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Evaluating Vitamin C Vaginal Tablets Effect on *Gardnerella vaginalis* Infection

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ABSTRACT

Introduction: Bacterial vaginosis (BV) is the most common vaginitis in childbearing women. Despite the availability of various treatments, recurrence rates are high. This study investigated the effects and side effects of vitamin C vaginal tablets in patients with *Gardnerella vaginalis* infection based on Amsel criteria and the culture. **Methods:** A double-blind, randomized clinical trial was conducted on 48 non-pregnant women aged 15-40 years referred to Alawi Hospital in Ardabil Province, Iran. After the diagnosis of *G. vaginalis* infection based on Amsel criteria and culture methods, the patients were randomly assigned to receive either intravaginal 250 mg vitamin C (intervention group, n=24) or 250 mg metronidazole tablets (control group, n=24) for eight nights. The patients were evaluated 10 ± 2 and 30 ± 2 days before and after the treatment. **Results:** Culture and Amsel criteria showed significant improvement in both groups during the first and fourth weeks of treatment. However, the intervention group that received vitamin C showed a more substantial improvement (P<0.002, P<0.001). Intravaginal vitamin tablets were more effective than metronidazole tablets in treating *G. vaginalis* infection. Furthermore, vitamin C tablets improved the abnormal growth of vaginal microflora and reduced the abnormally high pH of the vagina. This treatment effectively prevented the recurrence of *G. vaginalis* infection for one month. **Conclusion**: Vitamin C vaginal tablets showed to be a promising alternative treatment for *G. vaginalis* infection, but further research is needed to confirm these findings and address potential limitations.

INTRODUCTION

Vaginal infections and discharge are common complaints among patients visiting gynecology and obstetrics clinics. Bacterial vaginosis (BV) is a condition characterized by changes in the vaginal bacterial flora, resulting in a reduction in hydrogen peroxide-producing lactobacilli and overgrowth of bacteria with the predominance of the anaerobic bacteria *Gardnerella vaginalis* [1]. While many young childbearing women have clinical signs of vaginitis, *Trichomonas vaginalis*, and fungal cells are often overlooked in their discharge tests, leading to the diagnosis of nonspecific vaginitis.

Gardnerella vaginalis, a small Gram-variable bacillus, can cause vaginitis, and interactions between this bacterium and other anaerobic bacteria in the vaginal mucosa can result in various bacterial vaginosis [2]. BV is associated with an increased risk of endometritis after delivery and cesarean section, as well as pelvic diseases, vaginal cuff cellulitis after hysterectomy, abnormal cervical cytology, preterm delivery, low birth weight, amniotic fluid infection, chorioamnionitis, premature rupture of the membranes, postpartum fever, and intraepithelial neoplasia of the cervix [1].

G. vaginalis is killed by neutrophils, and the activation of complement alternative pathways results in opsonization, phagocytosis, and bacterial killing by neutrophils [2]. The Amsel criteria and the Nugent scoring system are commonly used diagnostic methods for diagnosing *G. vaginalis* infection. The Nugent scoring is a Gram-stain-based scoring system developed by Nugent and colleagues in 1991 and is considered the gold standard for diagnosing BV due to its high sensitivity. The Amsel criteria is a simple and accurate alternative method for diagnosing BV. BV is confirmed if at least three of the four Amsel criteria are positive, including homogeneous white or yellowish-grey vaginal discharge, vaginal discharge pH ≥ 4.5, positive whiff test, and presence of clue cells in the wet vaginal smear sample (at least 1 in 5 vaginal epithelial cells) [1, 3, 4]. Clue cells are vaginal squamous epithelial cells coated with anaerobic coccobacillus *G. vaginalis* on the surface and sides.

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Vitamin C vaginal tablets have been recommended for treating BV; this vitamin lowers the pH in the vagina to a level where bacteria cannot grow. Even lactobacilli that reproduce in pH less than 4 and adverse growth of anaerobes is considerably inhibited by the acidic vitamin C [5]. Maintaining an appropriate level of vitamin C in pregnant women can prevent rupture of the fetal membranes during childbirth and before premature birth, thereby reducing the risk of premature birth and improving pregnancy outcomes for both the mother and infant. Vitamin C also acts as an antioxidant and protects connective tissue, increasing the strength of embryonic membranes through its role in maintaining and building collagen [6].

