



Comparison of the effects of Mycocin vaginal cream and Metronidazole vaginal gel on treatment of bacterial vaginosis: A randomized clinical trial

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ABSTRACT

Metronidazole is recommended as the first-line treatment for Bacterial Vaginosis (BV). With respect to various side effects of metronidazole and increased antibiotic resistance, it seems necessary to find a medication with fewer side effects. The present study aimed to compare the effects of Mycocin vaginal cream (made of garlic and thyme) and metronidazole vaginal gel on treatment of the patients with BV. This clinical trial which was conducted on 120 married women aged 18-44 years whose infection with BV was diagnosed through clinical Amsel criteria. The participants were randomly divided into two groups of 60 receiving Mycocin vaginal cream or metronidazole vaginal gel for seven days. Clinical Amsel criteria and the patients' complaints were assessed again 7 days after completion of the treatment period. The results showed a decrease in the patients' complaints and clinical Amsel criteria after the treatment with Mycocin vaginal cream and metronidazole vaginal gel. However, no significant difference was observed between the two groups regarding clinical improvement in Amsel criteria ($P < 0.05$). Mixed Garlic and Thyme vaginal cream (Mycocin) seems to be an appropriate alternative for metronidazole vaginal gel in treatment of BV.

Keywords: Garlic, Metronidazole vaginal gel, Mycocin vaginal cream, Thyme, Bacterial vaginosis

INTRODUCTION

Bacterial Vaginosis (BV) is one of the most common causes of vaginal discharge in women of reproductive ages. The prevalence of this problem varies from 11-48% in various communities [1] and its incidence rate has been estimated to be 16-20% in pregnant women [2]. The prevalence of BV in the United States was reported to be 29.2% and 25% among 14-49-year-old and pregnant women, respectively [3]. In Iran, the prevalence rate of BV ranged from 16.2% to 39.9% in different urban areas [4-7].

BV can increase the risk of various complications, including amniotic fluid infection, premature rupture of fetal membranes, low birth weight, premature birth, abortion, vaginal cuff cellulitis after hysterectomy, endometritis, cervicitis, urinary tract infection, cervical intraepithelial neoplasia, HIV, ectopic pregnancy, and infertility [8].

Among different methods, Amsel's clinical criteria and Nugent Scoring System (NSS) are more commonly used for diagnosis of BV [9]. NSS which was presented by Nugent et al. in 1991 is based on Gram stain [10]. Because of high sensitivity, NSS is considered as the gold standard for diagnosis of BV [1, 11]. However, this method is time-consuming and expensive and requires laboratory facilities; therefore, most physicians tend to use simple but precise clinical criteria, such as the one proposed by Amsel et al., instead of NSS [12, 13].

Based on the recommendation of the Center for Disease Control and Prevention (CDC), metronidazole can be considered as the first-line therapy for BV [3]. Nevertheless, several complications have been detected following oral and vaginal metronidazole treatments, including diarrhea, vomiting, metallic taste, appearance of vaginal candidiasis, headache, dizziness, and rarely pancreatitis [14, 15]. Besides, use of metronidazole is not recommended in pregnancy and lactation and it has shown contraindications in the first trimester of pregnancy [16, 17]. Oral forms of metronidazole have also shown gastrointestinal complications in pregnant women [14].

Furthermore, a steady rise in the number of drug-resistant pathogens has produced serious risks for public health. Thus, all the aforementioned problems led to performance of researches for alternative treatments for BV, such as using probiotics and herbs.

Garlic, with the scientific name *Allium sativum*, can be used as an alternative herbal medication for BV [18]. Durairaj et al. [19] in 2009 demonstrated inhibited growth effects of garlic extract on Gram-positive and Gram-negative bacterial strains in vitro. Tea tree oil, garlic boric acid douching, and yogurt can be used as alternative herbal treatments for BV and yeast vaginitis [20]. In the study by Gutman et al., great discharge was the most common complaint among the BV patients. Additionally, they showed that both treatments (garlic vaginal cream and metronidazole vaginal gel) had the same effect on reducing the discharges [12].

