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Short communication

Genital Human Papillomavirus Infection in Women from the Zagreb Region

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A B S T R A C T

Human papillomavirus (HPV) infection is the most common sexually transmitted infection, especially among young, sexually active individuals. As persistent infection with oncogenic types may lead to cervical cancer, HPV testing is a useful tool to screen for women at risk for subsequent development of cervical cancer. The aim of the study was to determine the prevalence of high-risk HPV (hrHPV) infection in different age groups of cytologically selected women from the Zagreb region, and to evaluate the frequency and results of repeat hrHPV testing. During a one-year study period (November 2005 to November 2006), a total of 3,440 cervical samples from women attending gynecological services of public and private health care systems were received. They were tested for 13 hrHPV genotypes by the polymerase chain reaction based AMPLICOR HPV test (Roche Molecular Systems). The overall prevalence of hrHPV was 34.6%. Most samples were obtained from women aged 21–30 years (44.2%), followed by the 31–40 (27.6%), 41–50 (15.7%), 51–60 (5.3%) and ≥61 (2.4%) age groups. Out of 3,227 cervical samples obtained from women of known age, 4.9% were obtained from the group of girls younger than 21, in which the highest prevalence of hrHPV (49.4%) was found. A similar prevalence was observed in women aged 21–30 (45.1%). The prevalence gradually decreased with age. During the study period, repeat hrHPV testing was performed in samples from 66 women at different intervals. Out of 28 women that were hrHPV negative on initial testing, only five women turned positive on repeat testing. Out of 38 women that were positive on initial testing, in one-third hrHPV could not be detected on repeat testing. As expected, hrHPV infection was highly prevalent in female adolescents and young women. Further investigation on repeat hrHPV testing is needed to assess virus clearance and rate of newly acquired infection.

Key words: *High-risk human papillomavirus (hrHPV), prevalence, AMPLICOR HPV Test, repeat hrHPV testing*

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted infection[1]. Among more than 100 known HPV genotypes, there are 40 that affect anogenital mucosa[2]. According to their potential to increase the risk of cervical cancer, they are divided into low-risk group, which is usually associated with benign lesions such as condylomata acuminata, and high-risk HPV (hrHPV) genotypes, which have a role in cervical carcinogenesis[3]. High-risk HPV types are demonstrated in almost 100% of cervical carcinomas[4]. The infection is especially prevalent among sexually active adolescents and young adults, but usually of short duration in these age groups[5]. Only persistent infection, which is more common in older women, may lead to cervical cancer and its precursor lesions, cervical intraepithelial neoplasia 2/3 (CIN 2/3). The precise role of HPV in the etiology of cervical cancer is unknown and the host immune system is considered to be of crucial importance in HPV clearance or development of persistence after primary infection[6,7].

HPV testing has a recognized role in improvement of cervical-cancer screening programmes, evaluation of women with unclear or low-grade cytological abnormalities, and follow-up of patients treated for CIN[3,8,9]. The only test currently approved by the U.S. Food and Drug Ad-

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ministration for HPV detection is Hybrid Capture 2 HPV DNA test (HC2) (Digene Corporation, Gaithersburg, MD, USA). This signal amplification assay detects 13 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and five low-risk (6, 11, 42, 43 and 44) HPV types. Although evaluation of its laboratory performance has confirmed its reliability and reproducibility, several recent studies showed a significant analytical inaccuracy, mainly due to the cross-reactivity of its high-risk probe cocktail[10–12]. In 2003, a newly developed polymerase chain reaction based AMPLICOR HPV test (F. Hoffmann-La Roche Ltd., Basel, Switzerland) was marketed. This target amplification test detects the same 13 hrHPV types as HC2 assay, with simultaneous detection of human β-globin gene, which allows assessment of cellular adequacy, extraction and amplification of each processed specimen. Some recent studies demonstrated the HC2 assay and AMPLICOR HPV test to give comparable results, both being suitable for routine use[13,14]. In a recent study, the AMPLICOR HPV test demonstrated even higher analytical sensitivity and specificity[13]. While the higher analytical specificity of AMPLICOR HPV in comparison to that of HC2 can be considered clinically beneficial, the clinical importance of the higher analytical sensitivity of AMPLICOR HPV is still a matter for extensive professional discussion[13,15].

The aim of the study was to determine the prevalence of hrHPV infection in different age groups of women from the Zagreb region using AMPLICOR HPV test, and to evaluate the frequency and results of repeat hrHPV testing in the same population.