Several studies have investigated using vitamin C to treat and prevent BV complications. For example, Andal *et al*. (2002) concluded that vitamin C had a more significant therapeutic effect than metronidazole in preventing preterm birth in women with BV [7]. Also, Hoh *et al*. (2006) found that intravaginal use of vitamin C was influential in reducing vaginal pH and treating vaginitis in pregnant women [8].

The present double-blind, randomized clinical trial evaluates the effect and side effects of vitamin C vaginal tablets on Amsel criteria in patients with *G. vaginalis* infection. This study will provide further insights into the potential use of vitamin C as a treatment for BV and help to inform clinical practice.

MATERIAL AND METHODS

Patients. This study was performed on women with vaginal discharge referred to the gynecological clinic of Alawi Hospital in Ardabil, Iran, from 2016 to 2019. The study was approved by the Ethics Committee of Ardabil University of Medical Sciences (code no. 4395/89587) and was registered in the Iranian Registry of Clinical Trials (code no. IR.AROMS.RFC.B95-96).

The inclusion criteria for women were being married, non-pregnant, and non-lactating, aged 15-40 years, and diagnosed with *G. vaginalis* infection based on Amsel criteria and culture method. Exclusion criteria included having chronic diseases, HIV, genital herpes, or other vaginal infections, unexplained vaginal bleeding, using vaginal medications and vaginal douches, and taking antibiotics including rifampin, phenytoin, phenobarbital, or any anticoagulants during the last two weeks [2, 9, 10].

***G. vaginalis* diagnosis by Amsel criteria.** Diagnosis of *G. vaginalis* infections was performed based on the presence of at least three out of the four Amsel criteria, including 1) homogeneous thin dark gray discharge, 2) vaginal pH $\geq$ 4.5, 3) positive whiff test, and 4) presence of clue cells (the criterion in all positive cases) [1, 3, 4]. Under the supervision of a gynecologist, patients were first examined for vaginal mucosa using a sterile speculum. The amount, color, consistency, homogeneity, and pH of the patients' discharges were measured and recorded.

Vaginal sampling. Sampling was carried out using two sterile swabs. The first swab was placed in 1 ml of normal saline and immediately transferred to the laboratory for culture. The second swab was smeared on three slides. The first slide was examined for clue cells by Gram staining using microscopy with 100x magnification. The second slide was stained by the Papanicolaou (Pap) staining method and examined by a pathologist. On the third slide, a drop of normal saline was added, a cover slip was placed on it, and looked for motility. Trichomonads were investigated using a microscope with a 40x magnification. On the fourth slide, a KOH 10% drop was added to the smear of vaginal discharge, and the release of the fishy smell was investigated. Then, a cover slip was placed on the slide and examined under a microscope with 40x magnification for *Candida*, especially in the form of hyphae. Samples that were positive for *Trichomonas* and *Candida* were excluded from the study.

***G. vaginalis* culture.** Physiological serum from the first swab was cultured onto plates containing Columbia CAN Agar. The plates were incubated in an anaerobic candle jar at 35 °C for 48-72 h. After colony growth, differential tests were to diagnose *G. vaginalis*, including smear preparation from the colony, Gram staining, and observation of coccobacilli or diphtheria-like thin bacilli but Gram-negative or -variable. Furthermore, the catalase and oxidase tests were thoroughly evaluated [2].

Administration of Vitamin C. Of the 762 women referred to the outpatient infirmary with abnormal vaginal discharge, 427 patients did not meet the inclusion criteria, and 249 did not have the Amsel criterion and culture score. Thirty-two patients did not have spouses' consent and were excluded from the study. After confirming the diagnosis of *G. vaginalis* infection, 48 patients were included in the study and were randomly divided into two groups of 24. One group received 250 mg vitamin C vaginal tablets daily; the other received 250 mg metronidazole vaginal tablets for eight nights. Vaginal vitamin C tablets were prepared in collaboration with a pharmacist at Ardabil University of Medical Sciences using the tablet formulation commonly used for antiseptics such as microcrystalline cellulose, starch, lactose, and magnesium stearate and were packed as eight tablets. They were delivered to Sina Pharmacy of Ardabil according to the gynecologist's order.

The effect of the medications was evaluated for 10±2 and 30±2 days. The infection improvement was defined as the absence of all symptoms according to Amsel criteria) or the presence of at most one of the symptoms (except clue cells). Also, the presence of clue cells was considered based on the culture detection method and laboratory tests [2, 4, 11, 13, 14].

