Prevalence of HPV-DNA in Pap smears containing ASC and AGC performed within Population Programme of Prophylaxis and Early Detection of Early Cervical Cancer

Występowanie HPV-DNA w rozmazach cytologicznych zawierających ASC i AGC wykonanych w ramach Programu Profilaktyki i Wczesnego Wykrywania Raka Szyjki Macicy

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Additional key words:
ASC
AGC
HPV infection
Pap tests
HPV testing

Dodatkowe słowa kluczowe:
atypté komórki nabłonkowe - ASC
atypté komórki gruczołowe - AGC
zakażenie wirusem HPV
cytologia
testy na obecność wirusa HPV

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Aim: to assess the incidence of HPV-DNA in women with ASC/AGC compared to patients with normal Pap smears. Material and Methods: The study group consisted of 242 women (207 ASC and 35 AGC cases). The control group counted 200 age-matched women with negative Pap smears. Cervical samples collected from all the participants were tested for the presence of HPV-DNA using the Hybrid Capture-2 test. Results: Total HPV infection was significantly higher in the study than in the control group (43.0% vs. 14.0%) (p=0.005). There was no difference in the incidence of HPV-DNA between ASC and AGC groups. Prevalence of HPV-DNA ASC-H was significantly higher in ASC-US group (83.3% vs. 40.5%) (p=0.004). HPV positive endometrial AGC significantly outnumbered HPV positive endocervical AGC (88.9% vs. 26.9%) (p=0.003). Similar trends were observed for the high-risk type of HPV (p<0.001).

Conclusions: The significant difference in HPV-DNA incidence between the study and control groups suggests that HPV plays a role in the development of ASC and AGC. The implementation of HPV testing in all women diagnosed with ASC or AGC can lead to tailored therapeutic management and more careful follow-up care.

Background
Cervical cancer is the second most common type of cancer among women one a worldwide basis, accounting for 500,000 new cases and nearly 240,000 deaths annually. Nations with regular screening programs have, in fact, noted a significant decrease in cervical cancer mortality and morbidity rates. However, this widespread screening has also resulted in increased detection of lesser grade abnormal results. Of these, in terms of the Bethesda system-classified lesions, atypical squamous cells (ASC) and atypical glandular cells (AGC) are thought to constitute the largest and the most controversial groups, respectively [8,26]. The human papillomavirus (HPV) appears to be responsible for a large portion of these findings [9,12,15,23,30].
The aims of the study are to compare the frequency of HPV infection in women with ASC and AGC (study group) on the basis of atypia and patient age subgroups, to compare these findings with results obtained from age-matched women with normal cervical cytology (control group), and, finally, to compare the accuracy of HPV-DNA testing, in order to better triage women with atypical cell changes in cytological smears.

Material and methods

The experimental protocol was approved by the University Review Board and informed consent was obtained from all the study participants.

Cervical smears from 5000 women, aged 25-65 years, who participated in the 2002-2003 cycle of the cervical cancer screening program run by the Department of Obstetrics and Gynecology at the Jagiellonian University, were collected and analyzed. All the women diagnosed with squamous or glandular cell atypia were then placed in the study group, which eventually consisted of 207 (4.14%) cases of ASC and 35 (0.7%) cases of AGC. The control group was composed of 200 age-matched women with normal cervical cytology. A Hybrid Capture-2 (HC-2) test for the detection of 13 high-risk (16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68) and 5 low-risk (6, 11, 42, 43, 44) HPVs was carried out for all the study and control group subjects.

In accordance with the Bethesda 2001 system requirements, the ASC patients were divided into two subgroups: (A) atypical squamous cells of undetermined significance (ASC-US), and (B) atypical squamous cells where high grade squamous intraepithelial lesion (ASC-H) cannot be excluded. Similarly, two subgroups were established in the AGC group: (A) atypical endocervical cells, and (B) atypical endometrial cells. The effect of the women's age on the HPV-DNA detection rate was evaluated in both the study and the control groups.

