CLINICAL STUDY

Current possibilities of cervical precancerous lesions screening in Slovakia: prevalence of high risk human papillomavirus in patients with cytological diagnoses of atypical squamous cells of unknown significance

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Abstract: Introduction: It has been confirmed, that there is a causal relationship between persistent infection of high risk human papillomavirus (HR-HPV) and the development of cervical cancer. In population of women older than 30 years HPV infection becomes a significant etiological factor of precancerous lesion of the cervix, but HPV infection may spontaneously regress in the majority of the cases.

Patients and methods: The analysed study group consisted of 397 samples with cytological diagnosis of atypical squamous cells of unknown significance (ASCUS). All cases underwent HPV DNA testing using the Hybrid Capture 2 (HC2) assay. We analysed prevalence of HR-HPV and a viral load expressed as relative light units/cut off ratio (RLU/CO) in different age groups with cytological diagnoses of ASCUS.

Results: The prevalence of HR-HPV with cytological diagnoses of ASCUS was detected in 44 %. The prevalence of HR-HPV between patients aged 17–29 and between patients aged 30–40 was 55 % and 48 % respectively and we detected significant reduction of prevalence (28 %) in patients older than 41 years.

Conclusion: Based on the results of presented study we assumed that age the 40 and over is crucial for the development of serious precancerous lesions in Slovakia, thus this age group is the most suitable for HPV triage of ASCUS. As a refinement of that type of ASCUS triage we recommend to add to the algorithm quantitative measurement of viral load in the specimens in the form of RLU/CO ratio (Fig. 3, Ref. 27).

Key words: cervical cancer, ASCUS, management, HPV detection methods, clinical validation.

Cervical cancer is estimated to be the third most common cancer and the fourth most common cause of cancer deaths worldwide in women. Long-time incidence and mortality trends in Slovakia are similar to developing countries (1). Although Slovakia is ranked among the economically more advanced countries, up to now it has not succeeded in reducing mortality of cervical cancer due to the lack of interest of women in screening for the disease and the opportunistic character of its organisation. In the last published year 2007 (2), the occurrence of cervical cancer in Slovakia represented 627 cases (age-adjusted incidence to the World standard ratio (ASR-W) was 16.6/100 000) and 237 deaths from cervical cancer (ASR-W mortality was 5.7/100 000). Analysis of the age-specific incidence rates in y. 2003–2006 show, that cervical cancer did not occur before the age of 20. It started to increase after the age 25 with the highest incidence in the age group of 45–49 years (1). Persistent infection with HR-HPV is a necessary step in the development of cervical cancer and other anogenital carcinomas (3, 4). Infection with HR-HPV has also been etiologically linked with head and neck squamous cell carcinoma (5). The majority of HPV infections of the cervix are transient and undergo clearance or become latent within 1–2 years of detection, especially among younger women, while in population of women older than 30 it becomes a significant etiological factor of precancerous lesion of the cervix (6, 7). Incorporation of HR-HPV detection into cervical screening programs besides conventional cytological screening may improve the ability to identify women at risk of cervical cancer (8). According to our bibliographic research, there are only few studies about the prevalence of sexually transmitted infection in Slovakian women (e.g. urogenital chlamydial infection in women from eastern Slovakia) (9). Our study is the first analysis of prevalence of HR-HPV of patients with cytological diagnosis of ASCUS and also the first attempt to introduce the viral load of HPV in the specimen in the management guidelines of precancerous lesions in Slovakia.

Patients and methods

In the reporting period of 36 months we analysed 397 cervical cytology samples for the presence of HR-HPV. The age of patients

