

The importance of primary screening in early detection of cervical dysplastic lesions

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Abstract

The cervical cancer prevention involves the identification and the eradication of the precursor and invasive lesions, early, through cytological screening. The lack of screening determines an important increase of the cervical cancer rates in socially disadvantaged women. The cytological screening detects most of the cervical neoplasia in premalignant or early malignant stages, unknown stages of malignancy when evolution under treatment is optimal. The objective of the study was the early detection of the cervical dysplastic lesions with carcinogen potential, through cervical cytology and association with the human papillomavirus (HPV) testing. Regardless of the class of intraepithelial lesions, the most affected patients were aged between 30-39 years. We have found in small numbers, the existence of positive cases for Chlamydia trachomatis and Mycoplasma genitalium, pathogens involved in the progression of HPV infection. The treatment of the cervico-vaginal infections associated with HPV cervical lesions was specifically targeted, depending on the result of the antibiotic cultures. The cervical cytology examination after the specific treatment showed a decrease in the severity of the cytodiagnostic. Following the cytology study based on the Bethesda cytology, the low-grade squamous intraepithelial lesion class numbered more than half of the patients, 56% followed by the atypical squamous cell (ASC) of undetermined significance, high-grade squamous intraepithelial lesion and ASC which cannot exclude a high-grade lesion categories. HPV testing identified the low-risk HPV types 61 and 6, while the high-risk HPV predominated in types 16 and 18. The HPV testing in the primary screening may lead to early diagnosis of high-grade neoplastic lesions. The women with multiple risk factors for cervical cancer require inclusion in screening programs and the setting of prophylactic measures.

Keywords: cervical-vaginal cytology, epithelial cell abnormalities, HPV testing

Introduction

The cervical cancer prevention involves the identification and the eradicating action of the precursor and invasive lesions, early through cytological screening. It is estimated that half of the women diagnosed with cervical cancer did not perform cytological screening. The lack of screening determines the important increase of the cervical cancer rates in socially disadvantaged women. The cytological screening detects most of the cervical neoplasia in premalignant or early malignant stages, unknown stages of malignancy when evolution under treatment is optimal. In the countries with an organized screening program, there was a steady decline of 60-70% in the incidence and mortality rates of cervix cancer^(1,2). The specificity of the Papanicolaou test is high, approximately 98%, but its sensitivity is lower and variable. Although the incidence of the squamous cell carcinoma continues to decline, the incidence of the adenocarcinoma has increased, especially in women less than 50 years⁽³⁾. The adenocarcinomas and the adenosquamous carcinomas are more than 20% of the cervical cancers.

The combination of human papillomavirus (HPV) with the cytology testing increases the sensitivity of the Papanicolaou test for high-grade squamous intraepithelial lesion (HSIL) from 50-85% to 100%⁽⁴⁾. The lack of sensitivity in the detection of the cervical adenocarcinoma

encountered in the traditional cytology is in favor of the HPV testing in screening⁽⁵⁾. Some authors are in favor of the use of HPV testing in the absence of the cytology as primary screening for the cervical cancer prevention⁽⁶⁾. The HPV testing is twice as sensitive as the Papanicolaou test and leads to the early detection of high grade malignancies, but has a lower specificity, especially in young women^(7,8,9).

The objective of the study was the early detection of the cervical dysplastic lesions with carcinogen potential, by cervical cytology and association with the HPV testing, considering the high incidence of the cervical cancer in the world and in our country.

Methods

The study was conducted between September 2017 and May 2018 and it was performed on 122 selected patients, aged between 18 and 65 who had exfoliative cervico-vaginal cytology for the microbiological study and cytotumoral test. The HPV testing was performed at all the patients. The samples of the vaginal discharge were taken outside the menstrual period to avoid contamination with blood. The patients were informed before harvesting 24 to 48 hours, not to undergo sexual relaxation, not to use intravaginal pads or local contraceptives, not to perform vaginal irrigation to make cell leakage abundant. We recommended the treatment of

the colpocervicitis at the previous visits before harvesting the cervico-vaginal discharge.