The data were subjected to comprehensive analysis utilizing both descriptive statistics (mean and standard

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deviation) and analytical statistics (specifically, a comparison of the changes in pH level, vaginal secretions, and Clue cells between the metronidazole and vitamin C groups at 10 ± 2 and 30 ± 2 days after treatment). The Chi-square statistical test was employed for this purpose. Additionally, the comparison of the Whiff test was conducted using Chi-square, Fisher's exact, and McNemar tests. All statistical analyses were performed using SPSS Statistics 21.0.

RESULTS

Follow-up after treatment. In follow-up, one patient from the vitamin C group and one from the metronidazole group did not participate in the first follow-up sessions due to improved vaginal symptoms and decreased disease severity. Three patients from the vitamin C group and four from the metronidazole group did not participate in the second follow-up session due to travel and completion of treatment.

Demography of study patients. The demographic characteristics and midwifery history of participants in the two groups were comparable. In both the metronidazole and vitamin C groups, the age range of 31-35 years exhibited the highest frequency of infection, while the age range of 20-25 years exhibited the lowest frequency. Furthermore, the highest percentage of infection frequency was observed among individuals with educational backgrounds ranging from elementary to high school, while the lowest percentage was observed among university-educated individuals. Additionally, the highest percentage of infection was found among those with a duration of marriage between 1-5 years, whereas the lowest percentage was observed among those with a duration of marriage between 21-25 years. Lastly, the highest percentage of infection occurred among individuals with 1-2 pregnancies, while the lowest percentage was observed among those with 6-10 pregnancies. It is important to note that in this table, all variables were assessed in terms of frequency percentage. Only the age group variable was further investigated for mean and standard deviation values (Table 1).

Table 1. Demographic characteristics and midwifery history of participants in two groups receiving either intravaginal metronidazole (n=24) or vitamin C tablets (n=24) for treating *G. vaginalis* infection.

Variables	Metronidazole n (%)	Vitamin C n (%)	*P*-value
Age (years)			
15-20	2 (8.33%)	3 (12.5%)	
21-25	4 (16.67%)	5 (20.83%)	
26-30	6 (25%)	6 (%25)	0.832[a]
31-35	9 (37.5%)	8 (33.34%)	
36-40	3 (12.5%)	2 (8.33%)	
Mean ± SD	31.84±9.6	31.26±7.3	
Job			
Housewife	13 (54.46%)	12 (50%)	0.401[b]
Employed	11 (45.54 %)	12 (50%)	
Education			
Elementary to high school)	10 (41.66%)	11 (45.83%)	0.139[c]
Diploma	7 (29.17%)	8 (33.33%)	
University	7 (29.17%)	5 (20.84%)	
Duration of marriage (year)			
1-5	9 (37.5%)	8 (33.33%)	0.724 [c]
10-6	6 (25%)	8 (33.33%)	
15-11	3 (12.5%)	2 (8.34%)	
20-16	5 (20.84%)	4 (16.67%)	
25-21	1 (4.16%)	2 (8.33%)	
Number of pregnancies			
2-1	13 (54.17%)	12 (50%)	0.476 [c]
5-3	9 (37.5%)	11 (45.84%)	
10-6	2 (8.33%)	1 (4.16%)	
History of infection in the last year			0.523[b]
yes	13 (54.16%)	12 (50%)	
No	11 (45.84%)	12 (50%)	

IQR: Independent t-test[a]; Chi-square[b] and Chi-square for trend[c]

Culture. Following administering vaginal vitamin C tablets, 83.34% and 87.4% of patients were negative for coccobacilli 10±2 and 30±2 days later, respectively, compared to 79.2% -62.1% in the metronidazole group. The difference between the two groups was statistically significant (P <0.005) (Fig. 1).

