FREQUENCY AND RISK FACTORS OF CERVICAL HUMAN PAPILLOMA VIRUS INFECTION IN WOMEN IN MONTENEGRO

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Abstract - Cervical human papilloma virus (HPV) infection among women from 17 to 62 years of age in Montenegro was studied using in situ hybridization and cytological testing. Cervical HPV infection was diagnosed in 44.3% of women. The most common HPV genotype was 31/33/51 found in 56.9%. HPV positive samples for types 16/18 were identified in 41% HPV positive samples. The results showed that 57% and 45% of women who had had their first sexual intercourse before and after the age of 18, respectively, had cervical HPV infection. Cytological findings of Papanicolaou (Pap) test class III were significantly more common in women smokers. Cervical HPV infection among women in Montenegro is most commonly caused by HPV genotypes for which a prophylactic vaccine is available, or by phylogenetically related types, thus offering the opportunity of using vaccines to reduce the incidence of HPV infection.

Key words: human papilloma virus; cervical cancer; HPV vaccine

INTRODUCTION

Human papilloma virus (HPV) is considered an etiologic agent in almost all cervical cancer and cervical intraepithelial neoplasia (CIN). In the mid-1970s, hypotheses about the relationship between HPV infection and cervical cancer were made. In the 1990s, thanks to the methods of molecular biology and epidemiological studies, this relationship was proven (zur Hauzen, 1976; zur Hauzen, 2006). About 5 million new cases of cervical cancer are diagnosed every year in the world. Approximately 250 000 women die from this disease every year, out of which most (80%) deaths occur in developing countries, including Montenegro (Bosch, 2008). On average, 83 new cases of cervical cancer have been detected annually in the last 10 years in Montenegro (data from Cancer Council of Clinical Center of Montenegro).
The approximately 40-60 types of HPV that can infect the genital tract are divided into HPV types with high oncogenic potential and types with low potential (Munoz et al., 2006). HPV 16, 18, 45 and 31 are the most important oncogenic HPV types, but epidemiological studies show that there are geographical variations in the prevalence of different HPV types (Munoz et al., 2003). The prevalence of HPV infection in general population females varies with age (Bosch and Sanjoze, 2003).

Carcinogenesis is a complex and multi-stage process where, in addition to HPV infection as the most important, there are also other, more or less defined cofactors. The long duration of the process, knowing the factors and cofactors, gives the possibility of preventing the development of cervical cancer. The implementation of programs for cervical cancer prevention in developed countries has given very good results. Prophylactic vaccines for oncogenic HPV genotypes 16 and 18 have been available since 2006, expanding the range of prevention of this serious disease in women. Vaccines are applied in many developed countries. Although the current data on the effects of vaccination are encouraging, some issues remain open (Colgrove, 2006).

In Montenegro, HPV vaccine has not been implemented so far. A draft national program for the prevention of cervical cancer is in preparation. Such a program relies on local epidemiological data and is adjusted to the economic potential of the country and other resources necessary for its implementation.

This paper presents data on cervical HPV infection in women in Montenegro and some of the risk factors for the development of this infection. Such data are important in creating a strategy to prevent cervical cancer.

**MATERIALS AND METHOD**

**Materials**

The study involved 115 cervical smears from women aged from 17 to 62 years who visited the Clinic for Gynecology, Clinical Center of Montenegro, in the period from April 2000 to November 2001. The swabs were used to investigate the presence of HPV and for cytological testing. One woman was under 19 years of age, 37 were aged between 20 and 29, 31 from 30 to 39, 34 from 40 to 49, 9 from 50 to 59 and 3 from 60 to 69.

The presence of HPV in the cervical swabs was diagnosed by *in situ* hybridization (BioPap Human Papillomavirus In Situ Screening/Typing Assay for Cervical Specimens, Enzo Diagnostics, Inc. USA ). Three different HPV probe reagents were provided, one that detects both HPV 6 and 11, a second that detects both HPV 16 and 18 and a third that detects HPV 31, 33 and 51.

Cytological examination of the cervical epithelium was performed using the Pap test.

All examined women completed an anonymous questionnaire with questions about the age of first sexual intercourse, number of sexual partners and smoking habits.

**RESULTS**

Cervical HPV infection was diagnosed in 51 (44.3%) out of the 115 women included in this study. Pap test class III was found in 9 of the 51 women with cervical HPV infection and in 3 of 64 women without this infection. Data analysis showed that Pap test class III was significantly more frequent in women with cervical HPV infection than in those without such infection ($P = 0.032$).