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was between 17 to 71 years (n = 397), median age 32 years. Each patient had a cytological diagnosis of ASCUS diagnosed in our laboratory. None of the patients was vaccinated, duplicate examinations were excluded from the study group. All cases underwent HPV DNA testing using the commercially available Hybrid Capture 2 assay by Quiagen/Digene. Each sample was examined for the following high-risk types of HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68. During the sampling, transport, calculation of luminescence and interpretation of results we followed the instructions of the manufacturer. We used the original (Dynex Slovakia, Ltd.) diagnostic-kit. The Digene HC2 High-Risk HPV DNA Test was the first clinically validated HPV test on the market. The test is an in vitro nucleic hybridisation assay using an RNA probe mixture from 13 high risk (HR) HPV types, without distinguishing between them. The assay utilizes signal amplification technology: the semiquantitative viral load of a positive sample is indicated by a chemiluminescent signal measured in relative light units (RLU) of the sample to that of the cut-off (CO) value of the positive control. The manufacturer established an RLU ratio of 1.0 or higher as positive. The exact RLU values are not routinely reported as the test is used in a qualitative fashion (positive vs. negative). In our study group positivity was further divided to borderline positivity (1 ≤ RLU/CO ≤ 2), weak positivity (2.0 < RLU/CO ≤ 10.0), mild positivity (10.0 < RLU/CO ≤ 100.0) and high positivity (100.0 < RLU/CO).

Results

The number of analysed samples with cytological diagnosis of ASCUS was n = 397. Age categories: The total number of the analysed samples of patients up to 29 years of age was 128 (32 %), between the age of 30 to 40 it was 141 (36 %) and for ages of more than 40 it was 128 (32 %). From the total number of 397 patients negativity for HR HPV was detected in 221 cases (56 %), positivity was in 176 cases (44 %). We detected negativity for HR HPV in ASCUS patients in 54 samples up to the age of 29, 71 samples between the age of 30 to 40 and 96 samples in patients up to 41 years of age. Positivity for HR HPV was detected in 71 patients (55 %) up to 29 years old, in 69 patients (48 %) between 30 and 40 years of age and in 36 patients (28 %) older than 41 years (Fig. 1). During more detailed analysis of RLU/CO results, we detected high positivity in samples of 87 patients, mild positivity in samples of 53 patients, weak positivity in samples of 26 patients and borderline positivity in samples of 10 patients (Fig. 2). Figure 3 shows the number and the RLU/CO value of HR HPV positive ASCUS patients of different age groups. The number of patients with strong positivity for HR HPV before the ages of 29 and 40 years was the same, 36, the highest of all age groups.

Discussion

ASCUS is the most common abnormal cytological diagnoses within cervical cancer screening (10, 11). The risk of invasive cancer in patients with ASCUS cytology is low, ranging from 0.1 % to 0.2 % according to the literature (12, 13).

Emerson et al (13) analysed 15860 patients with cervical cytology a period of 9 years. Patients with previous dysplasia or carcinoma were excluded from analysis. A diagnosis of ASCUS was made in 1117 cases (7.0 %). After excluding 345 patients with previous dysplasia or human papillomavirus-related diagnoses and 129 patients with no follow-up specimens, 643 study patients remained. Squamous intraepithelial lesion (SIL) or dysplasia was subsequently diagnosed in 197 patients (30.6 %). High-grade squamous intraepithelial lesion (HSIL) or at least moderate dysplasia was diagnosed in 64 patients (10.0 %). In 21 cases (3.3 %) the high-grade dysplasia
developed more than 2 year after the first ASCUS diagnosis. Follow-up disclosed no cases of invasive carcinoma. Among ASCUS patients followed for up to 9 year, 20% develop only low-grade SIL or mild dysplasia and 10% develop HSIL or moderate or severe dysplasia. In 3.3% of patients HGSIL was found out within 2 years from ASCUS diagnosis, after 9 years of observation SIL or dysplasia occurred in 30.6% of cases, 10% out of which was HGSIL.

ASCUS-L SIL Triage Study Group (ALTS) pillar study (10) was undertaken to compare alternative strategies for the initial management of a cytological diagnosis of ASCUS. A total of 3,488 women with a community-based ASCUS interpretation were randomly assigned to immediate colposcopy, triage that was based on HPV DNA testing or conservative management based on repeat cytology. Prevalence of HR-HPV in ASCUS cytological diagnosis was 45%. The study end point was 2-year cumulative diagnosis of CIN grade 3. To compare triage tests, Solomon et al in ALTS (10) re-estimated the performance of HPV and cytology in successfully referring women with underlying CIN grade 3. A single enrolment HPV test identified 92.4% of the women diagnosed with CIN grade 3. Serial cytology, even at an ASCUS threshold, would have required two visits to achieve similar sensitivity (95.4%) and would have referred 67.1% to colposcopy.