The data (mean ± SEM) were analyzed using the Kruskal-Wallis test, the Chi² test, and the student t-test. A p-value of less than 0.05 was considered significant. The Kruskal-Wallis test and Chi² test were used to compare the findings between the study and control groups, subtypes of AGC or ASC and high-risk HPV and low-risk HPV infection. The influence of the patient’s age on HPV infection for the entire AGC group and ASC group, and for the respective subgroups in each case, was analyzed using the student t-test.

Specimen Collection and Interpretation

All specimens were obtained from a polyethylene brush (Cervex Brush, Rovers Medical Devices B.V, the Netherlands). The sample of cells collected was transferred to a glass slide, fixed with Cytofix (Cytofix, Samko, Poland) for 15 minutes, and labeled with the patient's ID. The cytological specimens were then stained automatically in Varistain Gemini automated slide stainer (ThermoShandon, Shandon Scientific Limited, England). Thus prepared, the slides were examined under a light microscope (Carl Zeiss, Germany) and classified by certified cytotechnicians in accordance with the Bethesda 2001 system.

Detection of HPV-DNA

Cervical scrapings were collected with the Hybrid Capture Cervical Sampler, placed in Specimen Transport Medium and examined for presence of HPV-DNA with the HC-2 assay (Digene Cervical Sampler, Digene Corp. USA).

Results

In the study group, the HPV infection was detected in 104 (43.0%) women. Of these, 69 (25.6%) cases were diagnosed within the ASC group, with 60 high-risk and 29 low-risk HPV infection cases (see table I). In the AGC group, HPV-DNA was detected in 15 (42.9%) cases. With 12 high-risk HPV and 3 low-risk HPV infection cases (see table II). The HPV was less common in the control group, where a positive HPV HC2 test was found in 28 (14.0%) women, with 20 high-risk and 8 low-risk HPV infection cases (see table III). On the basis of these results, the frequency of HPV infection in the study group was significantly higher than in the control group (43.0% vs. ASC-H cases) (p<0.001). Furthermore, the oncogenic potential of the HPV infection was significantly higher in the study group than in their control counterparts (36.0% vs. 10.0% of high-risk HPV cases) (p<0.001).

Within the study group, no significant difference was observed between the women with a diagnosis of ASC and AGC in respect of both the total frequency of HPV infection (43.0% of ASC vs. 42.9% of AGC diagnosed women) and the frequency of high-risk HPV types (29.0% of ASC vs. 34.3% of AGC cases) (see table I and II). The study group originally consisted of 248 women diagnosed with ASC. 41 (16.5%) of the women were lost during the follow-up. Of the 207 women subjected to further analysis, 94.2% were diagnosed with ASC-US and 5.8% with ASC-H. The total frequency of HPV infection in the women diagnosed with ASC-US was significantly lower than in the group diagnosed with ASC-H (40.5% of ASC-US vs. 83.3% of ASC-H cases) (p=0.004). There was also a significant difference in the subgroup distribution of the high-risk infection in women with ASC (25.6% of ASC-US vs. 83.3% of ASC-H cases) (p<0.001).

In the AGC group, which consisted of 35 women, 74.3% were diagnosed with endocervical atypia and 25.7% with endometrial atypia. The total frequency of HPV infection in the women with endocervical atypia was significantly lower than in their counterparts with endometrial atypia (26.9% vs. 88.9% cases) (p<0.001). Furthermore, high-risk HPV infection showed a significant difference in the AGC subgroup distribution (15.4% of endocervical vs. 88.9% of endometrial cases) (p<0.001).

The difference in frequency of HPV infection (p=0.768) between the endocervical AGC and the control group has not been stated.

