Vaginal Candidiasis & Lactobacillus flora

Original Article

The relationship between symptomatic vaginal Candidiasis and Lactobacillus flora, using methenamine silver staining method

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ABSTRACT

Objective: To determine whether there is a relationship between the presence of Lactobacilli and the growth of Candida.

Material and Methods: Out of 3762 vaginal smears examined cytologically in Al-Zahra Hospital, Isfahan from 2001 to 2006, 242 patients were diagnosed as having Candida. Among them, 215 satisfactory smears of patients who were normally menstruating, were subsequently selected for evaluation. For determining the flora type and maturation index, the vaginal parts of VCE smears were studied. The control group included 200 patients who had normal menstrual cycle, satisfactory smears and no infectious agents. Smears were stained with Methenamine silver staining method for determining Lactobacillus flora. Lactobacillus positive groups were classified into three types: Type 1: Lactobacillus overgrowth flora, Type 2: Lactobacillus flora, Type 3: mixed flora. To evaluate hormonal status, the cellular maturation index (CMI) was also determined. Data were analyzed by SPSS software using Chi-square and T-tests.

Result: Lactobacilli prevalence rates in Candida positive and negative groups were 194 cases (90%) and 175 cases (87.5%), respectively (p=0.7). In Candida positive group, Lactobacillus flora types included: Type I; (9%), type II; (84%) and type III; (7%) (p<0.0001). Superficial,
Intermediate and parabasal cells ratios were demonstrated in term of CMI. There were no significant differences in CMI between Candida positive and negative cases.

**Conclusion:** These results suggest that Lactobacillus does not have a protective role against vaginal Candidiasis. Considering the maturation index pattern, we can conclude that progesterone plays an important role in Candida infections. (Rawal Med J 2009;34:82-85).

**Key words:** Candida, lactobacillus, vaginal smears, methenamine silver.

**INTRODUCTION**

Vaginal Candidiasis (VC) is the second-most-common form of vaginitis. A subgroup of women with VC develops recurrent symptomatic episodes of vaginitis or even chronic VC.¹ Some 20% of healthy symptomless women during their reproductive premenopausal stage develop VC with a dramatic decrease after menopause.² Candida infections are prone to occur when the progesterone level is high, as in pregnancy or when contraceptive hormones are used. Infections are also common when bacterial equilibrium is disturbed, e.g. by broad-spectrum antibiotics or chemotherapeutic drugs. However in most patients, a precipitating cause is not found.³ It has been suggested that the normal vaginal bacterial flora and specifically Lactobacillus species play a critical role in the prevention of vaginal infections and the transmission of pathogens responsible for sexually transmitted diseases.⁴ There is a controversy on the relation between Lactobacillus flora and VC. In some studies, it has been considered as a protective barrier by producing of hydrogen peroxide (H₂O₂) and blocking epithelial receptors for blastospore⁵,⁶ and others considered it as an inducing factor.⁷,⁸ In this study, we compared the presence of Lactobacillus flora and patterns of this flora between candida positive and negative cases.

**MATERIAL AND METHODS**

The study was carried out in cytology and gynecology departments of Al-Zahra Hospital, Isfahan, Iran from 2001 to 2006. A total of 3762 vaginal smears were examined cytologically and 242 patients were diagnosed as having Candida (64.33 per 1000). Among symptomatic patients with normal menstruation, 215 satisfactory smears were selected for evaluation. The control group included 200 patients who had normal menstrual cycle, satisfactory smears and no infectious agents. We used VCE or Triple-Smear method of sampling in which material from vaginal pool, cervical transformation zone and endocervical canal was smeared in single slide.
For determining the flora type and maturation index, the vaginal parts of VCE smears were studied.

Table 1. Comparison of Lactobacillus flora types between Candida positive and negative patients

<table>
<thead>
<tr>
<th>Type Group</th>
<th>Lactobacillus overgrowth flora</th>
<th>Lactobacillus flora</th>
<th>Mixed flora</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida positive</td>
<td>18(9%)</td>
<td>163(84%)</td>
<td>13(7%)</td>
<td>194</td>
</tr>
<tr>
<td>Candida negative</td>
<td>44(25%)</td>
<td>122(70%)</td>
<td>9(5%)</td>
<td>175</td>
</tr>
</tbody>
</table>