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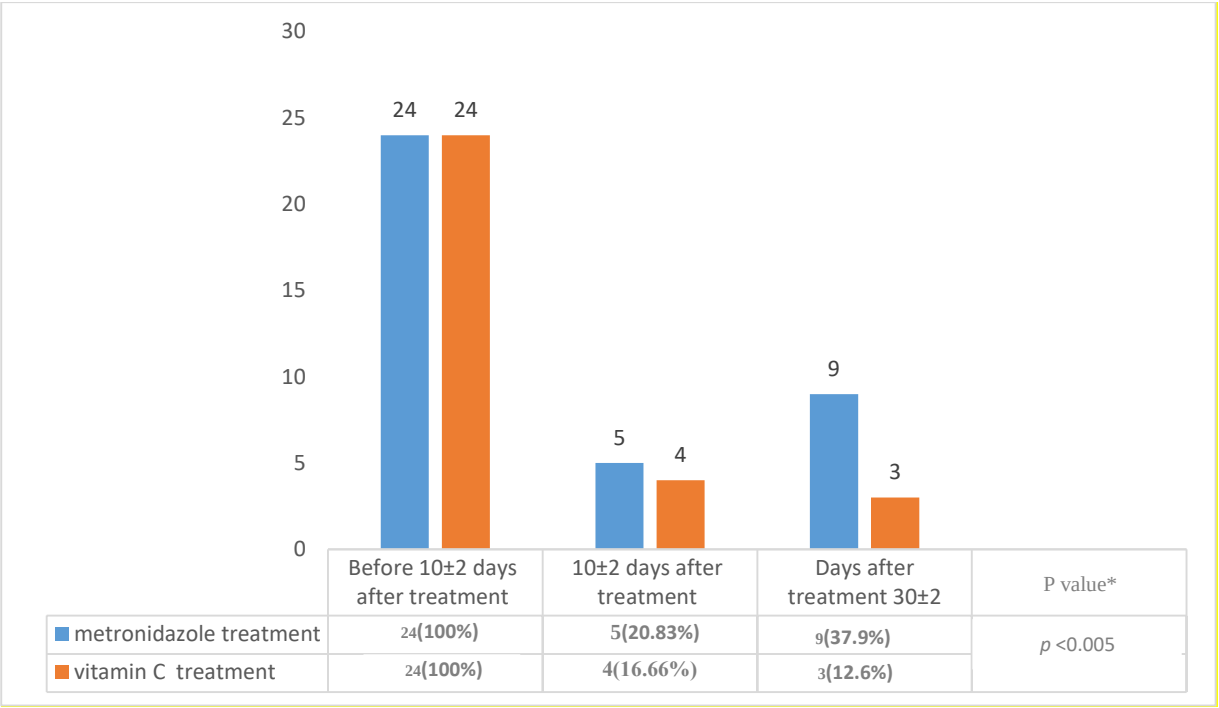


Fig. 1. Comparing the effect of treatments by cultivation in the study groups.

Amsel criteria following vitamin C administration. About 10±2 and 30±2 days after administration of the vaginal vitamin C tablets, 95.66% and 100% of patients had a vaginal discharge pH less than 4.5, compared to 75% and 80% in the metronidazole group. The percentages of patients with normal vaginal discharge and negative clue cells in the vitamin C group were also significantly higher than those in the metronidazole group (60%-80% vs. 12.5%-33.3% and 79.17%-100% vs. 50%-62.5%, respectively). The differences between the two groups were statistically significant (*P* =0.001 and *P* =0.005, respectively) (Table 2).

Evaluating patient satisfaction showed that 85% of the participants in the vitamin C group and 75% in the metronidazole group were satisfied with the treatment.

Table 2. Comparing the effect of metronidazole and intravaginal vitamin C tablets on *G. vaginalis* caused vaginosis by Amsel criteria.

Amsel-based treatment	Study time: Before treatment			10 ± 2 days after treatment			30 ± 2 Days after treatment		
	Metronidazole tablets N = 24 n (%)	vitamin C tablets N = 24 n (%)	P value*	Metronidazole tablets N = 24 n (%)	vitamin C tablets N = 24 n (%)	P value*	Metronidazole tablets N = 24 n (%)	vitamin C tablets N = 24 n (%)	*P*-value*
Vaginal discharge	24 (100 %)	24 (100 %)	0.976 [a]	9 (37.5%)	7 (30.43%)	0.005[a]	6 (00/25%)	5 (20.83 %)	0. 005 [a]
PH>4/5	24 (100%)	23 (95%)	0.990 [a]	7 (29.16%)	5 (20/83%)	0.002 [a]	9 (37.5%)	1 (4/54%)	0.001 [a]
Whiff test	23 (95%)	24 (100 %)	0.476 [b]	7 (30.43%)	6 (25.00%)	0. 005[ab]	6 (00/25%)	5 (20.13%)	0.005 [ab]
Clue cell	24 (100%)	24 (100 %)	0.261 [a]	8 (33.33%)	5 (20.83%)	0.001[a]	5 (520.13 %)	0 (0/00%)	0.001 [a]
The overall result			0. 475[a]			0.00 3[a]			0.001 [ab]

Chi-square [a], Fisher's exact and Mc Nemar tests [b]

In conclusion, the results of this study suggest that vaginal vitamin C tablets may be more effective than vaginal metronidazole tablets in treating *G. vaginalis* infection. The study has limitations, including a small sample size and a short follow-up period. Further studies with larger sample sizes and extended follow-up periods are needed to confirm these findings.