Dolatian et al. also revealed a significant decrease in patient complaints and Amsel's clinical criteria related to treatment of BV following treatment with garlic vaginal cream and metronidazole vaginal gel. They concluded that garlic vaginal cream was an appropriate alternative to metronidazole vaginal gel [21].

Thyme, with the scientific name *Zataria multiflora*, is another herb introduced as a new source for treatment of infections. Thyme is a member of the Labiatae family that grows wild in central and southern Iran, Afghanistan, and Pakistan [22]. Thyme has been introduced as an anti-parasitic, anti-fungal, and anti-bacterial agent and contains two important components, namely thymol and carvacrol, which have antimicrobial properties [23]. Thyme also has therapeutic effects on respiratory disorders (such as bronchitis, laryngitis, and tonsillitis), gastrointestinal disturbances, minor wounds, and common cold, and is used as an antibacterial agent in oral hygiene [24]. Simbar et al. [22] showed that patients' complications and Amsel's criteria significantly decreased after treatment with *Z. multiflora* similar to metronidazole vaginal gel ($P < 0.05$).

Considering the various side effects of metronidazole and increased antibiotic resistance associated with these chemical drugs, an alternative treatment with fewer complications is needed to be introduced. Therefore, the present study aims to compare the effects of Mycocin vaginal cream (made of garlic and thyme) and metronidazole vaginal gel on treatment of patients with BV.

MATERIALS AND METHODS

The present randomized clinical trial (Identifier: IRCT2014062718187N2) aimed to compare the therapeutic effects of Mycocin vaginal cream made of *A. sativum* and *Z. multiflora* (Goldaru, Esfahan, Iran) and metronidazole vaginal gel on BV. Mycocin was prepared as a cream in a 50g tube containing 19-22 mg allein of *A. sativum* and 8-12mgthymolof *Z. multiflora* (registration no. 1228103469). Besides, metronidazole was made by Parsdarou Company, as in a 0.75% g vaginal gel (serial No. 2625).

This study was conducted on 120 married women in reproductive ages (aged 18-44 years) who suffered from BV. The participants were randomly divided into two groups of 60. The study was performed in the infertility clinic of Mother and Child hospital affiliated to Shiraz University of Medical Sciences which is a large referral center in south of Iran. The exclusion criteria of the study were pregnancy, breast feeding, consumption of antibiotics and anti parasitic, anti-coagulant, immunosuppressive, or vaginal drugs during the last 14 days, alcohol consumption, recognized medical diseases, abnormal uterine bleedings, recurrent vulvo vaginitis, and having several sexual

partnerships. The women were also excluded from the study in case trichomonas or candida was there in their samples. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences. Besides, the entire participant signed written informed consents for participation in the study and completed the demographic information questionnaire.

Any signs of vaginitis and abnormal discharge were observed and examined vaginally using a speculum. Vaginal secretion samples were collected from the upper-lateral side of the vaginal wall using a sterile swab. Then, the samples were applied onto three slides. Normal saline was added to the first specimen to observe the probable existence of clue cells, *Trichomonas vaginalis*, and *Candida* (hyphae or mycelium). One drop of 10% KOH solution was added to the second specimen to carry out the Whiff test (the release of an amine (fishy) odor upon addition of 10% potassium hydroxide to the vaginal fluid) and to assess the possible presence of *Candida* (hyphae or mycelium) microscopically. Finally, the third specimen was fixed by methanol to be used for Gram stain and diagnosis of BV by NSS. The samples' pH was evaluated by pH-meter paper (Nagel- Germany-Machery Co.).

