INTRODUCTION

Cervical cancer is an important public health problem worldwide, considering that this condition is the second cause of mortality through cancer in women, following breast cancer, causing yearly over a quarter of a million deaths globally.\(^1,2\)

In developed countries, where the screening programs using the cytological examination Babes-Papancioloacou are correctly and completely implemented, the detection and treatment of asymptomatic precancerous lesions, which are curable in almost 100% of the cases, is possible.\(^3\) Still, five of six women with cervical cancer live in developed countries and 80% of them are diagnosed in a late stage of the disease.\(^4\)

The persistent infection with approximately 15 carcinogen genotypes of human papillomavirus (HPV) determine practically all the cases of cervical neoplasia, as well as precancerous lesions and in situ carcinoma.\(^5\)

The vaginal ecosystem is a complex and dynamic system of microorganisms which interact with local factors to maintain the balance of this environment.
The endogenous microflora comprises a variety of microorganisms of which we can mention aerobic, anaerobic and optionally anaerobic ones. Of these, Doderlein bacilli (Lactobacillus acidophilus) represent a special category in the vaginal ecosystem, having a role in maintaining the acidity that characterizes the vaginal secretion in physiological conditions and plays a vital role in avoiding the colonization of the vagina with pathogenic organisms, including those which produce bacterial vaginitis and candidiasis, infections of the urinary tract or sexually transmitted diseases. Aside lactobacilli, in the normal vaginal secretion there are also a number of aerobic bacteria of the diphteroid type, Streptococcus, Staphylococcus or anaerobic (Peptococcus, Peptostreptococcus, Bacteroides speciae), as well as mycolasma and isolated strains of Candida. Any change of the local conditions of the vagina (pH modification, temperature, hormonal impregnation etc.) can lead to colonization with possible or really pathogenic germs and the appearance of vulvo-vaginal infections.

It is also known that altering the vaginal biocenosis, through the presence of infectious factors at this level, is involved in the initiation of some cellular changes at local level, which in time can lead to a lesional cervix; there are studies which show a high incidence of cervical dysplasia in patients with bacterial vaginitis.\(^7\)\(^9\)

**MATERIALS AND METHODS**

This paper presents a retrospective study done on a cohort of 113 patients between the ages of 18 and 79 years, which presented to the Bega Clinical Hospital of Obstetrics and Gynecology, Timisoara, in the interval 01.07.2008-15.03.2009. The study included patients which had pathological cytologies, and for which we have monitored the association of infectious factors aside the infection with HPV, through bacteriological samples and cultures from the vaginal secretion.

All the patients included in the study had a Babes-Papanicolaou cytology done, and samples were taken from the vaginal secretion for HPV DNA testing, as well as for other cultures and bacterial tests in order to detect the possible associated infectious factors.

For the detection of the HPV infection the samples have been taken through brushing the cervix and have been tested for HPV DNA, through the polymerization chain reaction (PCR), for the following HPV genotypes:

- High-risk HPV strains – 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82 – of these, strains 16, 18, 31 and 45 have a very high oncogenic risk;
- Low-risk HPV strains – 6, 11, 40, 43, 44, 54, 70;
- Possible high-risk HPV – 26, 53, 66;
- Other types of HPV – 69, 71 and 74.

Also there were samples where the viral DNA was amplified, but the genotype involved could not be detected.

**RESULTS**

The cyto-bacteriological examination performed through the Babes-Papanicolaou cytology has provided the following results: out of a total of 113 samples, 74 have been classified LSIL according to the Bethesda classification (analogous to CIN I and cellular changes associated with HPV), 22 samples matched the HSIL changes, analogous to CIN II and CIN III, and 17 samples have been classified as ASCUS - atypical squamous cells. (Fig. 1)

![Figure 1. Distribution of the results obtained at the cytobacteriological test in the studies cohort.](image)

The HPV DNA results have shown the following: 30 samples have been negative and 74 samples (71%) have been positive for the infection with different HPV genotypes. Among the positive samples, in 15 cases we detected the simultaneous infection with multiple strains of HPV.

The detection of other infectious factors associated with the HPV infection, possibly involved in the appearance of the lesional cervix, has been done through the sampling of cultures and bacteriological tests from the vaginal secretion. In a large number of cases we have found the disturbance of the vaginal biocenosis with a variety of infectious factors. From the total number of 113 bacteriological tests, in 27
cases we have found the replacement of the normal vaginal flora formed by lactobacilli Doderlein, with a pathogenic flora represented by Gardnerella vaginalis, species of Candida, Trichomonas vaginalis or Lepthotrix vaginalis.

Thus, as presented in Table 1, where we have represented the distribution of results of the bacteriological exams of the vaginal secretion, the most frequent infection was with Gardnerella vaginalis (12 cases), followed by vaginal candidosis (present in 8 cases) and the associated infection with both Gardnerella vaginalis and Candida albicans which was detected in 3 cases.

Table 1. The results of the bacteriological testing of the vaginal secretion.

