Human papillomavirus infection in relation to mild dyskaryosis in conventional cervical cytology

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Summary

Purpose of investigation: To establish the prevalence and distribution of high-risk human papillomavirus (HPV) genotypes in Slovene women with repeat mild dyskaryosis, and to evaluate three molecular methods for the detection of HPV that could be used as a complementary method to cervical cytology.

Methods: In this prospective study 148 women with three subsequent cervical cytologic tests within two years showing mild dyskaryosis were enrolled. HPV infection was determined using three molecular tests: *Hybrid Capture II* and two variants of polymerase chain reaction (PCR-PGMY11/PGMY09 and PCR-CPI/CPIIG).

Results: HPV was detected in 17 of the 45 women aged ≤ 30 years and in 21 of the 103 women aged > 30 years (37.8% vs 20.4%, p = 0.04). The most common genotype was HPV 16 detected in eight (21.1%) women, the next were HPV 53 and HPV 51, each detected in five (13.2 %) women. The three molecular methods matched in 92.9%.

Conclusion: Low prevalence of HPV infections indicates that cervical screening programmes in Slovenia are overburdened with mild dyskaryosis. Repeat cytology is not reliable; HPV testing might be useful as a complementary method.

Key words: Human papillomavirus; Mild dyskaryosis; Cervical cancer.

Introduction

Persistent infection with high-risk human papillomavirus (HPV) genotypes represents the major etiologic factor for development of cervical cancer [1].

Screening for high-grade cervical intraepithelial neoplasia (CIN2+) is based on cytology in most countries [2]. In Slovenia, as well as elsewhere in the world, the follow-up of the women with repeat mild dyskaryosis represents a clinical and a public health problem, especially because of the low sensitivity of cytologic testing [3, 4]. Data in the literature shows that the sensitivity of cytologic tests in detection of CIN2+ ranges between 40 and 80% [3, 4]. Furthermore, cytological screening is not reliable in the detection of changes of the glandular epithelium and adenocarcinoma that also contribute to the increasing incidence of cervical cancer [5, 6].

For a proven close relation between infection with high-risk HPV genotypes and cervical cancer, some countries have added testing for infections with high-risk HPV genotypes to the follow-up of women with abnormal squamous cells and mild dyskaryosis [7, 8].

Because of the high prevalence of transitory infections with high-risk HPV genotypes in women younger than 30 years and spontaneous disappearance of the virus in 80% within the first year after infection [1], HPV testing has been recommended as a complementary test in cervical screening programmes or as a primary screening only in women over 30 years old [1, 9].

The aim of this prospective study was to establish the prevalence and distribution of high-risk HPV genotypes in Slovene women with repeat mild dyskaryosis. Additionally, we comparatively evaluated the appropriateness of different molecular methods for the detection of high-risk HPV genotypes that could be used as a complementary method to cytology.

Materials and Methods

In this study 148 women who had three consecutive cervical smears showing mild dyskaryosis within two years were enrolled. We divided the analysed population into two groups: the younger group, involving women aged ≤ 30 years (n = 45) and the older group involving women aged > 30 years (n = 103). In each woman, a cervical smear was taken for cytologic testing followed by HPV testing.

Cervical smears for HPV and cytologic testing were taken at the University Department of Obstetrics and Gynecology in Ljubljana. For HPV testing, a commercially available *Digene Specimen Collection Kit®* (Digene Corporation, Gaithersburg, USA) was used. The samples were stored at –20°C. Cytologic analyses were done as a routine procedure.

The presence of HPV infections was determined using three molecular tests: *Hybrid Capture II* (HCII) (Digene Corporation, Gaithersburg, USA) and two variants of polymerase chain reaction (PCR), PCR-PGMY11/PGMY09 and PCR-CPI/CPIIG, as previously set [10].

HPV genotypes were determined using the method of enzyme restriction of PCR products amplified using group-specific oligonucleotide primers PGMY11/PGMY09 [10].

By their oncogenous potential HPV genotypes were divided into [11]:

1) high-risk (HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 68, HPV 73 and HPV 82)

2) probable high-risk (HPV 26, HPV 53 and HPV 66)

3) low-risk (HPV 6, HPV 11, HPV 40, HPV 42, HPV 43, HPV 44, HPV 54, HPV 61, HPV 70, HPV 72, HPV 81 and CP 6108)

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4) undetermined risk (HPV 25, HPV 34, HPV 57, HPV 62, HPV 74, HP 70, IS 39, candHPV 89).

All cases with a double or multiple infection are referred to as having the highest risk genotype.

The study was approved by the national medical ethics committee.