Within the category of women of less than 30 years of age the HPV infections can reach prevalence of 50% which is a lot more than originally assumed (14). Even the incidence and prevalence of HPV infections between postmenopausal women increases (15). Fortunately, the majority (80–90%) of HPV infections are only of transient nature and they spontaneously disappear after several months leaving no morphological changes. During this transitional period we can observe only mild symptoms of HPV infection at the epithelium of the cervix (in the form of LG-SIL or CIN I) or the infection occurs not only clinically but also morphologically silently, i.e. without cytological abnormalities (14). Only persistently infected cervical cells have a substantial likelihood of gradually growing into diagnosable HSIL (16). This means that the fact of HPV presence (even the HR HPV) is not sufficiently serious and important information. It turned out that a viral “load”, i.e. number of copies of genome of high-risk type of HPV, plays an important role in the emergence and development of precancerous lesions of the cervix, beside the genotypes of HPV (14, 17). Several overview studies have shown that women with normal cytology but increased “load” of HR-HPV have an increased risk of developing serious CIN lesions. In patients with already developed CIN lesion and detected smaller amount (less than 10⁶ copies per sample) of HR-HPV types, the CIN lesion regressed. At a higher viral load the progression of CIN lesion to more serious lesions has been observed more frequently (17). Recent randomized controlled cervical screening trials have shown that HR-HPV testing on cervical scrapes can be also used as a primary tool for cervical cancer screening, given its superior sensitivity and negative predictive value for cervical pre-cancer compared with cytology (17). To make a distinction between HPV test performances for outcome measure “(pre)malignant disease” vs outcome measure “HPV presence” the terms “clinical sensitivity and specificity” and “analytical sensitivity and specificity” were introduced (17, 18, 19, 20). Many different HPV tests exist nowadays, which all have their technical, analytical and clinical properties. The choice for an HPV test for a given purpose should therefore not solely be based on practical considerations, but also on the intention of its use. If we e.g. expect detection of epidemiological studies of HPV incidence within certain region or an age group of population, methods with high analytical sensitivity will be more appropriate. These are able to detect very low amounts of HR-HPV (even less than 10 copies of viral DNA per sample) regardless of whether it has or has not any prognostic significance for a patient. Their disadvantage among others is their high susceptibility to contamination and, as a result of that, development of false positive results (17, 18, 19, 20).

However, in case of a patient with borderline ASCUS cytology to detect if there is a potentially prognostically significant amount of HR HPV in her lesion, it is recommended to use a method with high clinical sensitivity. High clinical sensitivity means that we detect clinically significant amount of HR-HPV, i.e. the amount that is capable of inducing development and progression of disease within the sense of precancerous lesion. The appropriate methods for such purposes are methods with sensitivity of 1pg HPV (approximately 50 000 copies per 1 ml sample). Methods with analytically lower sensitivity do not detect clinically insignificant amounts of HR HPV, e.g. “HPV presence”, they detect clinically relevant HPV infections causing “(pre)malignant disease” (17, 18, 19, 20).

The Digene Hybrid Capture 2 High-Risk HPV DNA Test (HC2), was the first FDA (Food and Drug Administration) approved clinically validated HPV test on the market (10, 20, 21).