The mean age of the women diagnosed with AGC and infected with HPV was significantly higher than that of both their ASC-diagnosed and control counterparts (AGC: 51.9 years vs. ASC: 40.0 years vs. Controls: 35.4 years) (p<0.001). A similar trend was observed with high-risk HPV infection, where the women diagnosed with AGC were significantly older than either those diagnosed with ACS- or the control subjects (AGC: 53.5 years vs. ASC: 41.5 years vs. control: 34.5 years) (p=0.03) (see Figure 1).

Discussion

The introduction of ASC and AGC was initially met with skepticism on the part of clinicians [11,13], since the abnormalities now described by the newly formulated nomenclature had previously been treated as benign lesions and grouped together under class II of Papanicolaou's classification. Yet, despite these modifications, both the actual clinical significance of squamous and glandular cell atypia, and the management of patients presenting with ASC and AGC, remained unclear [1,2,8,11,26]. A recently-published meta-analysis and new studies relating to the applications of HPV-DNA testing and other HPV detection methods (PCR, mRNA HPV) in triaging women with equivocal cytological abnormalities is of particular interest, given the growing number of innovative approaches to cervical cancer prevention [2,20].

Our results show that, of the 207 women diagnosed with ASC, 94.2% were diagnosed with ASC-US and 5.8% with ASC-H. The proportional distribution of abnormalities in our study mirrors the results obtained from the "ASCUS-LSIL Triage study", the largest investigation of the kind to date, sponsored by the National Cancer Institute [6,14]. Owing to the changes in nomenclature and the redefinition of certain terms, as well as the widespread use of non-standardized interpretation schemes, it is difficult to compare

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<th>Table I</th>
<th>HPV infection in group of women with ASC.</th>
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<tr>
<td>ASC</td>
<td>HPV</td>
</tr>
<tr>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>ASC US</td>
<td>195</td>
</tr>
<tr>
<td>ASC H</td>
<td>12</td>
</tr>
<tr>
<td>TOTAL</td>
<td>207</td>
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<th>Table II</th>
<th>HPV infection in group of women with AGC.</th>
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<tr>
<td>AGC</td>
<td>HPV</td>
</tr>
<tr>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>endocervical</td>
<td>26</td>
</tr>
<tr>
<td>endometrial</td>
<td>9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>35</td>
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<th>Table III</th>
<th>HPV infection in group of women with normal Pap smears.</th>
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<tr>
<td>CONTROLS</td>
<td>HPV</td>
</tr>
<tr>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>200</td>
<td>172 (86.0%)</td>
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the distribution of ASC in earlier studies [1,7]. Nonetheless, it proved possible to determine that atypical squamous cells of undetermined significance, classified as reactive, reparative or not otherwise specified, constituted 77-95% of the cytological diagnosis, while atypical favor neoplastic squamous cells of undetermined significance (ASC-US FN), recognized by the Bethesda system between 1988 and 2001, appeared in 5-23% of cases [1,9,22].

In our study, the percentage of HPV infection was found to be similar to that reported by other authors, namely 83.3% and 40.5% for ASC-H and ASC-US cases, respectively [23]. In their most recent study, Arbyn and colleagues applied meta-analysis to studies focusing on high-risk Hybrid Capture-2 probe utilization in triaging women with ASC-US and LSIL [2]. By applying a random-effect model for meta-analytical pooling, the authors analyzed the influence of covariates on the HPV positivity rate by meta-regression. On average, 43% (95% CI: 40-46%) of the women with ASCUS/ASC-US were high-risk HPV positive (range 23-74%). The authors concluded that, in women with ASC-US and LSIL, and, in particular, in younger women, reflex HPV triaging is of limited utility in these cases on account of the high HPV positivity rates [2,4].

