за гинекологија и акушерство, Медицински факултет, Универзитет „Свети Кирил и Методиј“, Скопје, Република Македонија

[2] Универзитетска клиника за радиотерапија и онкологија, Медицински факултет, Универзитет „Свети Кирил и Методиј“, Скопје, Република Македонија

[3] Институт за јавно здравје на Република Македонија, Медицински факултет, Универзитет „Свети Кирил и Методиј“, Скопје, Република Македонија

Вовед: Целта на студијата беше да ги споредиме резултатите од двете дијагностички техники за хуман папилома вирус (ХПВ): тестирањето хуман папилома вирус дезоксирибонуклеинска киселина (ХПВ ДНК) и тестирање хуман папилома вирус Е6/Е7 информативна рибонуклеинска киселина (ХПВ Е6/Е7 иРНК) кај жените со сквамозни клеточни абнормалности на грлото на матката.

Материјал и методи: Компаративната проспективна студија беше спроведена во периодот од јануари 2016 до јуни 2017 година, на 128 сексуално активни жени, на возраст од 20 до 59 години (40,50 ± 10,85) со цитолошки сквамозни клеточни абнормалност на грлото на матката. Кај сите пациентки направивме: тестирање ХПВ ДНК, тестирање ХПВ Е6/Е7 иРНК и колпоскопска цервикална биопсија со ендоцервикална киретажа за хистопатолошка анализа. Тестирањето ХПВ ДНК беше направено со помош на методите: мултипна полимераза верижна реакција и реверзна хибридизација. Тестирањето ХПВ Е6/Е7 иРНК беше направено со помош на полимераза верижна реакција во реално време.

Резултати: Анализата на податоците покажа асоцијација меѓу резултатите од тестирањето ХПВ ДНК и тестирањето ХПВ Е6/Е7 иРНК ($p < 0,0001$). Конкордантноста меѓу резултатите од двете тестирања беше умерена (55,47 %). Резултатите покажаа дека тестирањето ХПВ Е6/Е7 иРНК има поголема специфичност 88,89 % и поголема позитивна предиктивна вредност (ППВ) 93,59 % кај HSIL + инвазивен сквамозен карцином во споредба со тестирањето ХПВ ДНК, кое има специфичност 55,56 % и ППВ 84,61 %.

Заклучок: Резултатите од нашата студија потврдија дека тестирањето ХПВ Е6/Е7 иРНК е со поголема специфичност и поголема позитивна предиктивна вредност од тестирањето ХПВ ДНК и дека вирусните онкопротеини Е6 и Е7 се супериорни биомаркери за детекција на високоризичните ХПВ-асоцирани сквамозни интраепителни лезии на грлото на матката.

Клучни зборови: ХПВ, ДНК, иРНК, Е6/Е7, грло на матка