Materials and Methods

Patients and clinical specimens

During the one-year study period (November 2005 to November 2006), a total of 3,440 cervical cell specimens for hrHPV testing were received at the Laboratory of Molecular Microbiology, Zagreb Institute of Public Health. The specimens were obtained from cytologically selected women attending gynecological services of public and private health care systems in the Zagreb region. The median age of the women was 31 (range, 15–73) years. For 213 specimens no data on the patients' age were available. Cervical samples were collected by 46 gynecologists using Cervex-Brush (Rovers Medical Devices). Upon sampling, the brush was washed in a ThinPrep vial containing PreservCyt solution (Cytyc Corporation, Boxborough, MA, USA). During the study period, repeat hrHPV testing was performed in samples from 66 women at different time intervals. The indication for repeat testing was clinical evaluation of the patients.

Specimen preparation

HPV DNA was isolated from the PreservCyt solution using AmpliLute Liquid Media Extraction kit (AMPLICOR HPV test, Roche Molecular Systems) according to the manufacturer's instructions. Briefly, HPV DNA was released by lysing cervical cells under denaturing conditions at elevated temperatures in the presence of proteinase K, chaotropic agent and detergent, isolated and purified over columns with silica-based membrane using vacuum pressure, and eluted with elution reagent. During the procedure, the human β-globin gene was concurrently isolated, allowing assessment of cellular adequacy, extraction and amplification of each processed specimen.

Amplification

Polymerase chain reaction (PCR) based AMPLICOR HPV test (Roche Molecular Systems) is designated to amplify HPV DNA from 13 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The test amplifies 165 bp long nucleotide sequence within the polymorphic L1 region of the HPV genome with a master mix containing biotin labeled primers. An additional primer pair is added to allow simultaneous amplification of the human β-globin gene (268 bp amplicon). PCR was performed with a final reaction volume of 100 μL, containing 50 μL of AMPLICOR HPV master mix and 50 μL of isolated DNA, on GeneAmp PCR System 9700 with gold block (Applied Biosystems, Foster City, CA, USA) using AMPLICOR defined parameters: 2 min at 50 °C and 9 min at 95 °C, followed by 40 cycles of: 30 s at 95 °C, 45 s at 54 °C and 30 s at 72 °C, with a final extension at 72 °C for no longer than 1 h.

Hybridization and detection

After amplification, the amplicons were chemically denatured to form single-stranded DNA. Two separate 96-microwell plates, one coated with HPV high-risk probes and the other with β-globin specific oligonucleotide probes, were used for HPV and β-globin detection. After adding of AMPLICOR hybridization buffer and denatured amplicons to appropriate wells of both detection plates, hybridization reaction occurred. Following hybridization and washing procedures, bound hybrids were detected with a biotin avidin-horseradish peroxidase assay. The absorbance at 450 nm was measured immediately using an automated microwell plate reader (Anthos 2010, ASYS Hitech, BIOCHROM Group, Great Britain). According to AMPLICOR instructions, HPV absorbance reading of ≥0.20, accompanied with any value of β-globin result, was considered positive for the presence of hrHPV. HPV result of <0.20 and β-globin result of ≥0.20 was considered negative for the presence of hrHPV. Test result could not be interpreted if both (HPV and β-globin) absorbance values were <0.20. HPV DNA, if present, could not be detected because of inadequate cell content of specimen or inadequate extraction or amplification procedure. In that case, another aliquot of original specimen was retested and, if the same result appeared, new specimen collection was recommended.

Statistical methods

Proportions were compared by the χ^2-test. A p value <0.01 was considered statistically significant. The 95% confidence interval for a proportion was calculated ac-

cording the Wilson procedure with a correction for continuity[16]. The decrease of hrHPV prevalence with age was estimated by the χ^2-goodness of fit to rectangular distribution using STATISTICA 7.1 (StatSoft Inc., Tulsa, OK, USA).

Results

Results of this study conducted during the one-year period in 3,440 cervical samples tested for the presence of 13 hrHPV types by AMPLICOR HPV test showed the overall hrHPV prevalence to be 34.6%. Out of 3,227 cervical specimens from women of known age, hrHPV was detected in 1,120 (34.7%) samples. In four (0.1%) samples, HPV test results could not be interpreted. Out of 213 cervical samples from women with no age data available, hrHPV was detected in 70 (32.9%) samples (χ^2= 0.24, p>0.01).