It was not possible to determine HPV type in 10 (19.6%) of the 51 positive samples. The most common HPV genotype was 31/33/51 found in 29 (56.9%) HPV positive samples, followed by HPV 16/18 identified in 21 (41%) HPV positive samples (Fig. 1). The prevalence of HPV infection in the examined sample was highest in women under 29 years of age (53%). There was a second prevalence peak in the age group between 50 and 59 years (55.5%) (Fig. 2).
Fig. 1. The frequency of different HPV types in women with cervical HPV infection. Statistical analysis, performed using the statistical software package STATISTICA, the Fisher-Irwin test with a level of significance $\alpha = 0.05$, showed that the frequency of HPV types 31/33/51 ($t$) was significantly higher than HPV types 16/18 ($t$) and 6/11 ($t$) ($p=0.003$ and $p=0.000$, respectively). There was no significant difference in frequency between HPV types 16/18 and 6/11 ($p=0.381$).

Fig. 2. Prevalence of HPV infection and Pap test according to age groups. Analysis showed that cervical HPV infection (without PAP abnormal findings) ($\equiv$) was significantly more common in women aged $<29$ years as compared to other age groups: 30-39, 40-49 and 50-59 years ($p = 0.047$, $p = 0.01$, $p = 0.049$, respectively). Mutual comparison of other age groups (30-39 and 40-49, 50-59 and 30-39, 50-59 and 40-49) did not show significant differences ($p = 0.236$, $p = 0.762$, $p = 0.266$, respectively). Abnormal Pap findings (Pap class III) combined with HPV infection ($\equiv$) were significantly more common in women aged 50-59 years compared to the age groups 40-49 and 30-39 ($p = 0.015$, $p = 0.000$) and in women aged 40-49 years compared to women 30-39 years ($p = 0.001$).
The prevalence of Pap test class III had increasing tendency from younger to mature age, and had its peak in women aged 50-59 years.

Fourteen women had their first sexual intercourse before the age of 18, and 96 women had their first sexual intercourse at the age of 18 or over. Five women refused to answer this question (Table 1). HPV infection was more common in the women who had their first sexual intercourse before the age of 18 (57%), than in those whose first sexual intercourse occurred after the age of 18 (45%).

### Table 1. The age at first sexual intercourse and the finding of cervical HPV infection

<table>
<thead>
<tr>
<th>CERVICAL HPV INFECTION</th>
<th>AGE AT FIRST SEXUAL INTERCOURSE</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up to 18 y</td>
<td>18+</td>
<td>No data</td>
<td>TOTAL:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>negative</td>
<td>6</td>
<td>43</td>
<td>53</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>8</td>
<td>57</td>
<td>43</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>14</td>
<td>100</td>
<td>96</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

P = 0.281 > 0.05

### Table 2. Number of sexual partners and presence of cervical HPV infection

<table>
<thead>
<tr>
<th>CERVICAL HPV INFECTION</th>
<th>NUMBER OF SEXUAL PARTNERS</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>&gt;1</td>
<td>No data</td>
<td>TOTAL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>41</td>
<td>52.6</td>
<td>12</td>
<td>52.2</td>
<td>11</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>37</td>
<td>47.4</td>
<td>11</td>
<td>47.8</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>78</td>
<td>100</td>
<td>23</td>
<td>100</td>
<td>14</td>
</tr>
</tbody>
</table>

### Table 3. Smoking habits and Pap smears in examined women

<table>
<thead>
<tr>
<th>SMOKING HABITS</th>
<th>PAP TEST</th>
<th>TOTAL:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pap negative</td>
<td>Pap test class III</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>smokers</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>57</td>
<td>56</td>
</tr>
<tr>
<td>total:</td>
<td>102</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 4. Relative risks of changes in the cervical epithelium (Pap test class III) due to cervical HPV infection and smoking habits

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>RELATIVE RISK (RR)</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>4.0355</td>
<td>1.0068</td>
</tr>
<tr>
<td>Smokers</td>
<td>3.8723</td>
<td>0.9650</td>
</tr>
</tbody>
</table>
There were 23 out of the 115 women who had more than one sexual partner, and 14 women refused to answer to this question (Table 2). HPV infection was found in approximately the same percentage in women who reported having one sexual partner and those who had more than one sexual partner.