The secretion harvesting required visualization of the cervix for the detection of macroscopic lesions and the identification of the squamous-cylindrical junction and it was performed with the cervical brush, which, by clockwise rotation, collects the cells from the endocervical and exocervical canal due to longer bristles at the middle of the device and shorter on the flanks.

The microbiological examination of vaginal discharge has established the diagnosis for the presence of certain microorganisms and was accompanied by the antibiogram. The results of the cyto-tumoral examination were interpreted using the Bethesda system. HPV genotyping was based on the amplification of the target deoxyribonucleic acid (DNA) by the nucleic acid hybridization polymerase chain reaction technique.

Results

In the 30-39 age group, the majority of the investigated patients were summed up to 50 cases (41%), that being followed by almost the same frequency by the age group of 40-49 years - 29 cases (24%), and the 20-29 age group - 28 cases (23%). Beginning with the age of 50, there was a progressive decrease in the number of cases per age group, reaching 1% in the group of patients over 60 years - 1 case. The group of youngest patients under the age of 20 has summed up 4 cases (3%) (Table 1).

From a microbiological point of view, the vaginal secretion was sterile in 57 patients (47%) and there were various microbiological infections in 65 patients (53%). In these cases, the microbiological spectrum was variable, only 11 patients presenting only one germ infection (9%). In most of the cases, in 41 (34%), the vaginal discharge was positive for 2 microbial germs. The rarest was the polymicrobial infection with more than three

Table 1 Distribution of patients by age group

Age group	<20 years	20-29 years	30-39 years	40-49 years	50-59 years	>60 years	Total
Nr. cases	4	28	50	29	10	1	122
Percentages (%)	3%	23%	41%	24%	8%	1%	100%

Table 2 Distribution of patients by number of microbiological infections

Number of microbiological infections	0	1	2	>3	Total
Nr. cases	57	11	41	13	122
Percentages (%)	47%	9%	34%	10%	100%

