

Protective role of *Lactobacillus acidophilus* against vaginal infection with *Trichomonas vaginalis*

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Abstract:

Introduction: Vaginal colonization with lactobacilli species is characteristic of normal vaginal ecology. The absence of vaginal lactobacilli, particularly hydrogen peroxide producing isolates, has been associated with bacterial vaginosis and increased risk for sexually transmitted infection. In order to check the role of *Lactobacillus acidophilus* in vaginal infection when infected with *Trichomonas vaginalis*, in this study we mimic the vaginal condition in presence and absence of lactobacilli.

Methods: Forty *Trichomonas vaginalis* isolates from women referred to gynecology clinic were evaluated in this study. The parasites were isolated from vaginal discharge and urine in TYI-S-33 media. The attachment of parasites, as pathogen, to vaginal epithelial cells was examined in healthy women in presence and absence of lactobacilli as well as their excretory-secretory products specially those of *Lactobacillus acidophilus*.

Results: Maximum number of vaginal epithelial cells infected by *Trichomonas vaginalis* was 37% (at 50 min from co-incubation) in experiments with parasites and vaginal epithelial cells (1st group) against 47% (at 40 min) in tube containing parasite along with *Lactobacillus acidophilus* and vaginal epithelial cells (2nd group). However, parasites in presence of vaginal epithelial cells and excretory-secretory product of *Lactobacillus acidophilus* (3rd group) showed the least number of infected cells 20% (at 40 min). Difference between 1st and 3rd group as well as 2nd and 3rd group of these experiments were significant ($p < 0.001$). However, no significant difference were found between 1st and 2nd groups ($p = 0.28$).

Conclusion: Modifiable biological and behavioral factors in vaginal ecology are associated to *Lactobacillus* colonization. This study suggests intervention strategies to improve vaginal health in women with risk of pathogens.

Keywords: vaginal infection, *Lactobacillus acidophilus*, vaginal epithelial cells, *Trichomonas vaginalis*.

Introduction

Various microflora inhabit the genital tract of women. *Lactobacillus* is the chief microbe that acts as protectant against vaginal infections. Lactobacilli have been found to have a positive correlation with the health of the vaginal tract [1]. These microbes are gram positive rods that produce antimicrobial compounds, such as lactic acid and hydrogen peroxide (H_2O_2), substances that reduce pH offering a hostile environment to unwanted pathogens [2]. The lethal mechanism used against pathogens is the breakdown of their cellular DNA by hydroxide ions generated in presence of H_2O_2 . Myeloperoxidase and halides are plentiful in the uterine mucus, even more during ovulation. The conjunction with the acidic condition in the vagina, increases the effectiveness of the pathogens being destroyed [3]. Retaining a low pH environment is a paramount to impede specific pathogens propagation. Reports show that cases of vaginal ailments such as bacterial vaginosis (1) and

trichomoniasis increase when there is a degraded population of lactobacilli [2]. The most common symptoms in bacterial vaginosis is a smelly vaginal discharge and infected women with *Trichomonas vaginalis* present mal odorous vaginal discharge, pruritus and dyspareunia. *Trichomonas vaginalis* is a common sexually transmitted protozoan parasite that causes trichomoniasis. The latter was linked to Preterm birth, cervical cancer, infertility, pelvic inflammatory diseases and acquiring HIV [1, 2]. Different studies indicated that about fifty percent of the patients are asymptomatic [4]. Trichomonal cytoadherence to epithelial cells is a critical step in the initiation phase of the infection and subsequent pathogenesis. Surface proteins of the extracellular parasite are implicated in the initial adherence to mucosal tissue and are likely to play a critical role in the long term survival of this pathogen [5]. It is believed that lactobacilli safeguard their hosts against urogenital tract infections in several ways. Lactobacilli have a strong attraction to human

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uro-epithelial cells in vitro, both co-accumulate and competitively pathogenic bacteria are excluded from affixing to cells [6-8]. As such, this study is planned to perform in vitro studies with vaginal epithelial cells (VECs), *Trichomonas vaginalis* (T.v.), and *Lactobacillus acidophilus* (L.a.) in order to survey the role of *Lactobacillus acidophilus* in vaginal infection when infected with *Trichomonas vaginalis*.