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DISCUSSION

The study evaluated the effectiveness of vaginal vitamin C tablets compared to vaginal metronidazole tablets in treating *G. vaginalis* infection using Amsel criteria and cultivation detection methods. The recovery rate of patients in the vitamin C group was 79.17%-95.84% and 83/66%-87.4% at 10 ± 2 and 30 ± 2 days after treatment, respectively. Previous studies have reported improvements ranging from 66.7% to 86.2% in the vitamin C group using different treatment durations and diagnostic methods. The present study used Amsel criteria and cultivation detection methods, whereas some previous studies only used Amsel criteria. The study's use of both approaches provides two advantages. The discrepancies in the recovery rates observed in previous studies [16-18] and the present study may be due to differences in treatment duration and diagnostic methods, highlighting the importance of using multiple diagnostic methods to ensure accurate diagnosis and treatment.

In the present study, 10±2 and 30 ±2 days after administration of vaginal vitamin C tablets, 95.66%-100% of patients had a vaginal discharge pH less than 4.5, respectively; 75%-80% of the results of the Whiff test was negative; 60%-80% had normal vaginal discharge, and clue cells in vaginal discharge were negative in 79.17%-100% of patients. Petersen *et al.* (2004) reported that two weeks after administering vaginal vitamin C tablets, 83% of patients had a vaginal discharge pH of less than 4.7, which was consistent with the present study [17]. However, Goodarzi *et al*. (2011) [18] reported that one week after administration of vitamin C vaginal tablets, the vaginal discharge pH was less than 4.5 in only 40% of patients, which was inconsistent with the present study. This discrepancy could be due to differences in the duration of treatment. Petersen *et al*. (2004) also reported that two weeks after the administration of vaginal vitamin C tablets, 84% of patients had negative Whiff test results, which was almost consistent with the present study [17]. Goodarzi *et al*. (2011) reported that one week after administering vaginal vitamin C tablets, 66.7% of patients had normal vaginal discharge, and 86.7% were negative for clue cells [18]. These findings were consistent with the present study. Still, differences in the studied populations, research environment, sample selection methods, and severity of the infection could explain some of the variations in the results. Petersen *et al*. (2004) reported that two weeks after administering vaginal vitamin C tablets, 76.7% of patients treated with the placebo had no clue cells, which differed from the present study's findings [17]. The differences between the two studies could be due to variations in the duration of treatment, type of treatment, and type of diagnosis.

The purpose of treating *G. vaginalis* infection is to maintain a vaginal pH of 4.5 or less to prevent the overgrowth of pathogens until the normal vaginal flora is restored [13]. Recent studies have shown that intravaginal use of ascorbic acid (vitamin C) can effectively treat bacterial vaginosis (BV) by maintaining physiological acidity and a low vaginal pH through the production of lactic acid [14]. In this study, the intervention group's vaginal pH was 32% lower than the control group on day 30±2. Participants were also asked about their satisfaction with the treatment, with 85% of the vitamin C group reporting satisfaction compared to 75% in the control group. However, vaginal burning after taking vitamin C was reported as a reason for dissatisfaction in the intervention group. Polatti [19] reported high levels of satisfaction with treatment. Krasnopolsky *et al*. (2013) found that 75.3% of patients who received vaginal vitamin C described it as good or very good, consistent with the present study [20].

The strength of this study is the use of two diagnostic methods, theculture detection method, and Amsel's criteria. The main limitation is the short-term follow-up period. Further studies with more extended follow-up periods are recommended.

The results of this study demonstrate that intravaginal vitamin C tablets (250 mg once nightly for eight consecutive nights) are more effective than metronidazole vaginal tablets in treating *G. vaginalis* infection based on Amsel's criteria and culture method. Furthermore, this study showed that intravaginal vitamin C could improve the abnormal growth of vaginal microflora and reduce the abnormally high pH of the vagina, with a one-month follow-up period to prevent the recurrence of G. *vaginalis* infection. The mechanism of intravaginal vitamin C is thought to be through acidification of the vagina, which slows the overgrowth of pathogens and facilitates the re-establishment of normal vaginal flora. Intravaginal vitamin C has fewer side effects than oral antibiotics and can be an appropriate treatment option for *G. vaginalis* infection, particularly in vaginal dryness and increased vaginal discharge without systemic side effects.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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