HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL DYSPLASIA

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HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL DYSPLASIA (Abstract): Pap testing is considered to be the best screening tool for cervical cancer but there is currently great interest in the possible application of Human Papilloma virus (HPV) testing to supplement Pap screening for cervical cancer. **Aim:** To determine the prevalence of high-risk HPV types in the studied population and to explore the association between high-risk HPV types and cervical dysplasia. **Material and methods:** Cross-sectional study conducted at the Iasi “Cuza Voda” Obstetrics-Gynecology Hospital and Suceava County Hospital. 332 women who underwent colposcopy for cervical lesions between 2006 and 2011 were included in this study. **Results:** The overall prevalence of HPV was 57.23%. HPV prevalence differs significantly in the three age groups up to 50 years. It was highest in patients below the age of 40 and progressively lower with advancing age. The overall prevalence of cervical dysplasia was 56.62%. The prevalence of cervical dysplasia was highest in the age groups up to 40 years. The most important determinant of HPV infection is age. **Conclusions:** Persistence of HPV appears to be associated with progression to squamous intraepithelial lesion. Dysplasia is often missed in a cervical sample either because of human error in screening and interpretation, or because of suboptimal quality of Pap smear. Incorporation of HPV testing into the present Pap screening program has the potential of making screening for cervical cancer more effective, and a necessary prelude to assessing this is by determining the prevalence of the high-risk types. **Key words:** INVASIVE CERVICAL CANCER, PAP TEST, HUMAN PAPILLOMA VIRUS

Invasive cervical cancer is a disease that can be prevented by early detection and treatment. Recognizable precursor lesions generally precede it. The introduction of Pap screening programs has accelerated the decline in mortality due to cervical cancer in the past 45 years. Although this indicates a successful screening program, a series of studies revealed the sensitivity of the Pap smear to be only 51% and an unacceptable number of women who have been cytological screened develop invasive cervical cancer.

The objectives of the study were to determine the prevalence of high-risk HPV types and of cervical dysplasia in the studied population and to explore the association between high-risk HPV types and cervical dysplasia.

**MATERIAL AND METHODS**

In order to achieve these objectives, a cross-sectional study was conducted at the Iasi “Cuza Voda” Obstetrics-Gynecology Hospital and Suceava County Hospital. 332 women who underwent colposcopy for cer-
vical lesions between 2006 and 2011 were invited to participate. Written informed consent was obtained from the women being screened. Subjects were assured that their participation was voluntary and that their regular medical care would not be affected if they declined to participate.

The procedure for sample collection for HPV testing was incorporated into routine Pap testing. Pap samples were collected in a liquid based medium, which allowed both cytological diagnosis and HPV testing to be done on the same sample.

A cervical broom device was used to collect cells from the endocervix. The brush was then swirled immediately into a 20-ml container of buffered fixative to dislodge the cells into the medium. The sample was transported to the cytology laboratory of the Synevo laboratories in Romania. Pap test was always done first. Residual samples were forwarded for HPV testing. Pap smear reporting was based on the Bethesda System and the results were forwarded to the researcher. Cytology results were classified as follows: negative, benign cellular changes (BCC), atypical squamous cells of undetermined significance (ASCUS), low grade cervical squamous intraepithelial lesion (LSIL) and high grade cervical squamous intraepithelial lesion (HSIL). A pathologist routinely reviewed all abnormal Pap smears.

Colposcopy with cervical biopsy was performed in all cases. The findings of cervical biopsy histopathology were defined as HSIL, LSIL, negative (no colposcopic abnormality), others (including condyloma, benign atypia, HPV changes, squamous intraepithelial lesion unqualified, squamous intraepithelial lesion unqualified favoring HSIL or LSIL, immature squamous metaplasia with or without atypical, mature squamous epithelium, acute and chronic inflammation), adenocarcinoma in situ (AIS), and invasive cervical cancer.

Confidence intervals (the least frequency with which the interval will contain the true parameter) do much more than assess the extent to which the null hypothesis is compatible with the data. They provide simultaneously an idea of the likely magnitude of the effect and random variability. In this study 95% confidence intervals were used to assess statistical significance and random variability of the point estimate. Confidence intervals can also show that the data do not contain the information necessary for reassurance about an absence of effect. For a study to provide evidence of lack of an effect, the confidence limits must be near the null value. P-values were reported when analysis of trends were done.

