

Evaluation of the Therapeutic Effect of 15 ppm Silver Nanoparticle Spray Compared to Clotrimazole 1% on Candida Vaginitis: A Randomized Controlled Clinical Trial

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Abstract

Objective: In vitro and in vivo researches have shown that silver nanoparticles have more antimicrobial properties with a lower concentration than antifungal agents against candida vaginitis. Therefore, this study evaluated the therapeutic effect of silver nanoparticles (Nivasha spray 15ppm) compared to clotrimazole 1% vaginal cream on candida vaginitis.

Materials and methods: In this clinical trial study, 110 women with confirmed candida vaginitis randomly were divided into test (n=58) and control (n=52) groups. Silver nanoparticles spray with an applicator (Nivasha 15 ppm), and clotrimazole 1% were administered to test and control groups, respectively. Then, within ten days, post-intervention checkup and patient self-reported for treatment results were recorded in checklists and the data were analyzed statistically.

Results: The improvement rate in test group (98.0%) was 1.44 times higher than in control (67.9%). Moreover, disease symptoms after the intervention (including unusual secretions, itching and burning, redness) in test group were significantly less than in the control, but there was no significant difference in the ratio of edema in two groups (p=0.071). Furthermore, the average recovery time (days) of all symptoms in test group was lower than control (p<0.05). Finally, the rate of patients' satisfaction with the treatment process in the test group (76.9%) was more than control (46.6%) (p=0.004).

Conclusion: Nivasha spray had more effectiveness compared to the clotrimazole 1%. Therefore, it can be used as an alternative drug in the treatment of Candida vaginitis.

Keywords: Silver Nanoparticles; Candida; Vaginitis; Clotrimazole; In Vitro; In Vivo

Introduction

Vaginal infection or vaginitis is the most common

problem of women's diseases and is the cause of more than 10 million office visits per year (1). This disorder can occur at any age, but women of reproductive age are more susceptible to it. The most common symptoms of vaginal infection are itching,

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change in color and amount of odorless secretions, pain or burning during urination, pain during intercourse, bleeding or spotting (2). Normally, a healthy vaginal environment is acidic enough as 3.8-4.5 to prevent the growth of pathogens such as bacteria and fungi (3). Factors such as using inappropriate detergents change the acidity of the vagina and destroy the beneficial bacteria in the vagina which give chance for pathogenic agent to grow and cause infection in the place (4). In addition, factors such as long-term use of oral antibiotics that destroy the beneficial bacteria in the body, stress, sexual intercourse, hormonal changes (pregnancy, menopause), steroids, lack of personal hygiene, uncontrolled diabetes, and the use of drugs that are taken vaginally increase the risk of developing vaginal infection. Vaginal infection is associated with various side effects such as premature birth and low birth weight babies. The most common types of vaginal infections are fungal, bacterial and trichomonas vaginitis, which are mainly caused by candida species (*albicans*), bacteria (*gardenerlla vaginalis*), and parasites (*trichomonas vaginalis*), respectively (4). Bacterial vaginitis is caused by the entry and excessive growth of non-flora bacteria in the vaginal environment and is the most common infection of women of reproductive age (40 to 50%) (5). Trichomoniasis is transmitted through unprotected sex with an infected person and is more common in women than men (6). Candida vaginitis is the second most common cause of genital tract infection in women aged 20-50 years (7, 8). So that 75% of women suffer from vaginal candidiasis at least once and 45% of women suffer from vaginal candidiasis twice or more during their lifetime. A limited number of women (5%) may also experience relapse and chronic infection (9). The occurrence of this disease is estimated to be approximately 13 million cases per year (10), and the prevalence of this disease varies according to health, cultural and social factors. So that the rate of this infection has been reported in Turkey (17.04%) (11), Iraq (39%) (12) and Netherland (12.6%) (13) and in different regions of Iran (30-47%) (14). *Candida albicans* is responsible for 85-90% of candida vaginitis, and 10% of infections are caused by other types of fungi that are usually more resistant to treatment (15). *Candida* normally exists in different parts of the body, such as the mouth, throat and skin, digestive tract and vagina, and works in a balanced state with other microorganisms, but if the balance of the body's