Sherman et al. carried out a study examining the percentage of high-risk HPV infections in women with ASC-H (86%) and found it to be closer to that for women with HSIL (99%) than for those with ASC-US (99% and 63% respectively) [28]. Srodon et al. conducted a comparative analysis of Hybrid Capture 2 high-risk HPV positivity and frequency of histologically diagnosed HSIL for ASC-H and ASCUS to evaluate the performance of ASC-H as a cytocritic interpretation subcategory, and the potential utility of HPV testing for a colposcopy triage of ASC-H in routine practice [4,31]. The findings their data show that 64 of the 96 patients with ASC-H (66.7%) were HPV-positive compared with 484 of the 1079 patients with ASCUS (44.9%). Among the patients who had histologic follow-up, HSIL was identified in 18 of the 45 patients (40.0%) with HPV-positive ASC-H compared with 27 of the 266 patients (10.2%) with HPV-positive ASCUS (p<0.001) and 1 of the 22 patients (4.5%) with HPV-negative ASC-H (p=0.003); the latter result was similar to the finding for HSIL in 5 of the 86 patients (5.9%) with HPV-negative ASCUS. The frequency of HPV-positive ASC-H in our study (67%) was lower than that obtained in the ALTs for ASC-H (86%), but higher than that for ASCUS in both our investigation (45%) and the ALTs (51% for all ASC; 63% for ASCUS, equivocal for LSIL). Underlying HSIL was detected in a similar percentage of patients with HPV-positive ASC-H in both our study and the ALTs (41%) [31].

Wu et al. and Bansai evaluated the use of the high-risk human papillomavirus DNA test in the triage of women with ASC-H [4,36]. HPV DNA testing using the Hybrid Capture 2 method was carried out on 88 women with ASC-H diagnosed by means of the Thin Prep test. The HPV DNA test showed an overall positive rate of 67% and negative rate of 33% in 88 patients with ASC-H. The high sensitivity (100%) and negative predictive rate (100%) in detecting HSIL demonstrated in Wu's and in Bansai studies provide strong evidence that, instead of automatic referral to colposcopy, HPV DNA testing may be used as an alternative triage method for women diagnosed with ASC-H by means of the Thin Prep test, particularly for women over thirty [4,36]. All these results suggest the possibility of using the HPV test as a method which allows the unnecessary follow-up of HPV negative women with ASCs to be dispensed with [34]. In seven studies in which the investigators used the PCR system for HPV-DNA testing, the sensitivity for this diagnostic method was lower, compared to the HC2 assay, but the specificity was higher for CIN2+ in women with ASC-US [2]. Unfortunately, the different primers used by different authors mean that this conclusion cannot be generalized [2]. The contemporary study published in 2009 by Wentzensen et al., which analyzed the performance of Amplicor for detecting carcinogenic human papillomavirus infections and cervical pre-cancer in women with ASCUS and compared the results with Hybrid Capture 2, demonstrated that Amplicor was more analytically specific for detecting targeted carcinogenic HPV types than HC2, but missed more diseases in respect of non-targeted types, while HC2 was more likely to miss disease in terms of targeted types [35]. The study by Waldstrom showed good and equal clinical sensitivities of the Linear Array HPV genotyping test (LA) detecting HPV DNA from 37 oncogenic and non-oncogenic HPV types and the Aptima HPV assay detecting E6/E7 mRNA from 14 oncogenic HPV types for detecting CIN3+, but the Aptima HPV assay had significantly higher specificity for detecting CIN2+ and CIN3+ in women aged 30 years or older with ASC-US [34].

In the 5000, unselected population of women included in the cytological screening program, atypical glandular cells were stated in 35 (0.7%) of the cases. In the literature, the incidence of abnormalities defined as atypical glandular cells ranges between 0.1-2.1%, with an average of 0.5% [7,13,21,22,32]. The manifestation of equivocal cytological abnormalities of glandular origin in cytological smear is a substantial diagnostic and therapeutic problem. The significance of HPV infection in women with AGC is not yet known [16,37]. In our study group, the HPV infection was found in 42.9% women in the AGC group. In a study carried out by Ronnett et al., the use of HPV tests enabled the identification of 94.1% of the women with clinically significant abnormalities discovered during further follow-up [22]. Schnatz et al. evaluated the role of the HPV positive test in the management of 224 women with AGC [25]. Among these women, 30.4% tested positive for HPV. The rate of HPV-associated disease among cases testing positive for HPV was 40.0% compared with 4.0% among HPV-
negative cases. The sensitivity of HPV testing for HPV-associated disease was 81.3%. Women positive for HPV antigen or HPV DNA were less likely to have endometrial or ex- traterine disease (1.5%) than HPV-negative women (7.4%) [25].