**RESULTS**

Data on patients’ age and educational level were obtained from laboratory requisition form and clinical documentation. Mean age of patients was 36.9 ± 11.57 years with a median of 28 years. Most of the patients (176 – 53.01%) followed a medium education (high school). Age-group prevalence of HPV infection is presented in tab. I.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of subjects</th>
<th>HPV prevalence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>106 (31.93%)</td>
<td>58.49%</td>
<td>42.6-65.8</td>
</tr>
<tr>
<td>31-40</td>
<td>146 (43.97%)</td>
<td>59.59%</td>
<td>52.5-68.6</td>
</tr>
<tr>
<td>41-50</td>
<td>50 (15.06%)</td>
<td>54%</td>
<td>50.5-57.9</td>
</tr>
<tr>
<td>&gt;51</td>
<td>30 (9.04%)</td>
<td>56.66%</td>
<td>48.9-60.0</td>
</tr>
</tbody>
</table>

HPV prevalence differs significantly in the three age groups up to 50 years. It was highest in the patients under 40 and progressively lower with advancing age.

HPV prevalence did not differ signifi-
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Significantly with education. Sixty-four patients (19.69%) did not provide information on their education level and prevalence in this group was about the same as for those with known education.

HSIL accounted for 88 (26.5%), LSIL for 92 (27.71%), atypical squamous cells of undetermined significance (ASC-US) for 69 (20.78%) and squamous cell carcinoma (SCC) for 5 (1.5%) of the referral Pap smears in women with cervical dysplasia. Pap smear was negative in 78 cases (23.49%).

Colposcopically directed cervical biopsies reported no pathological abnormality (negative) in 64 (19.28%), HSIL in 105 (31.62%), LSIL in 83 (25%) and other lesions in 80 (24.1%) women. A fair agreement between Pap smear and cervical biopsy was proved by the Kappa test (0.5) when analyzing for squamous intraepithelial lesions (SIL).

The prevalence of cervical dysplasia was highest in the age groups up to 40 years. At age >41, the prevalence was half of that at the younger ages, and dropped even lower among those over 50.

Although not statistically different, prevalence of cervical dysplasia was slightly higher among those with high school education than in those with no or primary education, and higher again among those with a higher education.

In women with the cytological diagnosis of SIL, over 90% had HPV. Twenty-one percent of the women who were negative for cervical dysplasia (including BCC) were positive for HPV.

Women younger than 40 years had the highest odds ratio (OR) for HPV infection. The OR was significantly lower (0.63) in the 41-50 year-old group and dropped even lower (0.22) in those over the age of 51.

When comparing the OR for HPV infection in the groups by education, the lowest association (fully adjusted) was for the group with high school education, while the strongest association was in those with no, primary or gymnasium education had. Those with unknown education had the same OR as those with secondary education.

### TABLE II

<table>
<thead>
<tr>
<th>Age group</th>
<th>Basal OR</th>
<th>Basal 95% CI</th>
<th>Fully adjusted OR</th>
<th>Fully adjusted 95% CI</th>
<th>HPV adjusted OR</th>
<th>HPV adjusted 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>1.0</td>
<td>Referent</td>
<td>1.0</td>
<td>Referent</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td>31-40</td>
<td>1.02</td>
<td>0.6-1.7</td>
<td>1.03</td>
<td>0.6-1.7</td>
<td>1.5</td>
<td>0.8-2.7</td>
</tr>
<tr>
<td>41-50</td>
<td>0.45</td>
<td>0.2-0.9</td>
<td>0.43</td>
<td>0.2-0.9</td>
<td>1.3</td>
<td>0.6-2.8</td>
</tr>
<tr>
<td>&gt;51</td>
<td>0.33</td>
<td>0.1-0.7</td>
<td>0.32</td>
<td>0.1-0.8</td>
<td>0.66</td>
<td>0.3-2.2</td>
</tr>
</tbody>
</table>

HPV was very strongly associated with cervical dysplasia (OR: 28.6) even after adjustment for age, education and smoking status. Even though the confidence intervals were large (95% CI: 12-76), it was evident that HPV was significantly associated with cervical dysplasia.