Smears were stained with Methenamine silver staining method for determining Lactobacillus flora. Bacteria and fungi stained in black which could be easily studied for more details besides their relation with cells (Fig.1). Lactobacillus positive groups were classified into three types: Type 1, Lactobacillus overgrowth flora: The bacteria were rod-shaped, slender and long. There was an abundance of bacteria outside of epithelial cells, often with marked cytolysis of the glycogen-rich intermediate squamous cells. No coccoid bacteria were detected (Fig.2). Type 2, Lactobacillus flora: The number of lactobacilli was much lower than in compare with the first type. There was no cytolysis. In some smears, limited number of coccoid bacteria could also be found. Type 3, mixed flora: A mixture of lactobacilli and various other short, plump or round bacteria could be observed. Occasionally clue cells, squamous cells covered with cocci, could be found. To evaluate hormonal status, the cellular maturation index (CMI) was determined. For this purpose, 200 to 500 squamous cells were counted and the percent of parabasal cells, intermediate cells and superficial cells were determined. Data were analyzed via SPSS using Chi-square and T-tests. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Lactobacilli prevalence rates in Candida positive and negative groups were 194 cases (90%) and 175 cases (87.5%), respectively (p=0.7). The mean age of case and control groups was 35±8.5 and 35.3±7.25 years. Frequencies of all types of Lactobacillus microbial flora in both Candida positive and negative groups are shown in table 1 (p<0.0001). According to Chi-square test, there was no significant difference in CMI between Candida positive and negative cases (Table-2).
Table 2. Comparison of CMI between Candida positive and negative patients.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Case n=215</th>
<th>Control n=200</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Superficial</td>
<td>42.5</td>
<td>22.4</td>
<td>45.1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>57.3</td>
<td>22.5</td>
<td>54.5</td>
</tr>
<tr>
<td>Parabasal</td>
<td>0.18</td>
<td>0.7</td>
<td>0.28</td>
</tr>
</tbody>
</table>

N.S: No significant

Figure 1: High – power (*400) photomicrograph showing yeast of Candida spp.

**DISCUSSION**

The advantage of cytologic evaluation of the vaginal flora for bacteria and fungi in direct smears instead of using microbiological culture is that they can be observed as they occur in their natural habitat. Contaminant fungi are occasionally a source of confusion. Because the bacteria are well stained in silver staining method, the vaginal flora can be classified depending on the shape and form of the bacteria. Since Doderlein’s description in 1892, the presence of Lactobacilli in
vaginal secretions has been considered as normal flora. It has been suggested that Lactobacilli have a protective role in the vagina, preventing the transmission of pathogens responsible for sexually transmitted diseases.\textsuperscript{11} In Schroder study, VC was more accompanied with intermediate flora instead of Lactobacilli free flora.\textsuperscript{12}

The defense mechanisms for lactobacilli have been proposed as production of bacterial toxins like hydrogen peroxide a decrease in the available energy source, inhibition of epithelial receptors for blastospore and production of inhibitory substances.\textsuperscript{6,13} However, Lactobacilli inhibitory and toxic effects against the Candida have been documented.\textsuperscript{4} The organism colonization may be due to normal flora loss, cellular immune deficiency, metabolic changes in vagina, or idiopathic mechanisms.\textsuperscript{13} Vaginally administered Lactobacilli to prevent Candidiasis following antibiotic therapy have been unsuccessful.\textsuperscript{14,15}

\textbf{Figure 2:} High – power (*400) photomicrograph showing Lactobacillus overgrowth flora with marked cytolysis

Despite of many in- vitro studies, in an in-vivo one performed by Demirezen, which was similar to ours, Lactobacilli was known as a provoking factor of Candida infection and there was a significant difference between Candida positive and negative groups in terms of Lactobacilli presence.\textsuperscript{8} Probably in-vivo, Lactobacilli can provide a favorable environment regarding acid pH.\textsuperscript{7,8} Although in our study, no significant difference was observed between case and control
groups in terms of Lactobacilli presence, this can be from differences in age groups, demographic features, and in the method of seeing microbial flora. In Demirezen study, the routine staining method called Papanicolaou was used but we used silver staining method to see microbial flora with a better visualization. With respect to this fact that Candida grows in acid pH, VC is expected to be found more frequently with type I flora (Lactobacilli overgrowth) which makes a favorable environment regarding cytolysis and acid pH. However, Candida showed a statistically significant coexistence with type II Lactobacillus flora. This indicates that Lactobacilli has an inhibiting effect through toxic substance production and stimulates Candida growth by decreasing the vaginal pH.

There is a higher risk of Candida in women consuming anti-estrogen drugs such as Tamoxifen. Acute episodes of VC often occur during pregnancy and during the luteal phase of the menstrual cycle, when levels of progesterone and estrogen are elevated. We found no significant difference among different CMIs; but like Demirezen study, the most prevalent index in Candida infected smears was intermediate type. Cytohormonal analysis in vaginal smears in each person can have different tissue sensitivity toward hormones and the pure effect of the hormone can’t be studied. Thus, it is recommended to determine the VC risk based on estrogen and progesterone serum concentrations. In conclusion, these results suggest that Lactobacillus do not have a protective role against vaginal Candidiasis. Considering the maturation index pattern, we can conclude that progesterone plays an important role in Candida infections. It may stimulate Candida growth by decreasing the vaginal pH. This should be considered in therapeutic procedures against chronic Candida.

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REFERENCES


