

## Effect of ozonated water on normal vaginal microecology and *Lactobacillus*

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*To the Editor:* The female vagina is a complex microecology system. Generally, the vaginal microbiota of healthy women is dominated by H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus*, which decomposes glycogen in vaginal epithelial cells into lactic acid, maintaining the normal acidic environment of the vagina.<sup>[1]</sup> And the change of the dominant position of the *Lactobacillus* will cause a variety of vaginal infections. The conventional treatment of vaginitis mainly through antibiotics to kill the pathogenic microorganisms. Meanwhile, *Lactobacillus* is sensitive to cephalosporin antibiotics, penicillin, clindamycin, and so on.

Ozone is a reactive oxygen species consisting of three oxygen atoms produced by ultraviolet light and high-pressure diatomic oxygen, recognized as a strong oxidative antimicrobial agent. Ozone therapy has received increasing attention in recent years and is widely known for its good effects on infection, reperfusion injury, cancer, and dental caries.<sup>[2,3]</sup> Currently, ozone therapy is a new concept in clinical treatment of vaginitis. The medical integrated ozone therapeutic apparatus uses an ozone generator to prepare a certain concentration of ozone, and mixes with the filtered tap water to form ozonated water. Ozone and active molecules are in a liquid state play a role in sterilization in the vagina.

Although ozonated water is gradually used in clinical treatment of vaginitis, the influence of ozonated water lavage on the dominant position of *Lactobacillus* and vaginal microecology balance after vaginitis treatment are not clear. This work, by evaluating the microecology contents of vaginal secretions after vaginal lavage, including aerobic vaginitis (AV) scores, Nugent score, microbial density, microbial diversity, dominant flora, Lactobacillary grades, hypha, leukocytes, and vaginal microbial community diversity and abundance, aimed to

study whether ozonated water would cause changes in vaginal microecology and *Lactobacillus*.

Thirty female volunteers without vaginal infection, including AV, bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomonas vaginitis (TV), who were recruited to the gynecological clinic of Beijing Tsinghua Changgung Hospital from April 2016 to October 2016, completed the informed consent form approved by the Medical Ethics Committee of Beijing Tsinghua Changgung Hospital. Inclusion criteria were as follows: (1) non-pregnancy, non-lactation, and non-menstruation fertility women (18–50 years old) without vaginal infection; (2) no abnormal vaginal bleeding; (3) almost 3 days of asexual life before enrollment; and (4) no systemic or vaginal antibiotics for nearly 2 weeks.

All volunteers received ozonated water from the Medical Integrated Oxygen Therapy Instrument (XYK-6000D type; produced by Zhuhai Xinyike Medical Technology Co., Ltd., Zhuhai, China) for vaginal lavage for 5 min per day during non-menstrual periods for 3 consecutive days (ozone concentration: 80 mg/L, 3.0 L of ozone solution per lavage). The vaginal secretions were taken respectively by two swabs before the first vaginal ozonated water lavage and the first week, the second week, and the third week after the last lavage. A swab was used to prepare a dry slide for Gram staining, under 400-fold magnification for visual detection, to test for AV, BV, VVC, and TV. In addition, some indicators related to vaginal microecology, including microbial density (+, ++, +++, and ++++), microbial diversity (+, ++, +++, and ++++), dominant flora, the lactobacillary flora (lactobacillary grades I and IIa, IIb, III),<sup>[4]</sup> leukocyte ( $\leq 10$ /high power field [HPF],  $> 10$ /HPF and  $\leq 10$  per epithelial cell, and  $> 10$  per epithelial cell),<sup>[4]</sup> yeast blastospores and pseudohyphae was visual assessment by microscopic observation. The total DNA of vaginal flora from the second swab was extracted, and the

