

HPV IMMUNOHISTOCHEMICAL TESTING AND CERVICAL DYSPLASIA

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Abstract

Background and aim. HPV (Human Papilloma Virus) infection represents a necessary condition for cervical carcinogenesis. The purpose of this study was to evaluate the efficiency of HPV testing using an immunohistochemical staining kit with implications upon both diagnosis and treatment of cervical intraepithelial neoplasia (CIN).

Methods. Seventy-nine patients and eighty-six controls were enrolled in the study. Each patient had completed a physical examination, gynecological examination with cervical sampling using a liquid-based cytology system and also colposcopy. The cervical samples were analyzed according to Bethesda terminology and HPV-HR immunohistochemical staining was performed. In all the patients with high-grade lesion a surgical excision procedure was performed followed by pathological examination of the specimen. The collected data were analyzed using statistical software.

Results. The colposcopic examination has detected acetowhite modifications of the cervical epithelium in 47% of patients with ASC-US (Atypical squamous cells of undetermined significance) in 71% of patients with LSIL (Low grade squamous intraepithelial lesion) and in 100% of patients with HSIL (High grade squamous intraepithelial lesion). The biopsy confirmed the diagnosis of LSIL in 27% of biopsy specimens in patients with ASC-US and in 79.99% of patients with LSIL respectively. In all patients with HSIL the diagnosis was CIN II or higher. The percentage of HPV-HR (Human Papilloma Virus – High Risk) positivity porportionally increased with the severity of cytological diagnosis: 30% in ASC-US, 42.86% in LSIL and 75% in HSIL patients. The sensitivity of detection of HPV-HR was 50% with CI 95% [17.45;82.55] for ASC-US, 77.77% with CI 95% [51.91;92.62] for LSIL and 81.81% with CI 95% [58.99;94.00] for HSIL.

Conclusion. HPV testing can be an important screening tool for cervical dysplasia. The HPV testing targeting high risk types is indicated for ASC-US and LSIL triage. The present work sustains the idea of introducing HPV testing as a primary screening tool for cervical cancer.

Keywords: HPV, cervical dysplasia, pap smear, immunohistochemistry, colposcopy

Introduction

Cervical cancer still represents an important factor of morbidity and mortality worldwide [1]. Cervical cancer represents the fourth most common cancer in women worldwide [2].

HPV (Human Papilloma Virus) represents a necessary condition for the development of cervical cancer, but most of the HPV cervical infections vanish without any treatment [3]. HPV has more than 120 types [4], but only fifteen high-risk types [5] are involved in carcinogenesis. The detection of a high risk type is associated with an increased risk of squamous cell carcinoma and adenocarcinoma [6-7] and need future surveillance [7]. The virus can be located either in the nucleus in an episomal state encountered in low grade cervical lesions, or integrated in the nucleus in high grade lesions [8]. Theoretically it is impossible to have cervical cancer without having an integrated HPV and the synthesis in large amounts of E6 and E7 oncoproteins that dysregulate the cellular cycle leading to neoplasia [8]. Therefore it is important to test for the presence of HPV in patients with abnormal cytologies.

Cervical cytology has been proved to be a powerful tool since its introduction, but has important limitations [9]. Cervical sampling using a brush, a sponge or an Ayre spatula takes the exfoliative cells from the surface of the cervix raising a cytological suspicion, but the final diagnosis can be made only after colposcopy, biopsy or on pathological specimen obtained after surgical removal. In order to avoid unnecessary treatment - it is known that a percentage of HSIL even can cure spontaneously in the absence of any treatment [8], - supplementary markers are needed.

Could HPV be such a marker? Unfortunately the exact role of HPV testing in both screening and therapy is far

for being established. The aim of this study was to analyze the exact place of HPV testing by immunohistochemistry staining in the diagnosis and treatment of cervical intraepithelial neoplasia.

Materials and methods

The present research was designed as a prospective case-controlled study including 79 patients and 86 controls. The patients were recruited at the 1st Clinic of Obstetrics and Gynecology Cluj-Napoca, Romania, between 1st of February 2010-30th June 2012. A written consent was signed by all the participants. For each patient a gynecological examination was performed, followed by a cervical sampling and then by a colposcopic examination with/without biopsy. The cervical sample was obtained using a brush (Cytobrush®) which after cervical sampling was immersed in a cytology vial (Cytofast®). The samples were stored in the freezer at 4-8°C. The cytology slides were obtained observing the producer instructions for the liquid based cytology system. The Pap smear was interpreted by the same cytologist using the Bethesda classification [10] with a Papanicolaou staining. Regarding their Pap smear results the patients were included either in study cases, or controls. For each patient the HPV-HR was performed using an immunohistochemical staining kit (ViroActiv HPV High Risk Kit®) according to the producer instructions. Image from a HPVHR patient is presented in figure 1, while immunohistochemical negative image is showed in figure 2. For a few cases the specific HPV genotype was detected using PCR types. All the HSIL cytological cases were confirmed by biopsy.

The statistical analysis was performed using Microsoft Excel 2007®.