All the laboratory procedures were performed in the laboratory of Mother and Child hospital. Presence of three out of the four Amsel's criteria was used for diagnosis of BV as follows: 1- presence of clue (vaginal epithelial) cells in wet smear and vaginal samples, 2- a homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls, 3- a vaginal pH higher than 4.5, and 4- positive Whiff test result [23, 24]. Also, Gram stain diagnosis of BV was based on obtaining a score between 7 and 10 in NSS.

The two study groups were required to use Mycocin and Metronidazole for 7 nights. The patients were asked about regular usage of the drugs and the probable drug complications through a phone call 4–7 days after the treatment period. In the follow-up visit, the whole diagnosis procedures were repeated and the BV treatment was considered successful if no Amsel's criteria and negative Gram stain results were detected.

Statistical analysis

The data were entered into the SPSS statistical software (version 18.0) and were analyzed using student's T test, Chi-square, Fisher's exact test, and McNemartest. McNemar was used to compare the signs of BV and Amsel's criteria before and after the treatment. Besides, Fisher's exact test was used to compare the results between the study groups. P-values < 0.05 were considered as statistically significant.

RESULTS

In this study, 4 patients were excluded within three days after the beginning of the study: one due to bleeding during the study and three because of intolerance to metronidazole. Nevertheless, they were replaced by other patients. Thus, a total of 120 women, 60 in each group, completed the study.

Table 1. Demographic and reproductive characteristics of the participants

| Group | | Mycocin (n=60) | Metronidazole (n=60) | P-value |
|-------------------------------------|----------------------|----------------|----------------------|---------|
| Age (years) | Individuals | 29.9 ± 6.4 | 30.1 ± 7.3 | 0.83 |
| | Husbands | 33.6 ± 7.2 | 35.7 ± 10.2 | 0.19 |
| Age at marriage | | 20.7 ± 3.3 | 18.5 ± 4.2 | 0.3 |
| Occupation n (%) | Housewife | 44 (73.3) | 45 (75) | 0.83 |
| | Employee | 16 (26.7) | 15 (25) | |
| Education level n (%) | Illiterate | 3 (5) | 7 (11.7) | 0.45 |
| | Under diploma | 14 (23.4) | 17 (28.3) | |
| | Diploma | 34 (56.6) | 25 (41.7) | |
| | Bachelor's and above | 9 (15) | 11 (18.3) | |
| Intercourse per week (median (IQR)) | | 2 (1-4) | 2 (1-3) | 0.3 |
| Contraception n (%) | | 37 (61.7) | 42 (70) | 0.3 |
| Abortion | | - | - | - |
| Repeated infection (median (IQR)) | | 2 (1-2) | 1 (1-2) | 0.2 |
| Pregnancies (median (IQR)) | | 1.5 (0-5) | 2 (0-6) | 0.1 |
| Cesarean delivery | | - | - | - |

The study results revealed no significant difference between the two groups in terms of demographic and reproductive characteristics, such as age, age at marriage, occupation, education level, frequency of intercourse per

week, frequency of repeated infection, number of pregnancies, number of abortions, number of cesarean deliveries, and frequency of using contraceptive methods ($P>0.05$) (Table 1).

The results showed a significant decrease in Amsel's criteria after treatment with Mycocin vaginal cream and metronidazole. Clinical improvement in Mycocin and metronidazole groups was 86.66% and 78.33%, respectively, but the difference was not statistically significant. In other words, Mycocin and metronidazole were equally effective in terms of clinical improvement. Nonetheless, most of the clinical improvement criteria, including KOH, clue cell, malodor vaginal secretion, itch, and abdominal pain, showed greater improvement in the patients under Mycocin treatment in comparison to the metronidazole group although the difference was not statistically significant (Table 2).