Material and Methods:

This study consisted of 40 *T.vaginalis* samples that were obtained from high-risk behavior women attending the gynecology clinics of Tehran province penitentiaries. TYI-S-33 media was used to check the vaginal discharge and urine samples from the patients, the parasites *T. vaginalis* were then isolated [9] grown in the same media with antibiotics (pen/strep 1%, Sigma) and underwent sub-culturing at different time intervals. The ATCC 4356 strain of *L. acidophilus* was maintained overnight in screw capped tubes containing Lactobacillus de Man, Rogosa Sharpe (MRS) broth in 5% of CO₂, and temperature was regulated to 37°C. Harvesting was accomplished by centrifugation at 500×g for 10 minutes and washing in PBS (pH 7.2) three times. The final pellet was suspended in PBS and adjusted to 2×10⁶ cells/ml [10]. Vaginal epithelial cells were taken from consenting, healthy women who attended family planning by sterile vaginal swap and kept in sterile PBS (pH 7.2). They were transferred to the laboratory and checked for absence of *T. vaginalis*. The cells were further processed by washing three times in PBS and centrifuged at 250×g for 5 minutes. Fresh VECs were used in all of these cytoadherence assay experiments. In order to find out the role of *L. acidophilus* against vaginal infection with *Trichomonas vaginalis*, three methodological groups were designed. The first group consisted of VECs and *T. vaginalis*, the second group contained VECs, *T. vaginalis* and *L. acidophilus*, and the third group had *T. vaginalis*,

VECs and excretory secretory product (ESP) of *L. acidophilus*.

The experimental methods in this section were used as previously reported by Alderete et al. [11], with some modification. To gage vaginal epithelial cell adherence by *T. vaginalis* in the presence of *L. acidophilus*, microtubes containing 200 µl of VECs (4 ×10⁴/ml), 200 µl of *L. acidophilus* (2×10⁶ /ml) and 200 µl of live motile trichomonads (2 ×10⁵/ml) were incubated at 37°C. Following incubation, wet mounts were prepared at different time intervals from ten up to ninety minutes. The percentage of VECs attached to trichomonads was determined by counting one hundred VECs in various fields under the microscope. Each isolate was subjected to three adhesion assays and the mean readings were documented. Controls without *L. acidophilus* were also processed simultaneously with each isolate.

To measure the effect of ESP of *L. acidophilus* against the attachment of *T. vaginalis* to VECs, the supernatant of *L. acidophilus* (2×10⁶/ml) was grown in MRS medium and incubated for one hour and half at 37°C in 5% of CO₂. To the suspension of *T.vaginalis* (2×10⁵/ml) and VECs (4×10⁴/ml), 100µl of ESP of *L. acidophilus* were added and incubated at 37°C for different times. Percentage of VECs attached by *T. vaginalis* was counted along with the control tubes without ESP.

Statistical method: Statistical test were done by SPSS 16.0 and P<0.05 was considered statistically significant. Differences between experiments were analyzed by one-way ANOVA - test and the groups were compared by LSD.

Results and Discussion

Results

Vaginal epithelial cells, *L. acidophilus* and *T. vaginalis* are show in (Figure.1) under light microscope, stained with Giemsa.

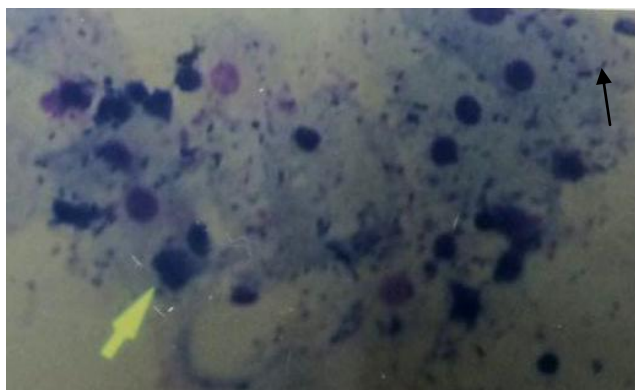


Figure 1. Vaginal epithelial cells showing attachment of *T. vaginalis* under light microscope (oil immersion), stained with Giemsa, yellow arrow showing *T. vaginalis* and showing *L. acidophilus*.

