

THE IMPORTANCE OF IDENTIFICATION OF HIGH RISK DNA HPV IN EVALUATION OF PATIENTS WITH ASCUS

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Abstract

Infections with high risk HPV cause cervical cancer when they are present in cases with ASCUS (atypical squamous cells of undetermined significance). A majority of these anomalies regress spontaneously, but some others no. While cytology is an effective method for cervical screening, HPV test has the potential to refine this method and to determine which patients need clinic evaluation. HPV test is proved to be useful in patients with equivocal results as ASCUS which is approximately half of anomaly results. Studies have shown that patients with LSIL and ASCUS in cytology, who can hide a high grade of CIN, can be identified through HPV test. Utility of HPV test in the detection of the illness is higher in low grade anomalies. A positive HPV test is used as a triage for colposcopic and histologic examination when the test is positive, or for normal control of cervix when the test is negative, waiting that cytology of these patients regresses to normality.

Material and Methods: This is a study of 43 cases selected from 241 patients who have done Pap test during the period January 2011-january 2012. Including criteria were all cases with repeated ASCUS in Pap test and all patients were 17- 35 years old. HPV test was applied using method of Hybrid Capture -2 (QIAGEN). Type of high risk HPV identified from kit was HPV 16,18,31,33,35,39,45,51,52,56,59,68.

Results: 43 patients were selected according to the above criteria, 11 patients (25.6%) of these, resulted infected with one or more high risk HPV. So, one to 4 women are infected with high risk HPV. There is a significance difference between patients with ASCUS and high risk HPV positive and those with ASCUS and high risk HPV negative (d 50% CI 95% [40.5-59.5] $p < 0.01$). Colposcopic and histologic results of patients infected with high risk HPV were: 9 of patients (81.5%) results with CIN2 and 2 patients (18.2%) were with CIN3. Results of patients with high risk HPV negative were: 2

cases (6.2%) with CIN 2. So patients infected with high risk HPV have relative risk 13 time higher having CIN 2 in biopsy compared with those with high risk HPV negative (RR 13 CI 95% [10.5-14.5] $p < 0.05$).

Conclusion: Incorporation of HPV test in patients presented with repeated ASCUS is useful and helpful for the identification of patients with risk for developing cervical cancer. This test is very important for detection, prognosis and treatment of patients with ASCUS.

Keywords: ASCUS, LSIL, HSIL, high risk HPV etc.

Introduction

Despite high incidence and mortality, cervical cancer is totally prevented and curable, because of early detection of preinvasive and microinvasive status of illness. Many factors are accused for cervical cancer etiology, but oncogen effect of HPV is confirmed. Epidemiologic, clinic, molecular and histologic data has recently accused HPV infection as causative for cervical cancer, especially for epithelial cervical cancer. HPV is an exclusive epitheliotrophic virus which infects all kinds of epithelial surfaces of vulva, vagina, cervix anus etc (1).

Special types of HPV cause special pathology with different clinic sign. So HPV 16,18, etc cause cervical cancer and dysplasia. Over 130 type of HPV are identified and over 30 of these touch female's anogenital tract (4). Infection begins when the virus wins access in basal epithelial cells. Minor traumas that are often caused by sexual act let virus to enter in target epithelial cells or in the transformation zone of the cervix. Neoplastic transformation induced by HPV, is associated with integration of a part of DNA-HPV genom, causes overrelease of E6 and E7 oncoproteina. These two oncoproteins interfere with rules of cell's cycle, inhibiting tumoral suppressor and causes damage and genetic instability and malign transformation (2).

The most of the patients, almost 80% are cleaned

spontaneously from infection, within 1 year. But if infection is caused from high risk type virus, organism is cleared slowly or not (3). High risk HPV are responsible for high grade lesions.

In the end of the 20-th century, specialist of cervical pathologies, used Bethesda system for classification of Pap test results (8,9). This system determines these categories of citologic analysis:

- Negative Pap test,
- Benign reactive cells change,
- ASCUS (atypical squamous cells of undetermined significance), ASCUS-high,
- AGUS (atypical glandular cells of undetermined significance),
- LSIL (low grade squamous intraepithelial lesions),
- HSIL (high grade squamous intra epithelial lesions),
- Cervical Cancer.

This system helps physicians to determine sufficient treatment for the patients.

High risk-HPV infections cause cancer when they are present in cases with LSIL and ASCUS.

ASCUS result is considered to be a little anormal.

ASCUS means: atypical squamous cells of undetermined significance.

A banal infection of cervix uteri, often can cause ASCUS in advanced ages, while in young females ASCUS is caused by HPV infections (1,14). ASCUS category is reserved for lesions in which there is not a clear difference between reactive and neoplastic cells (10,11). It is important to know that in low cervical lesions are present low risk and high risk HPV.

Colposcopic management of patients with LSIL and ASCUS is controversial. Managing these patients means to determine if anomaly is significant and looking what will happen in the future. A majority of these lesions regress spontaneously, thanks to normal immune system, while a minority of cases persist and can progress to dysplasia (12,13).