Table 2. Comparison of clinical improvement in the women with bacterial vaginosis before and after the treatment with mycocin and metronidazole

| Clinical index | | Before treatment | After treatment | P-value |
|---------------------------|---------------------|------------------|-----------------|---------|
| KOH | Mycocin ® n (%) | 44 (73.3) | 9 (15) | 0.001 |
| | Metronidazole n (%) | 47 (78.3) | 12 (20) | 0.001 |
| | P value | 0.522 | 0.471 | |
| Clue cell | Mycocin ® n (%) | 60 (100) | 8 (13.3) | 0.001 |
| | Metronidazole n (%) | 60 (100) | 14 (23.3) | 0.001 |
| | P value | - | 0.157 | |
| PH>4.5 | Mycocin ® n (%) | 53 (88.3) | 15 (25) | 0.001 |
| | Metronidazole n (%) | 49 (81.7) | 16 (26.7) | 0.001 |
| | P value | 0.306 | 0.835 | |
| Grayish-white discharge | Mycocin ® n (%) | 56 (93.3) | 13 (21.7) | 0.001 |
| | Metronidazole n (%) | 58 (96.7) | 18 (30) | 0.001 |
| | P-value | 0.402 | 0.297 | |
| Malodor Vaginal secretion | Mycocin ® n (%) | 40 (70) | 5 (8.3) | 0.001 |
| | Metronidazole n (%) | 40 (66.7) | 10 (16.7) | 0.001 |
| | P-value | 0.695 | 0.168 | |
| Itch | Mycocin ® n (%) | 32 (53.3) | 10 (16.7) | 0.001 |
| | Metronidazole n (%) | 30 (50) | 12 (20) | 0.001 |
| | P-value | 0.715 | 0.637 | |
| Abdominal pain | Mycocin ® n (%) | 27 (45) | 5 (8.3) | 0.001 |
| | Metronidazole n (%) | 32 (53.3) | 8 (13.3) | 0.001 |
| | P value | 0.361 | 0.378 | |
| Painful intercourse | Mycocin ® n (%) | 33 (55) | 11 (18.3) | 0.001 |
| | Metronidazole n (%) | 25 (41.7) | 8 (13.3) | 0.001 |
| | P-value | 0.144 | 0.453 | |

DISCUSSION

Garlic contains 33 sulfuric compounds; allein becomes a powerful anti-fungal and anti-microbial effects. Alleinase enzyme convert sallein into allicin. Allicin works through disruption of the thiol group of the bacterial enzymes and synthesis of bacterial RNA and proteins [25, 26]. A possible mechanism for the antimicrobial effect of allicin and garlic concentrate might be related to cross-linking of proteins and SH-enzymes. Allicin irreversibly inhibits SH-protease and NADP(+)-dependent alcohol dehydrogenase. Furthermore, the glutathione inside the mammals' cells can neutralize allicin activities. Sasso et al. [27] investigated the inhibitory effect of thymol on *Escherichia coli* and *Staphylococcus aureus* adhesion to human vaginal cells. They revealed that thymol inhibited bacterial adhesion and interfered with *E. coli* fimbriation fit a possible mechanism involving interference with surface bacterial appendages.

In the last decade, drug-resistant pathogens produced serious risks for public health. For instance, Mycoplasma and Mobiluncus, which play an important role in developing BV, are resistant to antibiotics [28]. Mastromarino et al. (2013) [29] in a literature review concluded that treatment of BV with recommended oral or vaginal antibiotics was often associated with treatment failure or high recurrence rates. In 2014, Mohammadzadeh et al. [30] demonstrated that the therapeutic effect of garlic tablet on BV were similar to that of oral metronidazole and Amsel's criteria were significantly decreased after both treatments. Nevertheless, they indicated that metronidazole was associated with significantly more complications [30]($P=0.032$). Verstraelen et al. [31] also reported that many gastrointestinal side effects, such as nausea, vomiting, and diarrhea, occurred in the patients under metronidazole treatment. Allicin

antimicrobial effects on the host cells without any adverse effects was one of the reasons for replacement of other chemical drugs with garlic [32].

In another study, 80% of the patients with mixed vaginitis were treated by using the vaginal creams which were made of garlic. Thus, they concluded that garlic vaginal cream was an appropriate alternative to metronidazole vaginal gel [21].