Out of 3,227 cervical samples obtained from women of known age, 158 (4.9%) were obtained from the group of girls younger than 21, in which the highest prevalence of hrHPV (49.4%) was recorded. Most cervical specimens were obtained from women aged 21–30 (1,425 samples, 44.2%); in this group hrHPV was detected in 642 (45.1%) patients. The prevalence of hrHPV gradually decreased with age (χ^2=1,531.3, df=5, p<0.01). In the 31–40 age group (27.6% of study patients), the recorded prevalence was 28.9%, followed by 19.3% in the 41–50 age group and 13.5% in the 51–60 age group. In women older than 60, the prevalence of hrHPV was 28.9% (Table 1). There was a statistically significant difference in the distribution of hrHPV genotypes according to age groups (χ^2=183.96, df=5, p<0.01), which was due to the higher prevalence of hrHPV in younger women (≤30 years).

Out of 3,440 cervical samples tested for hrHPV, 1,168 (34%) of samples were diagnosed as atypical squamous cells of undetermined significance (ASCUS), 468 (13.6%) of samples were diagnosed as dysplasia levis or CIN 1, 152 (4.4%) of samples were diagnosed as CIN 2 (n=107), CIN 3 (n=43) or carcinoma in situ (n=2), and for 1,652 (48%) of samples the informations regarding cervical abnormalities were not available. In the ASCUS group hrHPV was detected in 432 (37%) patients. The prevalence of hrHPV increased with the severity of cervical lesions. In the CIN 1 group hrHPV was detected in 201 (43%) and in the CIN 2/3 group in 111 (73%) patients (χ^2=158.1, p<0.01).

During the study period, repeat hrHPV testing was performed in samples from 66 women at different intervals. Out of 28 women found to be hrHPV negative on initial testing, only five women turned positive on repeat testing. Six women underwent retesting within three months of initial testing, all of them being negative for hrHPV again. Of twelve women that underwent re-testing within 3–6 months, hrHPV was detected in three cases. In seven women, repeat testing was performed within 6–9 months, one of them being positive for hrHPV. Three women underwent retesting within 9–12 months, with hrHPV detected in one case.

On repeat testing, hrHPV could not be detected in one third of 38 women found hrHPV positive on initial testing. Repeat testing within three months was performed in 11 women, two of them negative; within 3–6 months in 15 women, six of them negative; within 6–9 months in nine women, four of them negative; and within 9–12 months in three women, all of them positive.

Discussion and Conclusion

The present study assessed the prevalence of hrHPV types among cytologically selected women from a large, well-defined region in Croatia, tested during the one-year period at Zagreb Institute of Public Health. In 3,440 tested women with cytologically abnormal smears, the prevalence of hrHPV was 34.6%.

The prevalence of hrHPV is known to decrease with age, from 20% among women aged 20–30 to 5% in women aged >30, as estimated in previous studies in pregnant and non-pregnant women with cytomorphologically normal cervical smears[17–19]. Although the prevalence of hrHPV is considerably higher among women with cytologically abnormal smears, a higher rate of hrHPV is still recorded in women aged <30 than in those from older age groups irrespective of the grade of cytological abnor-

TABLE 1
RESULTS OF CERVICAL SAMPLES TESTED FOR 13 HIGH-RISK HPV GENOTYPES BY AMPLICOR HPV TEST ACCORDING TO PATIENTS AGE

Age (years)	Total n=3,227	NT[a]		Positive[b]		
		n=4	%	n=1,120	%	95%CI[c]
≤20	158	0	0	78	49.4	41.4–57.4
21–30	1,425	2	0.1	642	45.1	42.5–47.7
31–40	889	2	0.2	257	28.9	26.0–32.0
41–50	508	0	0	98	19.3	16.0–23.1
51–60	171	0	0	23	13.5	8.9–19.7
≥61	76	0	0	22	28.9	19.4–40.7

HPV – human papillomavirus, [a]NT – not possible to interpret, [b]χ^2=183.96, df=5, p<0.01, [c]CI – confidence interval

mality. A recent study of the distribution of HPV types in ThinPrep Papanicolaou (Pap) tests classified according to the »Bethesda 2001 terminology« in correlation with patient age detected one or more of 13 hrHPV types in 53% of samples diagnosed as atypical squamous cells of undetermined significance (59% of patients aged <30 and 45.5% of patients aged ≥30), 55.5% of samples diagnosed as low-grade squamous intraepithelial lesion (60% of patients aged <30 and 44% of patients aged ≥30), 80% of samples in which the high-grade squamous intraepithelial lesion could not be ruled out; and 87.5% of samples diagnosed as high-grade squamous intraepithelial lesion[20]. In the present retrospective study, most cervical specimens were obtained from women aged 21–30 (44.2%), in which group hrHPV was detected in 45.1% of patients. Limitations of the study included the unavailability of cytological, clinical and epidemiological data for all patients, and involvement of a great number of gynecologists and cytologists, which resulted in variable classification of cervical abnormality. Additional prospective investigation of samples cytologically diagnosed according to the »Bethesda 2001 guidelines« is needed.