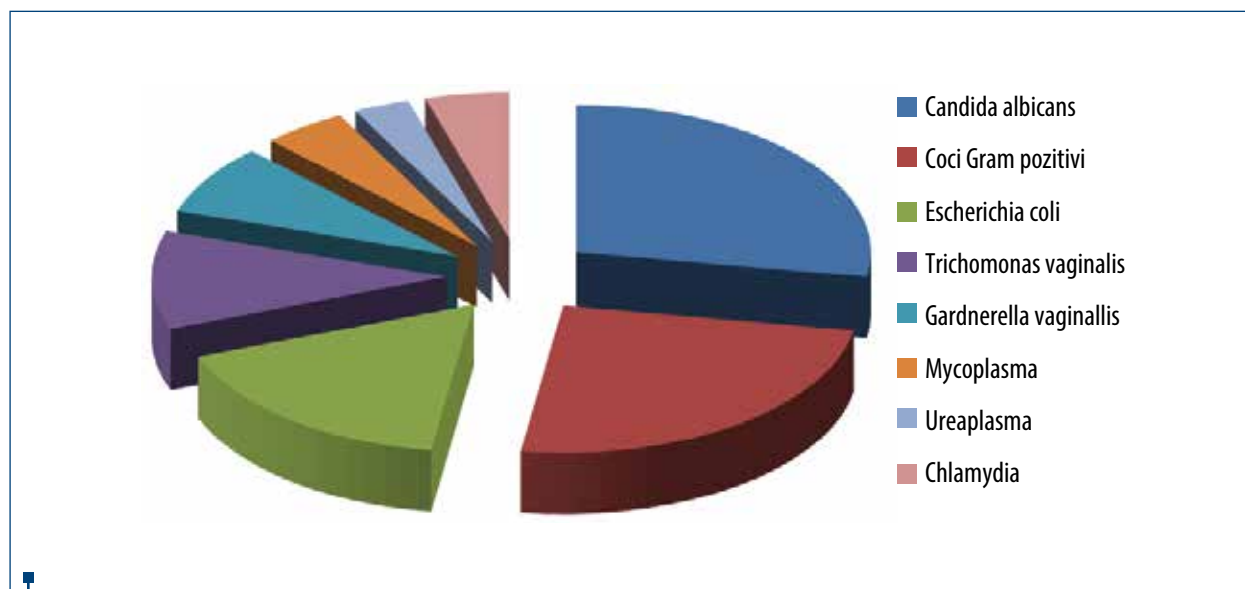


Figure 1. Distribution of patients by microbiological infections

germs, 13 patients (10%) (Table 2). Among the germs involved in the genital infections associated with HPV, the most common was *Candida albicans*, 18 patients (28%), followed by Gram positive (*Streptococcus* spp., *Staphylococcus* spp.) in 16 patients (25%) and *Escherichia coli* spp. cases (17%). *Trichomonas vaginalis* was identified in 7 patients (11%), commonly associated with *Gardnerella vaginalis*, 5 cases (8%). The pathogenic germs that can influence the evolution of HPV in female genitalia were *Mycoplasma genitalium*, 3 cases (4%), 2 cases of *Ureaplasma* (3%) and 3 cases of *Chlamydia trachomatis* (4%) (Figure 1).

The cytotumoral study revealed the predominance of low-grade squamous intraepithelial lesion (LSIL) type, 68 patients (56%), followed by atypical squamous cell of undetermined significance (ASC-US) 28 cases (23%), 18 cases (15%) high-grade squamous intraepithelial lesions (HSIL), 4 cases (3%) ASC-cannot exclude a high grade lesion (H), 3 cases (2%) squamous carcinoma and 1 case (1%) of atypical glandular cells (Table 3 and Figure 2).

In the HPV testing, 44 patients (36%) did not present infection, while 78 patients (64%) were infected. The most common type of low-risk HPV was type 61 with 12 cases (10%), followed by type 6 with 9 cases (7%) and other low risk types - 81, 11, 54 with 35 cases (28%). The most common type of high-risk HPV was type 16 with 8 cases (7%), followed by type 18 with 6 cases (5%) and other high risk types - 33, 52, 45 with 8 cases (7%) (Table 4 and Figure 3).

Discussion

The age average for the diagnosis of cervical cancer in the United States is 48 years, 10 years more than the cervical intraepithelial neoplasia⁽¹⁰⁾. Any cancerous lesion initially passes through the intraepithelial phase, where, if detected, it can be treated in most of the cases.

The microbiological study of the cervico-vaginal smears identified 53% of patients who had at least one genital infection. The genital infections cause an imbalance of the cervical-vaginal flora, resulting

Table 3 Distribution of patients by results of the cervical-vaginal cytology test

Cytology	ASCUS	ASC-H	LSIL	HSIL	SC	AGCs	Total
Nr. cases	28	4	68	18	3	1	122
%	23%	3%	56%	15%	2%	1%	100%
HPV	-	HPV 16	HPV 18	HPV-H	HPV 61	HPV 6	HPV-L
Nr. cases	44	8	6	8	12	9	35
%	36%	7%	5%	7%	10%	7%	28%

HPV= human papillomavirus; ASCUS= atypical squamous cell of undetermined significance; ASC-H= atypical squamous cell cannot exclude a high-grade lesion; LSIL= low-grade squamous intraepithelial lesion; HSIL= high-grade squamous intraepithelial lesion; SC= Squamous carcinoma; AGC= atypical glandular cells