Statistical analyses were performed using SPSS software for Windows (SPSS, Inc., IL). For comparison of the data between the two groups, a Student's t-test was performed. Differences were considered significant when p values were < 0.05.

Results

The analysis involved 148 cervical smears of 148 women with mild dyskaryosis in three consecutive cervical smears within the period of two years.

In the group aged ≤ 30 years there were 45 women, and in the group aged > 30 years there were 103 women. The mean age was 35.9 years (range 19-56 years).

The three molecular methods used (HCII and two variants of PCR) provided evidence of an existing HPV infection in 38 women (25.7 %) of the 148 women tested. The criterion for the presence of HPV infection in a particular sample was the positive result of at least one test used.

In the younger group, a HPV infection was found in 17 women (37.8 %) of the 45 women tested, and in the older group in 21 women (20.4 %) of the 103 women tested (p = 0.038).

In 37 women, at least one HPV genotype was identified. The unidentified genotype assessed by HCII was marked HPV X. HCII was positive in 32 women (25.7%), PCR-PGMY11/PGMY09 in 37 women (25.0%), and PCR-CPI/CPIIG in 30 women (20.3%) of the 148 women tested. The distribution of HPV genotypes is shown in Table 1.

High-risk HPV genotypes were identified in 28 (75.7%) women, probable high-risk genotypes in five (13.5%) women, low risk genotypes in three (8.1%) women and undetermined in one woman (2.7%) of the 38 HPV-positive women.

The most common genotype was HPV 16 found in eight (21.1%) women, the next were HPV 53 and HPV 51 each found in five (13.2%) women followed by HPV 31 in four (10.5%) women and HPV 18 in three (7.9%) women of 37 typed HPV genotypes. In one woman HPV infection was detected only with HCII.

The method recognised HPV infection in 32 of the 38 samples found as HPV positive. Negative results were obtained in six women infected by: low-risk genotypes HPV 6 (n = 1) and HPV 44 (n = 1); probable high-risk genotype HPV 53 (n = 2); high-risk genotype HPV 68 (n = 1), whereas one woman was infected by four genotypes HPV 66+HPV 72+HPV 73+HPV 53 (Table 1).

The results of the three chosen molecular methods for the detection of high-risk HPV genotypes matched in 92.9% of the cases.

Table 1. — *HPV infections detected by three molecular methods (HCII, PCR-PGMY11/PGMY09 and PCR-CPI/CPIIG), and HPV genotype distribution in women with repeat mild dyskaryosis in cervical cytology.*

Genotype HPV	No. of women	HCII	PCR-PGMY11/ PGMY09	PCR-CPI/ CPIIG
6	1	neg.	1	1
16	5	5	5	5
18	1	1	1	1
31	2	2	2	2
39	1	1	1	1
44	1	neg.	1	neg.
51	2	2	2	2
52	1	1	1	1
53	4	2	4	1
56	1	1	1	neg.
68	1	neg.	1	1
16+34	1	1	1	1
16+57	1	1	1	neg.
18+40	1	1	1	1
18+51	1	1	1	1
31+62	1	1	1	1
52+HP70	1	1	1	1
51+59	1	1	1	1
53+62	1	1	1	1
25+**31+73**	1	1	1	1
25+**31**+candHPV89	1	1	1	1
25+**58**+IS39+candHPV89	1	1	1	1
66+72+**73**+53	1	neg.	1	neg.
45+73+X	1	1	1	1
61+81+X	1	1	1	1
16+58+39+X	1	1	1	1
25+**51+73**+X	1	1	1	1
74	1	1	1	1
X	1	1	neg.	neg.
Total	38	32	37	30

X = unidentified HPV genotype; neg. = HPV test negative; High-risk HPV genotypes are marked in bold.

Discussion

Invasive cervical cancer is the third most frequent cancer in women in developed countries, and is a consequence of persistent HPV infection with high-risk genotypes [7, 8]. This is the only malignancy in which the detection and treatment of precancerous lesions are efficient in preventing the development of an invasive disease. These facts therefore justify the development and implementation of sensitive HPV testing to improve the results [9, 12].

The use of cytologic testing of cervical smears has unfortunately not resulted in complete deletion of cervical cancer from the list of oncologic diseases either in Slovenia or in the countries with more reliable screening programmes. On the contrary, in some developed countries the incidence of cervical cancer is increasing, especially in younger women, who represent an extremely important group of women for both their families and society [5].

Information on the prevalence and distribution of HPV genotypes in a country is crucial for choosing appropriate screening tests and making an appropriate and effi-

cient cervical screening algorithm for detection of CIN2+. In the future information on HPV genotype distribution will also play a role in choosing the appropriate vaccine for prevention of HPV infections.