While cytology is an effective method for cervical screening, HPV test has the potential to refine this methods determining which patients need clinical evaluation. HPV test is proved to be important in the *triage* of citologic ASCUS results, which are about half of Pap test results. If we applied a HPV test we can determinate if the anomaly is related or no with the presence of virus and the possible sources of future dysplasia. The studies have shown that patients with LSIL or ASCUS can hide a high grade lesion and these patients may be identified with HPV test (12).

Utility of HPV test in the detection of illness is higher in low grade anomalies (7).

A positive HPV test is referred as a *triage* for colposcopic and histologic examination, or for citologic examination of cervix when it is negative, waiting that cytology of these patients regress to normality. If HPV is not present, dysplasia is not present too, so it is not necessary to continue monitoring of these patients (6).

Colposcopy is very sensitive procedure for the detection of prevalent HSIL, but it must be adequate and evaluated from colposcopists with experience.

Many patients with ASCUS have normal colposcopic findings (11).

Conservative treatment consists of citologic follow up, but patients are not comfortable with waiting tactic for ASCUS outcome. On the other part Pap test is limited, because it depends from laboratory performance and has low sensitivity. So a immediately strategy is too attractive because it reduces the number of patients referred for colposcopic examination. This new strategy is Hibrid Capture test. This test evaluates quantitative load of HPV and detects oncogen or non oncogen type of HPV. This simple test to perform and positive results are associated with dysplasy. Combined test minimize colposcopic examination in normal woman.

AIM of this study is the evidention of the importance of DNA-HPV test in follow up of patients with ASCUS results in citologic examination.

Material and method

This is a study of 43 selected cases from 241 patients which are examined with Pap test during January 2011-January 2012, in Regional Hospital of Durres.

Inclusive criteria are: all women with repeated ASCUS in Pap test and women 17-45 years old.

HPV test is applied using method of Hibrid Capture-2 (Qiagen). High risk HPV type identified from kit is HPV 16,18,31,33,35,39,45,51,52,59,68.

Results

51 of patients (21.1%) result with ASCUS (categorized according to the Bethesda system).

After 6 months only 43 patients (17.8%) of the result again with ASCUS, but this difference of ASCUS result is not significant in percentage $d=3.3$ CI 95%] -3;10 [$p>0.01$.

Table nr.1 Cases with ASCUS according to the high risk HPV presence

	ASCUS	
	Nr	%
High risk HPV +	11	25.6
High risk HPV -	32	74.4
Total	43	100

Table one expresses spread of cases with ASCUS according to the presence of high risk HPV. 11 above patients (25.6%) are infected with one or more high risk HPV. So, one in four women are infected with high risk HPV. There is a significant difference between percentage of persons infected with high risk HPV and

the others that are not infected (d =50% CI 95%) 40.5-59.5[p<0.01).

Table nr.2 Cases according to the histopathologic examination

High risk HPV +				High risk HPV-			
CIN 2		CIN 3		CIN 2		CIN 3	
nr	%	nr	%	nr	%	nr	%
9	81.8	2	18.2	2	6.2	-	-

Table nr.2 expresses spread of cases with ASCUS and the presence of one or more high risk HPV virus, according to the histopathologic examination.

We applied colposcopic and than histopathologic examination according to the fact that biopsy is considered "golden standard". In histopathologic examination 9 of cases (81.8%) result with CIN2 and 2 of cases (18.2%) result with CIN3.

9 patients with CIN2 and 1 patient (nullipare) with CIN3 were treated with LEETZ (low electrical excision of transformation zone). While we applied total hysterectomy to the other patient with CIN3.

32 of patients (74.4%) included in this study are not infected with high risk HPV. Only 2 of these patients (6.23%) had anormal transformation zone with mosaic finding, punctuation and aceto white epithelial surfaces. Exocervix surface biopsy results CIN2. We applied LEETZ to these patients.

Patients resulted with high risk HPV positive have relative risk 13 times higher (having CIN2 in biopsy) compared with patients resulted with high risk HPV negative (RR=13 CI 95%) 10.5 -14.5[p< 0.05).

According to the histologic confirmation of CIN 3 there is not odds difference to have CIN 3 in histologic

examination, although of HPV positive or negative results.

Discussion

Management of patients with ASCUS is a common problem of physicians, because a little but important number of these patients results' with HSIL (high grade intraepithelial lesions) or even with cervical cancer.

Utility of HPV test in low grade anomalies is important to detect early malignant cervical pathologies (7).

In general patients with ASCUS have trends to result negative for the presence of high risk HPV.

Patients who result negative for high risk HPV have low risk to develop CIN2. These patients go on with normal follow up protocol, with continued Pap test after 6 months, until being totally negative. So routine monitoring of these patients is not necessary.

While patients with ASCUS and positive HPV test, need follow up with colposcopic examination and directed biopsy, because they have high risk for CIN 2 in histopathologic examination.

HPV test is used as a *triage* or for colposcopic and histopathologic examination when test is positive, or for cervix surveyance when it is negative, waiting to be citologically normal.

Follow up with HPV test is optimal protocol in patients with ASCUS repeated in cytology, because of its high sensitivity and negative predictive value.

Conclusion

Incorporation of HPV test in patients with ASCUS persistent is useful and helps the determination of high risk patients for having cervical cancer. So, this test is an important component of protocol for detection, prognose and treatment of patients with ASCUS.

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