Results of mean number of three groups adhesion experiments are shown in Figure 2. Before

the adhesion experiment, the viability of parasites was checked and more than 95% of them were in a

good condition of motility. The wet mounts prepared after every 10 minutes showed a time dependent attachment. Maximum mean numbers of VECs attached by *T. vaginalis* were found to be 37% (50 min) in *T.vaginalis* +VECs experiments (1st group) and 90 min from starting experiment the parasites were still alive but detached from epithelial cells. In the 2nd group, mean number of cells attached by *T.vaginalis* was 47% (40 min). However parasites in presence of VECs and excretory secretory product of *L. acidophilus* (3rd group) showed the least attachment i.e. 20% (40 min). Statistical analysis showed that difference between 1st and 3rd group as well as 2nd and 3rd group of these experiments were

significant ($p<0.001$), but no significant difference were found between 1st and 2nd groups ($p=0.28$). Then with increasing the time all three groups showed similar trends as such the peak of attachment were seen in 40 and 50 min of experiments, then the numbers of *T. vaginalis* attached to VECs were decreased and ranged from 1% to 6%.

When the numbers of VECs attached by *T. vaginalis* in all three experiments were compared with each other, results showed that *T. vaginalis* and VECs in presence of ESP of *L. acidophilus* showed significant drop in number of VECs attached by parasites ($p<0.01$).

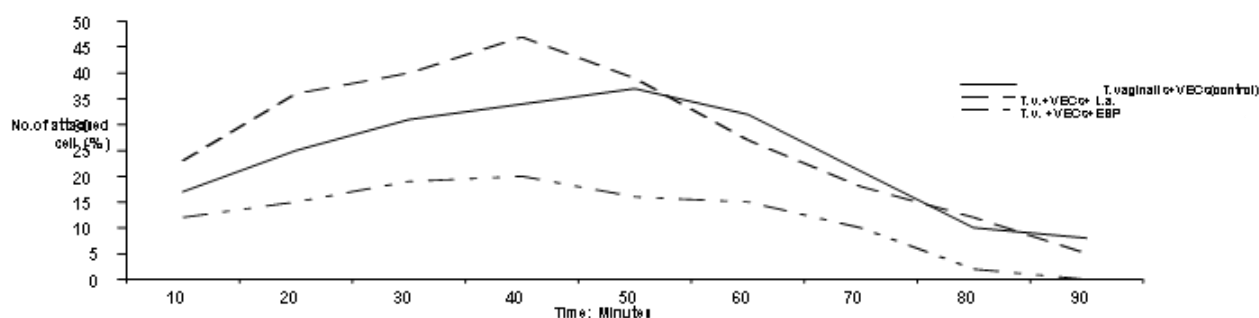


Figure 2. The attachment of *T. vaginalis* to vaginal epithelial cells (%) at different time intervals.

During the parasitism of VECs, the parasite changed from pear shape to amoeboid form (Figure 3) as observed under the microscope. After 70 min of incubation, there was almost no alive parasite in order to attach to VECs. Therefore, adding ESP of bacteria has deleterious effect on *T. vaginalis* isolates when compared with the other two experiments.

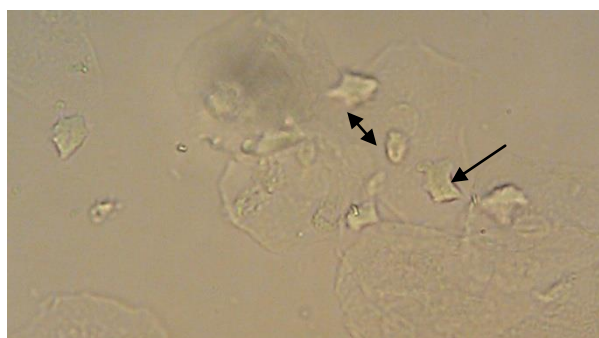


Figure 3. Attachment to vaginal epithelial cells and amoeboid form (←) of *Trichomonas vaginalis*. Arrows show the *T. vaginalis* attached to VECs (↔)