Sharpless et al. analyzed the findings from seven studies to determine the value of human papillomavirus (HPV) testing for HPV-associated cervical disease and atyp- ical glandular cell-associated cervical disease in women with AGCs [27]. This analysis included a search of the literature dating from January 1993 to September 2007 using various AGC-related terms. The rate of AGC-associated cervical disease for 661 cases of AGC with concurrent HPV testing was 23.3%. The rate of HPV-associated cervical disease was higher in HPV-positi- tive cases than it was for negative (53% vs. 3% respectively). The human papillomavirus-positive versus negative sta- tus predicted a higher likelihood of a cervi- cal intraepithelial neoplasia 2/3 lesion (odds ratio = 39.6, 95% CI=17.9-87.4, p <0.001).

The rate of HPV non-associated cancers was significantly higher in HPV negative patients than it was for those who were posi- tive (4% vs. 0.4%; p=0.016). Human papillomavirus testing had an overall sensi- tivity of 90%, a specificity of 79%, a 53% positive predictive value, and a 97% nega- tive predictive value for cases of HPV- asso- ciated cervical disease [27].

Our results allow the advancement of the thesis that the use of the HC2 test to detect HPV genetic viral material of HPV in combi- nation with the evaluation of the cytopathological features of atypical glandu- lar cells permits the identification of the group of women requiring colposcopic as- sessment because of the increased risk of HSIL or AIS [14]. Both our findings and those of other authors distinguish a group of women at higher risk of cervical disease and requiring close following. We would thus recommend that [8,20,26,37].

The fact that the HPV infection, and, in particular, high-risk HPV, was present to a high statistical significance more often in the study group than the control group indicates the possibility of using the HC2 test in the qualification of women for appropriate fol- low-up. Simultaneously, mapping only for high-risk HPV appears to be both sufficient and cost-effective [2,9,18,21,23]. However, Khan et al. confirmed a substantial increase in the risk of detecting CIN3 or cancer in women with positive HPV-16 results [14]. In contrast, 88% examined the impor- tance of detecting only HPV-16 DNA in women with ASC-US, mRNA HPV and HPV-DNA tests were positive in 21% and 25% of the subjects, respectively [19]. These results suggest using RNA HPV testing in women with equivocal cytology results and a posi- tive HPV-DNA test; however, it would appear to be more effective to use mRNA tests with the broad spectrum of HPV types [3,9,33].

It is interesting that we observed no differ- ence in the frequency of HPV infection between the endocervical AGC subgroup and the control group. This may be related to observations to the effect that, in cases of the presence of these abnormalities in pap smears, the histologic verification usu- ally reveals benign lesions, the most of- ten occurring being cervical polyps. Their presence is not related to HPV infections or carcinoma- genesis [5,16,17].

In summary, the evidence provides by the results of our study gives us a basis for saying that, in women with endocervical AGC, HPV testing may be an additional di- agnostic tool, whereas further triage may be dispensed with for HPV negative. Further- more, the data support the need for wider studies to be conducted.

Conclusions

The significantly higher frequency of HPV infection in women diagnosed with ASC/AACG, as compared to those with non- neoplastic cervical cytology, implicates this virus in both the development of a substantial por- tion of these abnormalities and the process of carcinogenesis.

The use of HPV-DNA testing may be helpful in the planning of further diagnostic and therapeutic follow-up. Identification of high-risk HPV infection is especially useful in women of over 30 years.

References