Women aged 40 and younger had the highest OR for cervical dysplasia and it was less than half in the women over 41. With age, OR became insignificant in the fully adjusted model and in fact it was HPV that mostly influenced this change.

There seemed to be a pattern of decreasing OR estimates for cervical dysplasia in smokers, with those who were former
smokers having a lower OR (0.70) and current smokers having an even lesser OR (0.62); however, none of these were statistically significant.

The age-adjusted odds of having cervical dysplasia increased significantly and dramatically with increasing viral load. The p-value for this trend is <0.0001. The prevalence of high-risk HPV types was about 55% and did not differ significantly by level of education. However, it did differ significantly by age showing a dramatic decrease in prevalence from 62.1% in the less than 40 year-olds to 48% in the over 41 year-old.

Prevalence of cervical dysplasia was 56.62% and did not differ significantly by highest level of education in the household. It too differed significantly by age between under 40 (59.7%) and over 41 year-olds (53.9%). Over 90% of women with a cytological diagnosis of SIL had high-risk HPV types.

Basal and age adjusted OR's between HPV and the five selected variables showed age as the only variable significantly associated with HPV. Age was also significantly associated with cervical dysplasia but the OR estimates shifted toward the null when controlling for HPV and the association changed to become insignificant after that adjustment HPV was strongly associated with cervical dysplasia with a fully adjusted OR of 28.6 (14.7, 55.5). The association between cervical dysplasia increased markedly with increasing viral load (p<0.0001).

DISCUSSION

Cervical dysplasia is a broad term describing the precursor lesions that are considered to be indicators of the progression to cervical cancer. These lesions were classified as cervical intra-epithelial neoplasia (CIN).

Numerous studies have consistently reported the association between sexual behavior and cervical cancer, and since the 1980's, attempts have been made to try to understand the role of HPV as the sexually transmitted agent responsible. The poor sensitivity and specificity of the methods used for HPV testing have led to varying estimates of the relationship between HPV infection and cervical neoplasia and this variability had led to considerable skepticism about the clinical role of HPV. A new generation of HPV testing has shown more consistent associations, and HPV DNA has been detected in over 99.7% of tumors from patients with invasive cervical cancer. HPV - negative cancers are virtually non-existent (5, 6).

This study found a strong association (OR: 28) between high-risk HPV types and cervical dysplasia. Comparison with other studies has been difficult because of the different techniques used for detection and the different combinations of strains being tested for, nonetheless, OR's of over 15 have been reported in a review of case-control studies that used reliable methods for HPV DNA detection. Cigarette smoking has been described as an independent risk factor for cervical neoplasia however this was not evident in this study. The OR estimates were actually lower in smokers but these were not statistically significant.

In examining indicators of socio-economic status, one study used low educational achievement and found it associated with increased odds ratios (OR's) of 3.4 (1.2-10.1) for CIN. Bosch et al also found a low level of education to be associated with cervical cancer (1).

The role of smoking is controversial. Some studies have shown a significant association between smoking and invasive cervical cancer; however, there has been uncertainty about whether the reported association...
was a causal or a confounding phenomenon reflecting such factors as sexual habits and infection with an unidentified infectious sexually transmitted agent (7).

Other risk factors that have been examined in the literature are oral and barrier contraceptives. Studies on oral contraceptives have shown protective, null, or weak associations with cervical neoplasia. Most recent studies have shown no association with barrier contraceptives have been shown to have a protective effect against cervical cancer: vaginal spermicides (OR = 0.28), diaphragms (OR = 0.67) and condoms (OR = 0.53) (8).

A series of studies have revealed that a unique feature of HPV testing is the ability to predict the subsequent development of cervical cancer precursors among cytologically negative women (9).

**CONCLUSIONS**

Persistence of HPV appears to be associated with progression to SIL. The high rates of sexually transmitted diseases (STD) imply that a potentially large number of women in Romania are at high risk for development of cervical cancer. Early detection could mean earlier treatment and a reduction of the number of women at risk for progression to invasive cancer.

Among patients with an abnormal Pap smear, women with HSIL require immediate colposcopy and histologic diagnosis. For patients with ASCUS or LSIL, women can be followed by either performing colposcopy immediately or by repeating the smears every six months for two years, or then performing colposcopy if LSIL persists.

**REFERENCES**