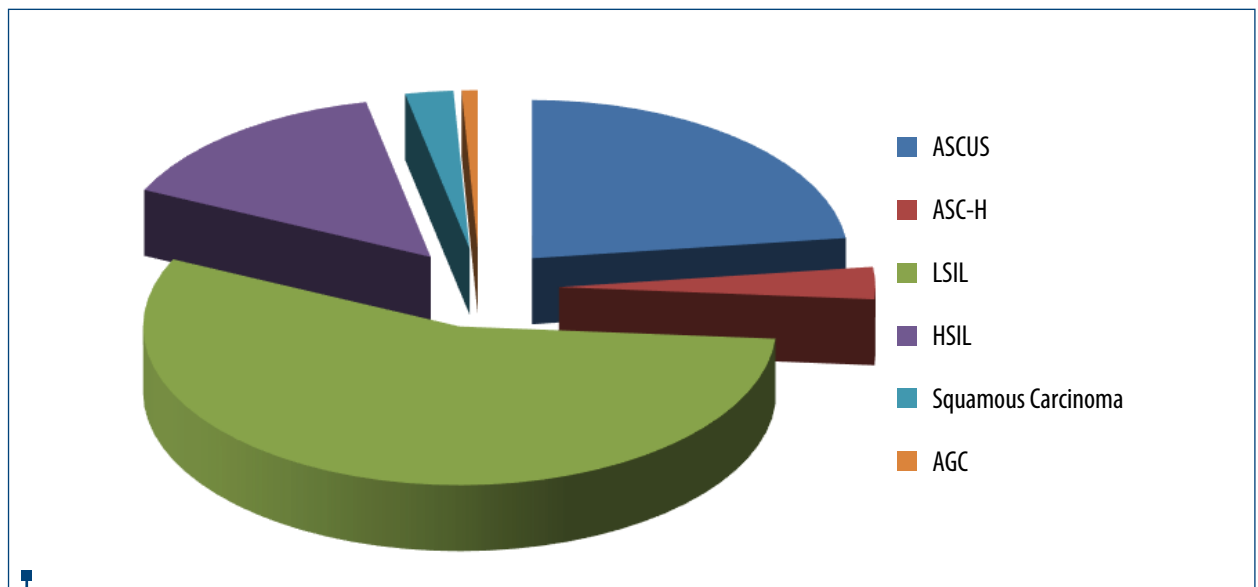


Figure 2. Distribution of patients by results of the cervical-vaginal cytology test

Table 4 Distribution of patients by type of HPV

HPV	Uninfected	HPV 16	HPV 18	HPV-H	HPV 61	HPV 6	HPV-L
Nr. cases	44	8	6	8	12	9	35
%	36%	7%	5%	7%	10%	7%	28%