Saeedian et al (22) compared development of dysplasia between HPV-negative and HPV-positive ASCUS patients. Overall only 22 of 1559 (1.4%) HPV-negative ASCUS women developed CIN grade 3 or worse during follow-up compared with 269 of 1767 (15.2%) HPV-positive ASCUS women (p < 0.001). HR HPV was also detected by the hybrid capture 2 method (HC2). The specificity of HPV testing (84%) was significantly higher than cytology using an ASCUS threshold (71%) (p < 0.001). Co-testing with cytology and HPV testing at 12 months resulted in even lower specificity (61%). The positive predictive value for CIN3 or worse was low for cytology (2.6%), Hybrid Capture 2 (3.8%), and co-testing with cytology and HPV testing (2.2%). The negative predictive value for all three management strategies was very high (99.70%, 99.82%, and 100% for HPV testing, cytology, or co-testing, respectively). The results show that the “HPV negative” patients with ASCUS cytological diagnosis should return to routine screening because the probability of the CIN2-CIN3 lesion formation is very low. However, if increased surveillance is chosen, a single HPV test for carcinogenic types at 12 months has significantly higher specificity and lower referrals than cytology.

Clinically validated HR HPV DNA test in combination with negative cytological examination has negative predictive value of 99–100% for the development of CIN 2 – CIN 3 (10, 16, 23).

Based on described and several other recent studies, the American Society for Colposcopy and Cervical Pathology (ASCCP) (24), updated the consensus screening guidelines and the management of abnormal cervical cancer screening tests and cancer precursor. For women “of general population” with ASCUS cytology reflex
HPV testing is preferred. For women with HPV negative ASCUS, whether from reflex HPV testing or co-testing, repeat co-testing at 3 years is recommended. For women with HPV positive ASCUS, whether from reflex HPV testing or co-testing, colposcopy is recommended. When colposcopy does not identify CIN in women with HPV positive ASCUS, co-testing at 12 month is recommended (not less than 12 months). For women with ASCUS cytology and no HPV result, repeat cytology at 1 year is acceptable. If the result is ASCUS or worse colposcopy is recommended, if the result is negative, return to cytology testing at 3 years interval is recommended. Endocervical sampling is preferred for women in whom no lesions are identified and for those with an inadequate colposcopy, but is acceptable for women with an adequate colposcopy and a lesion identified in the transformation zone. Because of the potential of overtreatment, the routine use of diagnostic excisional procedures such as loop electrosurgical excision for woman is unacceptable. There are different management guidelines for women aged 21–24 years, pregnant women, postmenopausal women and women aged 65 years considering exit from screening. For women aged 21–24 years with ASCUS, cytology alone at 12 month is preferred, but reflex HPV is acceptable. If HPV testing is negative, return for routine screening is recommended (cytology alone). If HPV is positive repeat cytology is recommended. Immediate colposcopy or repeat HPV testing is not recommended. Management guidelines for pregnant women with ASCUS are identical to those described guidelines for “general population”. Endocervical curettage is unacceptable. For pregnant women, who have no suspected CIN 2+ abnormality, postpartum follow up is recommended. Postmenopausal women with ASCUS should be managed in the same manner as women in the general population, except when considering exit from screening, when HPV negative ASCUS result should be considered as abnormal. Consensus guidelines is recommended repeat screening in year (co-testing preferred, but cytology is acceptable) (24).

European guidelines for quality assurance in cervical screening (2nd edition) (25) for the management of ASCUS are slightly different. For management for ASCUS three alternatives are recommended: HR HPV DNA testing, repeat cytology and referral for colposcopy. Reflex HR HPV testing is the preferred option when liquid based cytology is used. HPV positive cases should be referred for colposcopic evaluation. HPV testing can be repeated after 12 months when no CIN is found on colposcopy and biopsy. HPV negative women should be recommended repeat cytology after one year. A second acceptable option is repeat cytology. If it is negative, return to routine screening is recommended. If the first repeat cytology is again ASCUS, it should be referred for colposcopy. If any of the follow up smears is greater than ASCUS then referral colposcopy is advised. Referral for immediate colposcopy is another alternative, which many experts consider to be overmanagement. European guidelines recommended that clinicians should be guided by their own National guidelines (25).

In presented study group 56 % of samples of patients with ASCUS were negative for HR HPV. The HR HPV prevalence in our group with ASCUS cytology diagnoses was 44 %, which is in accordance with international literature (10, 21, 26). A significant decrease of positivity for HR HPV was detected after the age of 40 years (28 %). We assumed that this age will be crucial for the development of precancerous lesions within the Slovak population. Based on the results it can be assumed, that HPV infection in women of around 40 years of age is more likely to persist and precancerous lesion occurs. The hypothesis was indirectly confirmed also by the fact, that development of cervical cancer lasts approximately 10 – 12 years and the highest age specific incidence of cervical cancer in Slovakia is in 45 – 50 years of age (1).