Investigation into smoking habits as potential cofactors in carcinogenesis showed that Pap test class III was significantly more common in women who smoked than in those who did not (\( P = 0.042 < 0.05 \)) (Table 3). The existence of relative risk of occurring cytological changes in the cervical epithelium under the influence of the investigated factors was tested (Table 4). Applying logistic regression it was shown that the women who had cervical HPV infection or were smokers were at a four-fold higher risk of developing cervical cytological changes than those who did not have this type of infection or were non-smokers.

**DISCUSSION**

The test used in this study cannot provide precise data on the distribution of individual HPV types in HPV infection. However, it does not exclude the possibility of using the data obtained for various purposes, including consideration of possible strategies of cervical cancer prevention in Montenegro. HPV vaccines (bivalent and quadrivalent) protect against oncogenic HPV types 16 and 18. It has been shown that vaccines provide some cross-protection against HPV genotypes phylogenetically related to vaccine types, such as types 31, 33 and 35 (phylogenetically related to HPV16) and 45 (phylogenetically related to HPV18) (Bonanni et al., 2009). According to the results obtained in this study, the most commonly detected HPV genotypes were 31/33/51 found in HPV positive samples, followed by HPV 16/18. These data could induce about the consideration of introducing vaccination for prevention HPV infection.

However, the fact that the HPV vaccine is the most expensive ever produced should not be ignored. Three doses of the vaccine are needed for immunization. As the level of protective antibody titers is not known to date, there remains the question of whether revaccination would be needed or if vaccination offers lifetime protection. Cervical cytology screening is still necessary for vaccinated females.

The relationship between HPV infection and cervical cancer is stronger than that between cigarette smoking and lung cancer (Wallboomers et al., 1999). On the other hand, the development of cervical cancer subsequent to HPV infection is not inevitable. It is estimated that only 10% of women with HPV infection will develop cervical cell changes. Eight percent of women with cervical cell changes will develop carcinoma \textit{in situ}, and one in five women with carcinoma \textit{in situ} will develop cervical cancer. In addition to the fact that already developed lesions in the cervical epithelium may be removed and the epithelium recovered, the majority of HPV infections is transitory (more than 80%), i.e. self-eliminating mainly in young women. The risk of development and progression of cervical intraepithelial neoplasia is related to persistent HPV infection caused by one or more oncogenic HPV types (Bosch et al., 2002).

At the global level, HPV types 16 and 18 are the cause of half of high-grade cervical squamous intraepithelial lesion, 70% of all cases of cervical cancer and squamous adenocarcinoma and 25% of low-grade squamous cervical intraepithelial lesions (Arbin and Dillner, 2007). However, the oncogenic potential of certain HPV types is not defined, and there are those considered as likely to be oncogenic types, such as 26, 53 and 66 (Kocjan and Poljak, 2009).

In the case of mass vaccination, there is an open question about the potential impact on HPV ecology and whether such a vaccination will lead to a higher incidence of other oncogenic types. The vaccine is intended for girls aged 12-13. However, there is a possibility of vaccinating older females. The results of this study showed that the prevalence of HPV infection was highest among women under the age of 29, which is in agreement with literature data, indicating that the vaccine could be applied in the same way in Montenegro. The literature describes another prevalence peak at the age of 55 (Herero et al., 2000).
In this study, the peak was also observed in women aged 50-59 years. For the second prevalence peak, there has been no explanation so far.

Regarding the risk factor of acquiring HPV infection, early sexual relations and a number of sexual partners have not been shown as risk factors among women in Montenegro. Montenegro is a patriarchal country where sexual freedom is still limited by the traditional background, and the lack of response to this question reveals an unwillingness to talk about these issues even anonymously.

The World Health Organization recommends vaccination as a strategic approach to the prevention of cervical cancer in developing countries with limited financial capabilities and without developed cervical cytology screening.

During a five-year monitoring period, the effectiveness of vaccination in terms of reducing the incidence of CIN has been proven, but to evaluate the effectiveness in terms of reducing the incidence of cervical cancers, it is necessary to have post-vaccination monitoring for decades (Haug, 2009). Despite the vaccination, the women still need to continue cervical cancer screening because of the risk of exposure to other oncogenic HPV types and the unknown duration of anti-HPV immunity.

REFERENCES