normal flora is disrupted due to predisposing factors, an infection occurs (16). Also, fungal vaginitis, unlike the other two types of trichomonad and bacterial vaginitis, does not change the pH of the vagina (17) and is usually diagnosed by taking a history and examination, and in some cases by preparing a smear and culture from secretions (18). The treatment of vaginal infection depends on the cause of the infection. So that, tablets/cream/metronidazole gel or cream/ clindamycin gel are prescribed for bacterial infection, antifungal cream for yeast infection, and metronidazole or tinidazole tablets for trichomoniasis (19). In recent years, *Candida* species have appeared as important cases of invasive infections, and its resistance to standard antifungal drugs and the prevalence of this disease are increasing, and the risks arising from it seriously threaten women's society (20). This organism is a serious problem due to its tendency to relapse, since it is much more difficult to control recurrent cases of infection (21). Also, the toxicity of antifungal drugs, the development of resistance in fungi, and drug interactions indicate the importance of investigating the therapeutic effects of new drug combinations (22). Nano biotechnology is one of the branches of nanotechnology science, so that in the field of nanotechnology, the processes of nanoparticle synthesis in the appropriate size and chemical structure are important (23). Nanostructures and nanoparticles in particular have unique physical and chemical properties that can be used to facilitate the management of antimicrobial drugs and to overcome some limitations in traditional antimicrobial treatment methods (22). Meanwhile, silver nanoparticles are one of the commonly used nanoparticles in nanotechnology and have many applications in biosensors, pharmaceutical and medical sciences. So that, they can be used as antibacterial and anticancer agents (24). Silver nanoparticles are small in size and spherical in shape, which easily pass through the cell membrane and cause cytotoxicity against a wide range of cells through oxidative stress, destruction of mitochondria and DNA, and induction of apoptosis (25), but silver nanoparticles are harmless in specific doses (26). According to Lotfali et al. (27), silver nanoparticles showed more antifungal effect compared to amphotericin B and fluconazole and it can be used as an antifungal drug against different species of candida (*candida albicans*, *candida glabrata* and *candida tropicalis*).

Considering the physicochemical properties and

proving the antifungal effects of silver nanoparticles on clinical isolates of candida albicans cells in vitro and in vivo and since some common antifungal drugs have many side effects such as drug resistance, cancer and poisoning, silver nanoparticles are a new therapeutic option for the treatment of resistant cases of candidiasis, and its use can be considered as a drug candidate in the treatment of fungal vaginitis. Therefore, the present study was conducted to investigate the antifungal effect of 15ppm silver nanoparticles (Nivasha spray) compared to clotrimazole 1% cream on patients with candida vaginitis.

Materials and methods

In this clinical trial study, 110 definite candida vaginitis patients were studied from the Isfahan province, the center of the Islamic Republic of Iran. Random sampling was performed to complete the sample size. All patients were registered as known cases of candida vaginitis at the health and treatment centers and midwifery offices of the Isfahan University of Medical Sciences. The University's ethics committee approved the study according to the current version of the Declaration of Helsinki with code number of IR.MUI.MED.REC.240084. Randomization of patients was done according to a preexisting list produced by a computer program. Then, patients were comprehensively briefed and each of them signed a letter of informed consent and baseline data was obtained by a researcher-made questionnaire. The evaluation checklist also included two parts: recording clinical observations and recording laboratory results. So that all patients were carefully examined by two obstetricians and gynecologists, and patients' complaints were recorded in the symptom registration form and the clinical observations of the checklist. Laboratory results also were recorded in the form. In order to determine the infectious agent in people who had candida vaginitis in terms of the history of the disease and complaints, the status of secretions and vaginal pH, a sample was prepared from the secretions of the upper part and the side wall of the vagina using a sterile cotton swab, and it was drawn on a slide and sent to the laboratory (28). After confirmation of candida vaginitis, 110 patients with candida vaginitis were selected based on inclusion and exclusion criteria randomly divided into test (n=58) and control (n=52) groups and patients with a negative test were excluded from the study.

Inclusion criteria were married women with

positive vaginal smear for candida vaginitis (no other vaginal infections) with at least two symptoms of candida vaginitis (itching, burning and irritation, cheesy secretions, burning urine, painful intercourse), being literate, not pregnant/breastfeeding, not menopause, without chronic diseases such as hypertension and diabetes, not using herbal/chemical drugs and corticosteroids during the last two weeks, and lack of sensitivity to clotrimazole 1% cream (28).

Exclusion criteria were pregnancy during treatment, menstruation at the time of the study, abnormal uterine bleeding, forced to use antibiotics to treat non-vaginitis systemic infections during treatment, failure to follow the correct method of treatment (forgetting to use the spray 2 times in a row and 4 times intermittent until the visit on the 10th day), occurrence of treatment complications, unwillingness to continue treatment and non-cooperation in the follow-up (28, 29).