In our study, we detected HPV infection in 25.7% of the women with repeat mild dyskaryosis. The prevalence of HPV infections that can be estimated for a population depends mainly on the choice of the method used for detection of infection and on the woman's age. The criterion for the presence of HPV infection in our study was a positive result provided by at least one of the three methods used (HCII, PCR-PGMY11/PGMY09 and PCR-CPI/CPIIG). After dividing the study population into two groups by age, a statistically higher incidence of HPV infections was found in the younger group (37.8%) than in the older group (20.4%) ($p = 0.04$). We agree with Cuzick *et al.* [3] that an appropriate screening algorithm for women younger than 30 years should be prepared, because of spontaneous disappearance of the virus in 80% within the first year after infection [1].

The prevalence of infections with high-risk HPV genotypes observed in our study (25.8%) was significantly lower than reported in similar studies (31%-80%) [9, 13, 14]. We enrolled only women with repeat mild dyskaryosis, which was based on our presumption that in these women the risk of development of CIN2+ is increased. In spite of a strict inclusion criterion, the prevalence of HPV infections in our study proved to be very low. This suggests that repeat cytological testing as a follow-up method does not prove to be reliable in the studied population. This conclusion agrees with the study of Monsonego [15] who found that the percentage of abnormal cytologic smears might be increased by three times, simply for the fear of false-negative results, which definitely increases the number of pelvic examinations and control smears, needlessly burdens women both physically and mentally, and consequently increases costs. Arnold [16] reports that abnormal cytology is found in 7-8% of all smears, depending on the percentage of women at risk involved in testing. In Slovenia, the proportion of mild dyskaryosis in the period 1998-2002 was very high, about 11-12% of all cervical smears (unpublished data).

Monsonego [15] recommends introducing HPV testing as a complementary method to cytological testing in cytologic laboratories that have an over 8% share of smears with abnormal squamous cells or mild dyskaryosis. We are of the opinion that Slovenia should implement the same recommendation for two reasons: a high share of cytological smears with mild dyskaryosis and a low prevalence (20.4%) of HPV infections in women over 30 years old found in the present study.

In the detection of CIN 2+, sensitivity of HCII testing is about 90% [17], and specificity between 64% and 88% [9, 12]. The major advantage of HPV testing is a high negative predictive value, being as high as 98%, and major disadvantage a low positive-predictive value [18].

Although we did not establish sensitivity and specificity of HPV testing for the detection of CIN2+ in our study, we presume that in Slovenia the cytological screening programme is overburdened with repeat cytological testing, which is very likely to have low sensitivity and specificity. Our presumption is based on three facts: a high percentage of cytological smears with mild dyskaryosis (11-12%) in the general population in Slovenia, low prevalence of HPV infection, established in women with repeat cytological smears with mild dyskaryosis (20.4%), and a high incidence of cervical cancer (19/100,000) [19].

In our study we used three molecular methods (HCII, PCR-PGMY11/PGMY09 and PCR-CPI/CPIIG) because we wanted to evaluate the value of the HCII method, which has been suggested as a routine method for detecting high-risk HPV genotypes. At the same time we were interested in the distribution of HPV genotypes in women with cytological smears showing mild dyskaryosis.

It is known that the number of HPV genotypes varies considerably from population to population, between 13 and 23 [20-22].

In our study we identified a wide spectrum of HPV genotypes, 26, in a relatively small population of women with repeat cytological smears showing mild dyskaryosis. Identification of 26 HPV genotypes in 38 women means that we chose sufficiently sensitive methods for the detection and genotyping of HPV infections.

The distribution of HPV genotypes also greatly depends on the studied female population. In the world [23], Slovenia being no exception, the most frequently identified genotype in the general female population is HPV 16, followed by HPV 53, HPV 31 and HPV 51.

The overall agreement of the three methods used for the detection of high-risk HPV genotypes in our study (HCII and two variants of PCR) was 92.9%. The HCII test provided a satisfactory agreement with PCR (only one high-risk HPV 68 was not detected by HCII), which means that it can be used in a cervical screening programme as a complementary method to cytologic testing, or may be better used as a primary screening method.

We can conclude that repeat cytological testing as a follow-up method in women with cytological smears showing mild dyskaryosis in the Slovene cervical screening programme is not reliable. We provide evidence that using HPV testing as a complementary method to routine cytological testing we could avoid unnecessary examinations. At the same time an identified HPV infection could be a useful clinical parameter for appropriate selection of women for colposcopy, as well as a control mechanism for evaluation of the quality of cytological testing.

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