The patients were classified also on the base of the intensity of positivity of the RLU/CO value. Our group showed significant positivity for HR HPV (above 100.1) recorded in 49 % for age group up to 40 years of age, in the age group of older than 41 years of age it was 17 % of patients. There are several studies analysed correlation between HC2 HR-HPV DNA test chemiluminescence intensity from cervical samples with follow-up histologic results. Jarboe et al (27) tested the hypothesis that HR-HPV positives with low positive RLU/CO ratios (1–10) had a lower prevalence of cervical intraepithelial neoplasia 2–3 on histologic follow up. RLU/CO ratios for 388 consecutive HR-HPV positive cervical cytological specimens interpreted as ASCUS were reviewed. Individual RLU/CO ratios were compared with outcome histologic diagnosis in cases with colposcopic follow-up and tissue sampling (biopsy and/or endocervical curettage; n = 236). An RLU/CO ratio of 10 or less was associated with a significantly lower prevalence of CIN2/CIN3 on biopsy outcome after result of ASCUS. Origoni et al (9) analysed a correlation between viral load, expressed as RLU/CO, and the severity of cervical lesions existed in 614 ASCUS cases. HC2 RLU/CO values, categorised into five classes, were correlated to clinical outcomes and statistically analysed. A significant correlation (p < 0.0001) was observed between increasing RLU values and the prevalence of high-grade CIN (CIN2/CIN3). The mean RLU/CO values were for negative cases 68.1, for low-grade 172.5 and for high-grade lesions 1020.0. CIN2/CIN3 ranged from 4 % for 0 < RLU/CO ≤ 1, to 5 % for 1 < RLU/CO ≤ 10, to 9 % for 10 < RLU/CO ≤ 100, to 23 % for 100 < RLU/CO ≤ 1000 and to 48 % when RLU/CO were > 1000 (p < 0.05). The HPV viral load in ASCUS cases significantly correlates with the severity of cervical cancer precursors. These data may have prognostic value, as they significantly correlate with the probability of a CIN2+ (11).

The number of patients with strong positivity for HR-HPV before the ages of 29 and 40 years the highest of all age groups, but we must not forget the fact, that the majority of HPV infections are transient and undergo clearance or become latent within 1–2 years.

In conclusion, 2012 American updated consensus guidelines of ASCCP for the management of women with ASCUS on cytology preferred reflex HPV DNA testing. HPV testing results determine the follow-up management of women, depending on the age. European guidelines are slightly different. Three options are recommended: HR-HPV DNA testing, repeat cytology and referral for colposcopy. Reflex HR-HPV testing is the preferred option when liquid based cytology is used. European guidelines recommended that clinicians should be guided by their own National guidelines. HPV testing results determine the follow up management of women. It is necessary to use clinically validated HPV
test and to make a distinction between HPV test performances for outcome measure precancerous disease vs outcome measure HPV presence. HC2 RLU/CO values seem to be a prognostic factor for detection of precancerous lesions of ASCUS patients. A significant decrease of positivity for HR-HPV was detected after the age of 40 years (28 %). We assume that this age will be crucial for the development of precancerous lesions within the Slovak population and thus the most suitable for HPV triage of ASCUS. As a refinement of that type of ASCUS triage we also recommend to add to the algorithm quantitative measurement of viral load in the specimens in the form of RLU/CO ratio. We assume that much lower prevalence of underlying CIN 2/CIN 3 in patients with low RLU/CO ratio (i.e. low viral load) may justify modification of the management of ASCUS in this group of women. Our statements need further studies and follow-up of much more patients.

According to our bibliographic research, this study is the first analysis of prevalence of HR-HPV of patients with cytological diagnosis of ASCUS and also the first attempt to introduce the viral load of HPV in the specimen in the management guidelines of precancerous lesions in Slovakia.

References


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