HPV= human papillomavirus

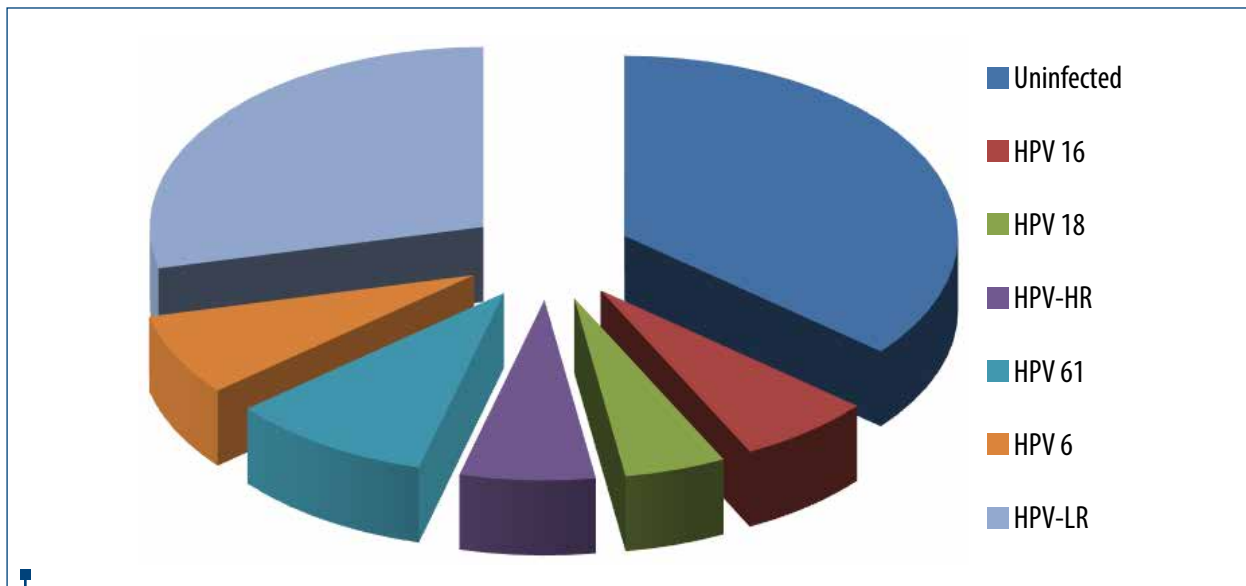


Figure 3. Distribution of patients by type of HPV

in increased susceptibility to the action of the other carcinogenic factors in the genesis of dysplastic lesions⁽¹¹⁾. The vaginal infections have not a direct effect on the onset of the intraepithelial lesions, but their presence causes a decrease in the cervical mucosal defense. Of particular importance is HPV co-infection with *Chlamydia trachomatis*, which by inhibiting cellular apoptosis and facilitating the integration of DNA-HPV into the cells, amplifies the severity of the HPV-initiated transformations^(12,13). It appears that the serotype G of *Chlamydia trachomatis* is associated with the development of severe dysplastic lesions and the development of cervical squamous carcinoma⁽¹⁴⁾. Another study has shown an increased risk of developing the cervical carcinoma in smokers who have been associated with HPV 16 infection and *Chlamydia trachomatis* infection⁽¹²⁾.

The category of ASC is considered to be the most common atypical epithelial cytology by most of the laboratories⁽¹⁵⁾. The obvious predominance of the LSIL cytoskeleton class versus HSIL suggests that the progression to high-grade lesions is rare, whereas HPV infection remains generally transient, aspect that was reported in similar studies^(16,17). In short periods of time the LSIL type of lesions regresses and only a small part progresses to high-grade lesions⁽¹⁸⁾.

The most common HPV types identified in relation to genital warts are 6 and 11, while types 16, 18, 45 and 31 are most commonly involved in the development of the high grade intraepithelial squamous lesions with important potential of malignancy^(11,19-22). The highest incidence of low-risk HPV types was in types 61 and 6, which were associated with the occurrence of plain or acuminate condylomas or associated with mild dysplasia. High risk HPV is the major etiological factor of pre-invasive and malignant lesions in the cervix.

Type 16 is the main type associated with the presence of the neoplasia being present in 40-70% of the invasive cancers in the world^(16,23-25). The prevalence of HPV type 18 is lower but is present in 25% of squamous carcinomas and even higher in the cervical adenocarcinomas and in the adenosquamous carcinomas⁽²⁶⁾. The strategy for monitoring a HPV positive test by cytology with or without genotyping for HPV 16 and 18 is still under further studies⁽²⁷⁾.

Conclusions

Regardless of the class of intraepithelial lesions, the most affected patients were aged between 30-39 years. We have found in small numbers, the existence of positive cases for *Chlamydia trachomatis* and *Mycoplasma genitalium*, pathogens involved in the progression of

HPV infection. The treatment of the cervico-vaginal infections associated with HPV cervical lesions was targeted, depending on the result of the antibiotic cultures. The cervical cytology examination after the specific treatment showed a decrease in the severity of the cytodiagnostic.

Following the cytology study based on the Bethesda cytology, the LSIL class numbered more than half of the patients, 56% followed by the ASCUS, HSIL and ASC-H categories. HPV testing identified the low-risk HPV

types 61 and 6, while the high-risk HPV predominated in types 16 and 18. The HPV testing in the primary screening may lead to early diagnosis of high-grade neoplastic lesions.

The women with multiple risk factors for cervical cancer require inclusion in screening programs and the setting of prophylactic measures. ■

Conflict of interests: The authors declare no conflict of interests.

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