Discussion

The use of antibiotics has been reported as a risk factor for loss of microflora; especially lactobacilli in the vaginal tract. Metronidazole was found to be used very frequently in treating bacterial vaginosis and trichomoniasis in suspected patients, without using proper lab diagnostic methods. Vaginosis is the cause of considerable morbidity and is the most cited cause of vaginal symptoms prompting women to seek gynecologists. Epidemiologic studies have demonstrated that bacterial vaginosis is associated with a distinct increased risk for acquisition of sexually transmitted infections [12]. The pH of vaginal environment after growth of *T. vaginalis*

changes to alkaline. The results of this study have demonstrated that the presence of *L. acidophilus* causes the death of pathogens. At the beginning of the experiment, the number of VECs attached by *T. vaginalis* had increased, but due to a low pH and production of H_2O_2 by *L. acidophilus*, the parasites detached from the cells and were ultimately incapacitated. Woojin et al. co-cultured *Lactobacillus acidophilus* with immortalized human vaginal epithelial cells (MS74 cell line), measured growth of *L. acidophilus* and the acidity of the culture medium. Their results indicated that *L. acidophilus* increases MS74 cell proliferation and viability, suggesting that lactobacilli may contribute

to the healthy environment for vaginal epithelial cells. They also documented that the growth curve of *L. acidophilus* and the pH values were relatively unaffected by co-culture with MS74 cells, confirming that *L. acidophilus* maintains a low pH in vaginal environment [13]. In another study on isolates from symptomatic and asymptomatic patients they found that in presence of *L. acidophilus* at one hour time period, an increase in attachment of *T. vaginalis* to VECs was observed followed by a gradual fall at two and three hours, ultimately leading to non-viability of *T. vaginalis* at four hours of co-incubation [4]. This study also got the same results but in different times of incubation. Report indicated that, the H₂O₂-producing lactobacilli were capable of increasing the activity of the host antimicrobial peptides (muramidase and lactoferrin) as well as the antibacterial activity of the epithelial cells [14].

The amoeboid transformation of *Trichomonas* during cytoadherence was shown in Fig 2. Moreno-Brito et al. showed that brief contact of trichomonads with vaginal epithelial cells (VECs), but not HeLa cells, produced dramatic changes in parasite morphology, as evident from the parasite transformation from an ellipsoid to amoeboid form. This suggests host-specific signaling of parasites in presence of VECs. Five different parasite surface proteins (AP120, AP65, AP51, AP33, and AP23) mediate adherence [15], and gene-encoding adhesions are unregulated during attachment to VECs [16].

Currently in Iran, the method of cleaning intravaginal environment is improved mostly due to personal hygiene or to prevent and treat an infection or prevent pregnancy. However, there is less agreement regarding douching for hygiene and relief of infection symptoms [17] since this process has been associated with adverse outcomes such as removal of normal vaginal flora permitting the overgrowth of pathogens. Pathogenic microorganisms may then ascend into the upper reproductive tract and lead to inflammatory scarring which is the principal cause of ectopic pregnancy, early miscarriage and infertility [18]. Vaginal washing, typically with water alone or with soap and water, was associated with an approximately 40% decreased likelihood of *Lactobacillus* and other H₂O₂-producing strains isolation [19].

Conclusion:

Modifiable biological and behavioral factors are associated with *Lactobacillus* colonization. These results suggest intervention strategies to improve vaginal health in women who are at risk of pathogens. Recently a probiotic drug named "Lactofem" was approved for women [20], made of vital lactic acid bacteria to be used in cases of vaginal microflora imbalance in order to protect and

increase the immune system effectiveness of the vagina.

Parasites in presence of vaginal epithelial cells and excretory secretory product of *Lactobacillus acidophilus* showed the least number of attachments. Further research is still needed to better understand the action of *L. acidophilus* by which *T. vaginalis* is eradicated and to identify the content of ESP.

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