<table>
<thead>
<tr>
<th>Positive bacteriological tests</th>
<th>27 (23.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardnerella vaginalis</td>
<td>12 (10.6%)</td>
</tr>
<tr>
<td>Candida</td>
<td>8 (7.1%)</td>
</tr>
<tr>
<td>Gardnerella vaginalis associated with Candida</td>
<td>3 (2.6%)</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Lepthotrix vaginalis</td>
<td>2 (1.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative bacteriological tests</th>
<th>86 (76.1%)</th>
</tr>
</thead>
</table>

The cultures taken from the vaginal secretion have shown the alteration of the vaginal biocenosis with a wide variety of infectious agents, of which the most frequently involved microorganisms were: Escherichia coli, Streptococcus beta haemolyticus, Klebsiella, Enterococcus faecalis, Staphylococcus aureus, Enterococcus spp. and Enterobacter aerogenes. Of the 113 cultures sampled, in 36 cases several pathogenically species developed, and 77 cultures have been negative. In Table 2 is presented the distribution of results obtained from the cultures sampled from the vaginal secretion.

We need to mention the fact that five patients with a positive culture had associated the presence of Gardnerella vaginalis, detected at the bacteriological test, and three patients with a positive culture has associated the presence of candidosis. Also it is very important to observe that for 31 patients with positive bacteriological test or culture, no lactobacilli Doderlein were detected at the bacteriological test. The study of the presence of infectious factors at the level of the vaginal tract has shown the alteration of the vaginal biocenosis in 47 of the patients in the cohort, in 13 cases there were associations between different bacterial species.

Table 2. The distribution of results obtained in the cultures taken from the vaginal secretion.

<table>
<thead>
<tr>
<th>Positive cultures</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>5</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>4</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>3</td>
</tr>
<tr>
<td>Associations between different bacterial species</td>
<td>3</td>
</tr>
<tr>
<td>Other bacterial species</td>
<td>7</td>
</tr>
</tbody>
</table>

The corroborated of the data obtained in the tests performed to detect the pathogenic microbial flora of the inferior vaginal tract, with the those from the cyto-diagnostic examination of the cervix, has shown the frequent association of the alteration of the vaginal ecosystem with modification of the Babes-Papanicolaou cytology classified HSIL and an association in a lower percentage with LSIL modifications or ASCUS. In other words, the presence of the infectious factors in the vaginal secretion was detected in 14 of the 22 patients with HSIL at the cyto-diagnostic examination, 29 patients with LSIL and only 4 patients with a cytology classified ASCUS. (Fig. 2)

![Figure 2. Percentual distribution of the presence of the infectious factors, according to the cyto-diagnostic result.](image)

DISCUSSIONS

Cervical intraepithelial neoplasia (CIN) and cervical cancer represent a major cause of morbidity and mortality for women and was several times suggested that bacterial vaginitis can play an important part in the development of CIN. It has been proven that the presence of HPV infection in the cells of the cervix is a major factor in the initiation of the cervical...
dysplasia processes, but not all patients infected with HPV develop cervical lesions, this indicating that there are additional factors which together lead to the initiation of displastic transformation processes. Moreover there are studies which have shown a greater incidence of displastic changes of the cervix among the patients with an alteration of the vaginal ecosystem. Bacterial vaginitis is a polymicrobial syndrome that affects the inferior genital tract, characterized by the replacement of the normal vaginal flora that comprises lactobacilli, with pathogenic flora mostly Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum, certain species of Mobiluncus or Prevotella, Chlamydia as well as other anaerobic organisms. Moreover, some Gardnerella vaginalis biotypes are more frequently associated with bacterial vaginits with clinical manifestation. In our study, the presence of the infectious factors in the inferior genital tract was detected in greater percentage of cases (41.6% of the patients included in the cohort had positive bacteriological tests or cultures), this fact proving the possible involvement of the alteration of the vaginal biocenosis, together with the HPV infection, in the development of the lesional cervix. Furthermore, in the cohort with HSIL cytology we saw the greatest percentage of positive samples for pathological bacterial flora (63.6% of the patients with HSIL cytology had a positive bacteriological test or culture), the lowest incidence of vaginal ecosistem alteration was observed in the patients with ASCUS cytology (23.5%). Of the 74 patients with a LSIL cytology, 29 cases have shown the presence of the infectious factors through the tests done (39.2%). The most frequent microorganisms involved in the alteration of the vaginal biocenosis at the patients in our cohort were: Gardnerella vaginalis (15 patients), Candida (11 patients), Staphylococcus aureus (8 patients), Escherichia coli (6 patients). What is more, 13 patients in the cohort (11.5%) have presented an alteration of the vaginal ecosistem through the association of two or more pathogenic agents.

Finally, it would be important to emphasize the fact that in what prevention and early detection of the premalignant and malignant cervical pathology are concerned, all women should have cytologies done every six months or at least once a year. Furthermore, the periodical colposcopic examination of the cervix should also be performed.

CONCLUSIONS

The increased incidence of infectious factors in patients with pathological results of the cytology shows that the statements, according to which these factors have an important part in the initiation of the displastic processes of the cervix together with HPV, have a real basis. Nevertheless, in the future, studies are needed to verify if this association exists, as well as research that helps in understanding the intimate mechanisms which initiate the dysplastic process. Until then, though, due to the frequent association of the pre-neoplastic associations with chronic vaginal inflammations, we deem extremely important the regular check-up with the detection and treatment of the genital infections, as a method of prevention of the appearance of the cancerous lesions.

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REFERENCES