Then, silver nanoparticles spray with an applicator under the Nivasha brand (containing 50 cc of silver nanoparticle solution with a concentration of 15 ppm and health code 9655068437941620, manufactured by Arad Nano Company) as a basic treatment for 10 consecutive days and twice a day and 3 puffs each time was administered to the test group (29) and control group used clotrimazole 1% vaginal cream (@Canesten, Bayer plc Company) (one applicator at night for 7 nights) (28).

Finally, the patients were asked to return to the center ten days after the completion of the intervention with a symptom registration form that was completed by the patients every day, and the effect of the spray and clotrimazole on the symptoms and signs of the disease was examined and recorded in the observation checklist and for the final confirmation of the presence or absence of candida albicans, re-smear from the patients was sent to the laboratory (28).

Statistical analysis: All data were analyzed with SPSS software version 23. The categorical variables were summarized by frequency (percentage) and quantitative variables by mean \pm standard deviation. The proportion of patients with clinical outcomes at the baseline was compared using chi-square test between test and control groups. Relative risk (RR) of the study outcomes (clinical outcome, cure, recurrence, and patient satisfaction) were calculated in test group compared to control. Mean time of improvement in terms of every outcome was calculated and compared using independent sample

T-test in two experimental groups. We used generalized estimating equation approach (GEE) to compare trend of self-reported symptoms improvement during ten follow-up days. The analyses of data were conducted based on the intention-to-treat perspective. P-value < 0.05 was considered significant.

Results

Comparisons of data of test and control groups: A total of 110 patients of Candida vaginitis (58 test and 52 control) were included in the study.

Mean age of all of patients was 37.57 and was not different between two groups, statically (p=0.754). Furthermore, the rate of have a job was 73.3% in test group and 82.7% in control group (p=0.264). Education level also was not statistically different between two groups (p=0.0588).

Demographic characteristics of participants are presented in Table 1. At the baseline, the percentage of symptoms including leucorrhoea, itching and redness was not statistically different between the groups (p<0.05), while the percentage of edema was higher in test group than control (p=0.009).

After intervention, according to the follow-up visit data, the cure rate was 98.0% in test group versus 67.9% in control group, so that the chance of cure was 44% more in test group (RR=1.44, p<0.0001). Although, after the intervention, the rate of all symptoms decreased in both group, the post-intervention rate of leucorrhoea, itching and redness was smaller in test group compared to control. However post-intervention edema rate was not statistically different between two study arms (2.0% in test group vs. 10.7% in control group, p=0.071).

Although, one person in the test group expressed dissatisfaction with the use of the drug due to increased burning and itching and one person in the control group due to skin redness an inflammation, satisfaction rate was statistically higher among the

patients allocated to test group compared to those allocated to control (76.9% vs. 46.6%, p=0.004) (Figure 1). Table 2 shows baseline (first visit) and follow-up (second visit) clinically-checked symptoms in the test and control groups.

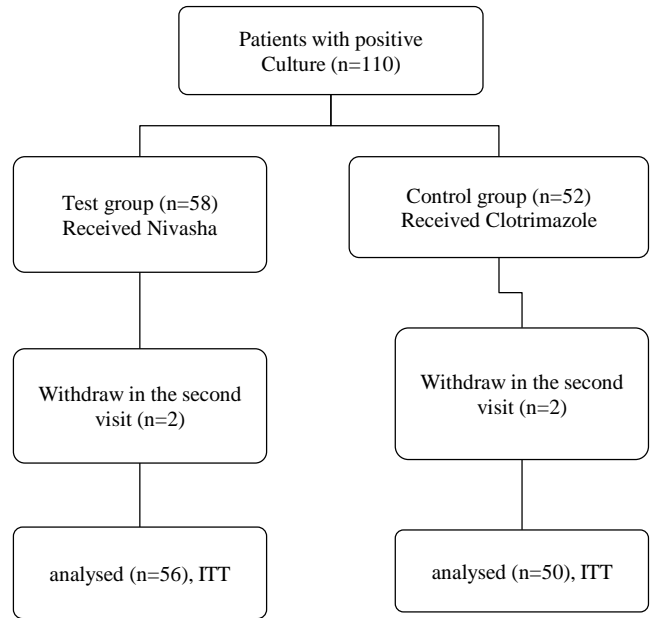


Figure 1: Flow Diagram

Among the patients who had complaint about each specific symptom at the baseline, mean time (day) of treatment was statistically fewer in test group compared to control group (p<0.05, Table 3). The mean time of relief from leucorrhoea, itching, redness and edema was 4.18±2.24 (day), 3.70±2.44 (day), 3.75±1.96, and 3.67±2.08 in test group while mean time of relief from these symptoms was more than 6 days in control group (p<0.05).