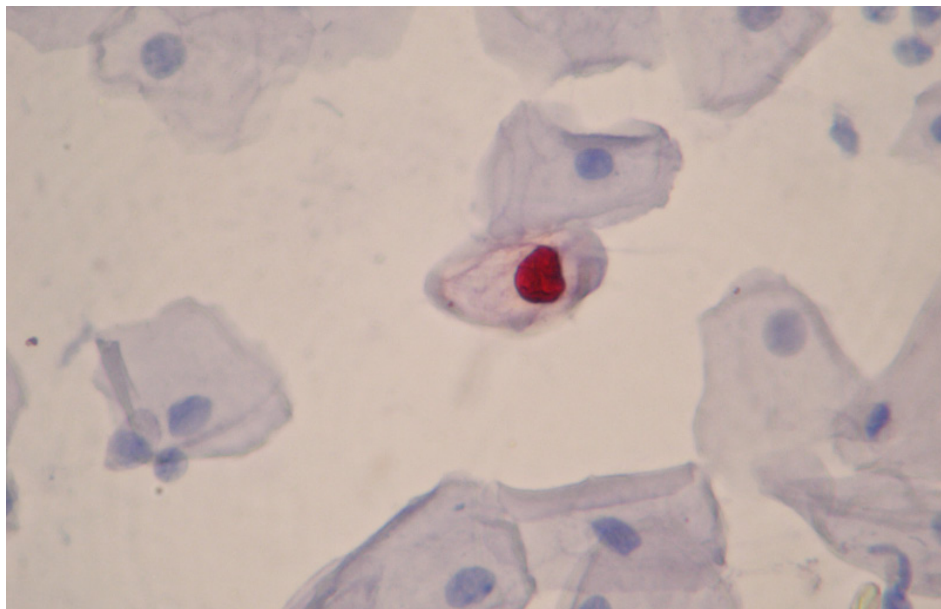


Figure 1. An image obtained from HPV-HRpositive patient (the nucleus is colored in red).

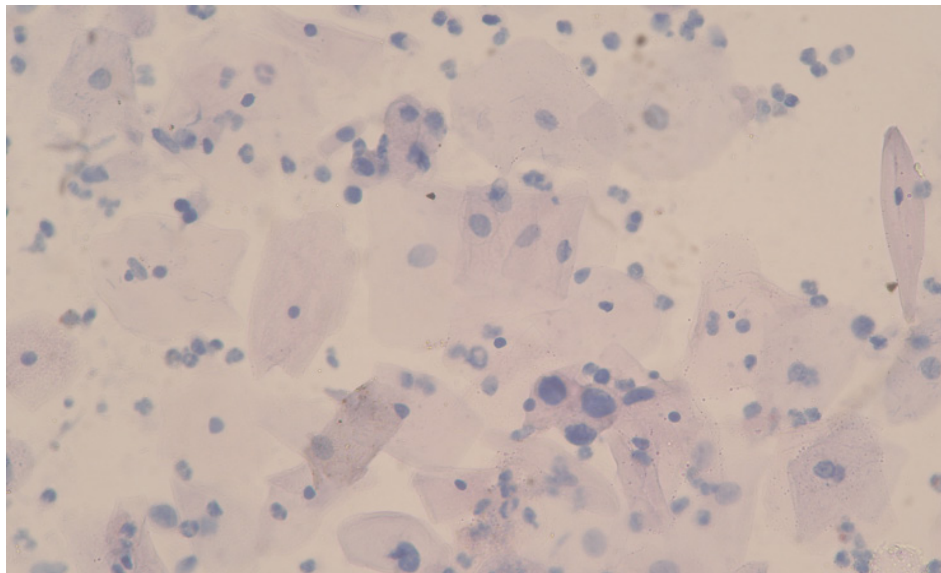


Figure 2. Image obtained from an HPV-HR-negative patient (none of the nuclei are colored in red).

Results

A total of seventy-nine cases and 86 controls were enrolled. The main cytological characteristics of the cases are presented in table I.

Both series contain comparable patients. The average ages for patients, controls and the main categories of the cervical dysplasia are shown in table II.

Due to the small number of cases the categories: AGC, ASC-H, CIS and suspicion of microinvasion have not been analyzed in the section below. The parameters associated to HR-HPV immunohistochemical testing are presented for the cytological diagnosis of ASC-US, LSIL

and HSIL in table III.

In 71% of LSIL and 47% of ASC-US an acetowhite or iodine-negative area was detected by colposcopy. The patient with ASC-US and LSIL were scheduled for follow-up.

All the patients with high-grade lesion had a surgical excision procedure (conization or hysterectomy) taking into consideration the extension of the lesion, future reproductive plans, age, comorbidities and last but not least, the patient’s choice. The high grade lesions were confirmed in all the cases by colposcopy and biopsy, and finally by the anatomical specimen.

Table I. Patients distribution according to the main classes of cervical dysplasia.

Cytological diagnosis	No of cases
AGC	1
ASC-H	4
ASC-US	7
LSIL	20
HSIL	41
CIS	5
Suspicion of microinvasion	1
TOTAL	79

Table II. Patients’ age.