As expected, hrHPV infection was found to be highly prevalent in female adolescents and young women from the Zagreb region. Similar findings were recorded in a study conducted in 1998 and 1999 in Croatia, when 466 women with minor and moderate cervical abnormalities were tested. The prevalence of hrHPV was found to be 66% in women aged <20, 57% in women aged 20–29, and 65% in women aged 30–39[21]. In this study, the decline of hrHPV prevalence with age was less pronounced because of the small number of subjects, especially those over age 50. In another study published in 2001, assessing the prevalence of HPV in cervical specimens obtained from 1,874 women with abnormal Pap smears collected during the 1996–1998 period, the HPV positivity rate declined with age[22]. In a more recent study, the same authors demonstrated the presence of HPV in cervical samples to significantly increase with the severity of cervical lesions[23]. The same was observed in the present study which is valuable because of the great number of women included, yielding relevant epidemiological data for this geographical region. However, properly designed, target studies are necessary to overcome the shortcomings of the present, retrospective study.

During the one-year study period, only 66 women were tested on multiple occasions, which cannot be considered a relevant sample, therefore further investigation with repeat hrHPV testing is needed to assess virus clearance and rate of newly acquired infection in correlation with patient age and cytological diagnosis.

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INFEKCIJA GENITALNIM HUMANIM PAPILOMAVIRUSIMA U ŽENA SA ZAGREBAČKOG PODRUČJA

S A Ž E T A K

Infekcija humanim papilomavirusima (HPV) najčešća je spolno prenosiva bolest, poglavito u mladih, seksualno aktivnih osoba. Kako ustrajna infekcija može dovesti do raka vrata maternice, testiranje na HPV je korisno sredstvo u probiranju žena koje su pod povećanim rizikom od razvoja karcinoma vrata maternice. Cilj ovoga rada bio je utvrditi prevalenciju infekcije HPV visokog rizika (hrHPV, engl. *high risk* HPV) u različitim dobnim skupinama citološki odabranih žena sa zagrebačkog područja, te analizirati učestalost i rezultate ponovljenih testiranja na hrHPV. U promatranom jednogodišnjem razdoblju (studeni 2005. do studeni 2006.) ukupno je zaprimljeno 3.440 uzoraka obrisaka vrata maternice žena iz ginekoloških ambulanata domova zdravlja i privatnih ginekoloških ordinacija. Uzorci su testirani na prisutnost 13 genotipova hrHPV testom AMPLICOR HPV (Roche Molecular Systems), koji se temelji na lančanoj reakciji polimerazom. Ukupna prevalencija hrHPV iznosila je 34,6%. Većina uzoraka zaprimljena je od žena u dobnoj skupini od 21–30 godina (44,2%), a slijedile su dobne skupine žena od 31–40 (27,6%), 41–50 (15,7%), 51–60 (5,3%) i ≥61 godine (2,4%). Od ukupno 3.227 obrisaka vrata maternice zaprimljenih od žena poznate dobi 4,9% je otpadalo na populaciju djevojaka mlađih od 21 godine. U toj dobnoj skupini utvrđena je najveća prevalencija infekcije hrHPV (49,4%). Slična prevalencija uočena je u žena dobne skupine od 21–30 godina (45,1%). U starijim dobnim skupinama prevalencija se postupno smanjivala. U promatranom razdoblju testiranje na hrHPV ponovljeno je u različitim vremenskim razmacima na uzorcima dobivenim od 66 žena. Od 28 žena koje su na prvom testiranju bile negativne samo ih je pet bilo pozitivno na ponovnom testiranju. Od 38 žena koje su na prvom testiranju bile pozitivne u jedne trećine se hrHPV se nije mogao utvrditi kod ponovnog testiranja. Kako se je očekivalo, infekcija hrHPV najučestalija je u adolescentica i mladih žena. Potrebna su daljnja istraživanja o ponavljanim hrHPV testiranjima kako bi se mogla procijeniti stopa iščezavanja virusa i stopa novo stečenih infekcija.