Thyme is an herbal medication affecting Gram-positive and Gram-negative bacteria [33]. Previous studies revealed the therapeutic effects of the essence of *Z. multiflora* cream on clinical improvement of candida vaginitis [34, 35] as well as its destroying effects on *Trichomonas vaginalis* in vitro [36], some Bacillus species [37], and *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* [38]. The phenolic components of *Z. multiflora* (thymol and carvacrol) could cause disturbance effects on membrane fatty acid components of microbial cells [39].

Simbar et al. [22] showed that the patients' complications and Amsel's criteria significantly decreased after treatment with *Z. multiflora* similar to metronidazole vaginal gel ($P < 0.05$). Hence, they supposed that *Z. multiflora* could be an appropriate choice for BV treatment, especially for the patients affected by metronidazole side effects.

The present study results demonstrated significant improvement in Amsel's clinical criteria after treatment with Mycocin vaginal cream and metronidazole. Additionally, no significant difference was observed between the two treatment methods regarding improvement of Amsel's clinical criteria. These findings were consistent with those of Dolatian et al. who found that micosin (made by garlic) vaginal cream had similar efficacy to metronidazole vaginal gel in improving Amsel's criteria. They reported 80% and 70% clinical improvement based on Amsel's criteria in micosin and metronidazole treatment groups, respectively [21]. Our results also showed 86.66% and 78.33% clinical improvement in Mycocin and metronidazole groups, respectively. In other studies, improvement rate of Amsel's criteria after treatment with metronidazole was reported as 76.8% [28] and 83.3% [40].

However, evidences has revealed several complications following application of *Z. multiflora*, including nausea, vaginal dryness, burning vagina [22], and skin and mucosal irritation [33]. Furthermore, several complaints associated with *A. sativum* intake included "garlic breath" and body odor, allergic reactions (such as allergic contact dermatitis, angioedema, pemphigus, and anaphylaxis), alteration of platelet function and coagulation (with a possible risk of bleeding), and burns (when fresh garlic is applied on the skin) which may enhance the pharmacological effect of anticoagulants (*e. g.* warfarin, fluindione) and reduce the efficacy of anti-AIDS drugs [41]. Our findings showed no complications following the application of mycocin. Thus, these complications may decrease if the combined form of *A. sativum* and *Z. multiflora* is used for BV treatment.

In the present study, all the patients (100%) in Mycocin and metronidazole groups had clue cells in their wet smear and vaginal samples. This finding is in agreement with that of the study by Gutman et al. [12] which indicated that 93% of the BV patients had clue cells in their wet prep examinations. Clue cells also showed a high specificity of 86% and 92.4% in the studies by Simoes et al. [1] and Amsel et al. respectively. Thus, it is considered as the most valuable criterion in diagnosis of BV [42].

Simbar et al. [22] demonstrated the prevalence rate of gray-white homogenous discharge to be 100% among the subjects in both metronidazole and oregano groups. Similarly, our results displayed the mean prevalence of gray-white homogenous discharge to be 95% among the study patients.

Another Amsel's criteria; i.e., Whiff test, was reported with a sensitivity of 33.9% and a specificity of 86.9% [43]. Finally, vaginal discharge pH was described to be of a high sensitivity (97%), but a low specificity (26%) [1].

In the present study, 75% of the patients showed positive results in Whiff test and nearly 85% of the patients in both groups had pH >4.5. Dolatian et al. reported that 84% and 74% prevalence of pH > 4.5 in vaginal discharge of the subject in mycosin group and metronidazole group, respectively [21].

In conclusion, practitioners can use herbal alternatives, such as garlic and thyme, which have similar effects to metronidazole on BV. Antibiotics have high probability of BV recurrence because of drug-resistance pathogens and numerous side effects. Thus, garlic and thyme can be offered as an appropriate alternative for treatment of BV.

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