Category	The average age (years)
LSIL	30.00
HSIL+CIS	40.00
Cases	36.80
Controls	37.00
Cases + controls	37.00

Table III. The sensitivity, specificity, negative predictive value, positive predictive values for ASC-US, LSIL and HSIL of HPV testing.

	ASCUS			LSIL			HSIL		
	Value	Lower Limit	Upper Limit	Value	Lower Limit	Upper Limit	Value	Lower Limit	Upper Limit
Sensitivity	50.00	17.45	82.55	77.78	51.92	92.63	81.82	58.99	94.01
Specificity	96.47	89.32	99.08	93.18	85.19	97.20	78.10	68.75	85.34
Positive Predictive Value	7.53	3.34	15.40	18.87	12.17	27.88	32.28	24.42	41.24
Negative Predictive Value	92.47	84.60	96.66	81.13	72.13	87.83	67.72	58.76	75.58

All the values from the table are expressed as a percentage.

Discussion

Exfoliative cervical cytology was discovered by Babeş [11] and later on by Papanicolaou [12]. The wide spread use of cytology as a primary screening tool in developed countries has led to an important decrease in the mortality and morbidity related to cervical cancer. Historically other methods have been proposed and used as screening tools for cervical cancer especially in low income countries such as VIA (visual inspection acetic acid), VILI (Visual inspection with Lugol's iodine) and cervicography and more recently Polarprobe [13] and speculscopy [14], but none of them succeed entering in the screening programs [13]. The above tools could be life-saving in low-income country where one might see a patient once in her lifetime and no cytology is available, especially if they are followed by see and treat therapeutic plan.

The recent advances in molecular biology have allowed a more comprehensive approach of the insights of the cervical neoplasia where HPV plays an important role [15]. HPV testing represents a new tool for the diagnosis of cervical neoplasia. HPV testing was used at the beginning in experimental studies and later on in clinical algorithm. HPV HR used as a screening tool has a better sensitivity (95%) than cytology (55%) in detecting high –grade lesions with comparable specificity (94% versus 97%) [16]. Therefore nowadays many voices are claiming the introduction of HPV test as the first screening tool for cervical pathology [17], our study supporting this idea.

In our series the sensitivity of HPV testing in ASC-US patients was 50% [17.45; 82.55] with a good specificity 96% [89.31;99.08] (table III). The value is comparable to that obtained by the ASCUS-LSIL Triage Study (ALTS study) – 55% on n=5060 [18]. For the HPV-HR positive cases the colposcopy is mandatory. The HPV-HR negative patient might have nuclear and cell shape modification due to reactive alterations, nonspecific or parasitic infection.

An even higher sensitivity was obtained for LSIL lesion 77.77% [51.91;92.62] with a very good specificity 93.18% [85.19;97.19] (table III). Due to the high proportion of positive patients the HPV testing is adding no benefit

to the cytology [18] alone in the diagnostic process, but it is very useful in the long term surveillance of these cases. All the patients with LSIL on cytology have been referred to colposcopy [19]. The detection of a high grade HPV is very important for the prognosis of the patient; a low grade HPV will probably have a self- limited evolution, but a LSIL HPV-HR positive lesion may also progress into a more severe high grade lesion, the HPV-HR being the most important risk factor for cervical cancer.

The sensitivity of HPV testing reached 81.81% [58.99;94.00] in HSIL (table III). Virtually there is no cervical cancer in the absence of HPV HR [3]. All the HSIL patients are referred for colposcopy and if a HSIL lesion is confirmed by colposcopy and biopsy an excisional procedure is proposed to the patient.

The high positiveness of HPV testing urges for the necessity of new tests more reliable for the evolution of these particular lesions. The new data suggest that the optimal triage tools beside referral for colposcopy is the dual staining p16/ki-67 that has a better sensitivity in detecting underlying cervical dysplasia sensitivity 81.8% versus 76.2% (dual staining/HPV testing) [20].

Immunohistochemistry has a comparable sensitivity for the detection of high-grade lesion with Hybrid Capture II: 81.81% versus 89.7% [21]; the sensitivity of PCR testing varies according to the primers that have been used ranging from 64% (MY09/MY11 primers – [22]) to 94% (GP5+/GP6+ primers [23]). From the clinical point of view the difference is acceptable taking into consideration the fact that the number of HPV copies in cervical intraepithelial neoplasia ranges between 1×10^5 to 1×10^7 [24].

Even if the sensitivity of immunohistochemistry is inferior to Hybrid capture II, it represents a valuable screening tool.

Conclusions

The HPV testing targeting high risk types is indicated for ASC-US triage and for LSIL evolution. No supplementary benefits are brought by HPV-testing for HSIL treatment, but HPV might be useful for postoperative

follow-up even in HSIL patients. LSIL HPV HR positive patients have a higher risk of progression. Colposcopy is a reliable method for ASC-US triage and also for the exclusion a high grade lesion in patients with LSIL but also for the confirmation of high-grade lesion. The golden standard for the diagnosis is the anatomical specimen obtained after conization or hysterectomy.

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