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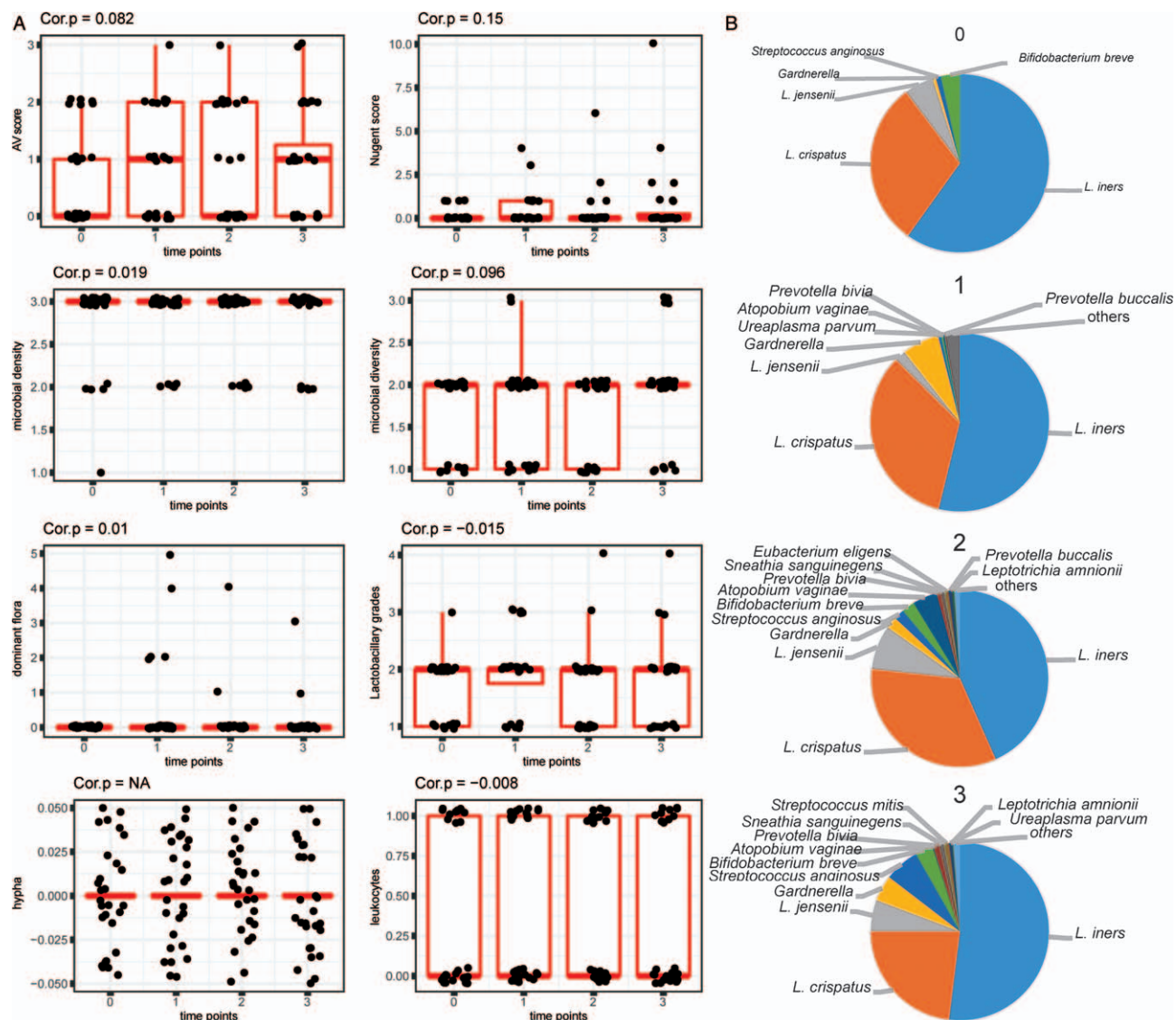
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**Figure 1:** (A) Correlation analysis on the contents of vaginal microecology, including AV score, Nugent score, microbial density, microbial diversity, dominant flora, Lactobacillary grades, hypha, leukocytes. The absolute value of the correlation coefficient (cor.p) was <0.2, indicating that there was no correlation between the above detection contents at the four time points of 0, 1, 2, and 3 before and after the ozonated water lavage. (B) Changes in vaginal flora composition ratio at different time points of 0, 1, 2, and 3 before and after vaginal lavage.

V1-V2 variable region of the 16S rDNA genes was sequenced by Illumina Miseq platform according to the 300PE MiSeq protocol.

Of the 30 female volunteers, 29 completed three follow-up visits and one was lost at the third week of follow-up. Before the first vaginal ozonated water lavage, the first week, the second week, and the third week after the last lavage were marked as 0-, 1-, 2-, and 3-time points, respectively. (1) The results of Pearson correlation analysis (cor.p) on the contents of vaginal microecology [Figure 1A], including AV score, Nugent score, microbial density, microbial diversity, dominant flora, Lactobacillary grades, hypha, leukocytes, the absolute values of correlation coefficients of the above items were all <0.2, indicating that the above contents were not changed at four time points. (2) As shown in Figure 1B, the top three bacteria at the 4-time points are three *Lactobacillus* spp.,

that is, *L. iners*, *L. crispatus*, and *L. jensenii*. The type and abundance of *Lactobacillus* are no significant change before and after lavage of ozonated water.

By evaluating the proportion and type of *Lactobacillus*, the dominant flora in the vagina of healthy women, and vaginal microecology before and after ozonated water lavage, we have confirmed that ozonated water lavage has no obvious side effects on intravaginal microecology and *Lactobacillus*. As far as we know, there are no reports on the effects of ozone water on *Lactobacillus* and vaginal microecology so far. The results of this study can be seen as extremely important evidence that ozonated water to treat vaginitis has a minor side effect on vaginal microecology and *Lactobacillus*.

Ozonated water is a new treatment for vaginitis, which not only kills pathogens but also protects *Lactobacillus* and

vaginal microecology from disorders. When serious vaginal secretion increases, accompanied by odor, a large number of inflammatory secretions stimulate patients with vulva and perianal, causing discomfort. Therefore, in the treatment of vaginitis, we can consider using ozone water to rinse the vagina, reduce the concentration of local vaginal pathogens, to help the recovery of vaginal dominant bacteria. As a relatively new treatment concept, ozone water anti-infection has been widely used in many industries, but there is still a lack of a large number of clinical evidence in the treatment of vaginitis. More basic and clinical studies *in vivo* and *in vitro* are needed to provide a theoretical basis for the extensive participation of ozone water in the treatment of vaginitis in the future.

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### Conflicts of interest

None.

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