Along with clinical baseline and post-intervention checkup, self-reported symptoms were also asked patients during 10 sequential follow-up days.

Table 1: Demographic characteristics of participants

Variables	Test (n=58)	Control (n=52)	Total	P-value
Age(year)	37.22+10.87	37.89+10.99	37.57+10.89	0.754
Job status				
Employed	33(73.3)	43(82.7)	76(78.4)	0.264
Unemployed	12(26.7)	9(17.3)	21(21.6)	
Education level				
Under diploma	15(31.9)	19(41.3)	34(36.6)	0.588
Diploma	16(34.0)	15(32.6)	31(33.3)	
Upper diploma	16(34.0)	12(26.1)	28(30.1)	

Table 2: The baseline and follow-up clinically-checked symptoms in two groups

Symptoms	Test (n=58)		Control (n=52)		chi-square	p	RR
	n. event/n. total	(%)	n. event/n. total	(%)			
Leucorrhoea							
First visit	46/52	(88.5)	49/58	(84.5)	0.37	0.544	0.13
Second visit	2/50	(4.0)	17/56	(30.4)	12.47	<0.0001	
Itching							
First visit	42/52	(80.8)	47/58	(81.0)	0.001	0.972	0.24
Second visit	3/50	(6.0)	14/56	(25.0)	7.08	.008	
Redness							
First visit	26/52	(50.0)	23/58	(39.7)	1.19	0.276	0.16
Second visit	1/50	(2.0)	7/56	(12.5)	4.17	.041	
Edema							
First visit	24/52	(46.2)	13/58	(22.4)	6.92	0.009	0.19
Second visit	1/50	(2.0)	6/56	(10.7)	3.25	.071	
Cure	49/50	(98.0)	38/56	(67.9)	16.31	<0.0001	1.44
Failure	1/50	1(2.0)	18/56	(32.1)			0.06
Satisfaction	40/46	(76.9)	27/45	(46.6)	8.51	0.004	1.65

RR: relative risk

Figure 2 shows the improvement rate of the symptoms among two study arms. GEE analysis revealed a steeper reduction rate of self-reported leucorrhoea (p for day × group effect = 0.007), itching (p for day × group effect = 0.02), redness (p for day × group effect = 0.023) and edema (p for day × group effect < 0.001) in test group compared to control.

Discussion

These days' works on nanoparticles are being carried out using it as a therapeutic agent and among different nanoparticles, nanosilver is the most mentioned nanomaterial and is evolving as the most effective nanoparticle with variety of applications (30). Silver nanoparticles possess increased antifungal activity against Candida species (31). For this reason, in this study for the first time, antifungal activity of 15ppm silver nanoparticle spray (Nivasha) was studied in comparison with clotrimazole and it was found that the rate of improvement in test group

is more than control group.

These findings are in agreement with the studies conducted by other researcher (22, 32, 33) that showed the strong antifungal effects of silver nanoparticles with a diameter of 10 nm by disrupting the membrane structure of candida albicans isolated from patients with candida vaginitis in laboratory conditions.

In vivo, similar results also were seen in 2021 by Jia et al study (34) explored the effect of silver nanoparticles (AgNPs) on fluconazole-resistant candida albicans and the synergism between AgNPs and fluconazole more thoroughly and extensively. So that, AgNPs and fluconazole co-treated mice had significantly lower average log₁₀ cfu/tissue compared with AgNPs or fluconazole-treated mice in all disseminated stains, and the overall survival rate was significantly improved.

In vitro, many studies also pointed to the effect of silver nanoparticles on candida albicans.

Table 3: Mean time (days) of remission in terms of all symptoms

Symptoms	Test (n=58)	Control (n=52)	Total	P-value
Leucorrhoea (number)	45	47	92	<0.001
	4.18±2.24	7.28±2.41	5.76±2.79	
Itching (number)	40	46	86	<0.001
	3.70±2.44	6.13±3.09	5.00±3.04	
Redness (number)	24	24	48	
	3.75±1.96	6.08±3.43	4.92±3.00	0.006
Edema (number)	24	14	38	
	3.67±2.08	6.79±3.81	4.82±3.18	0.002

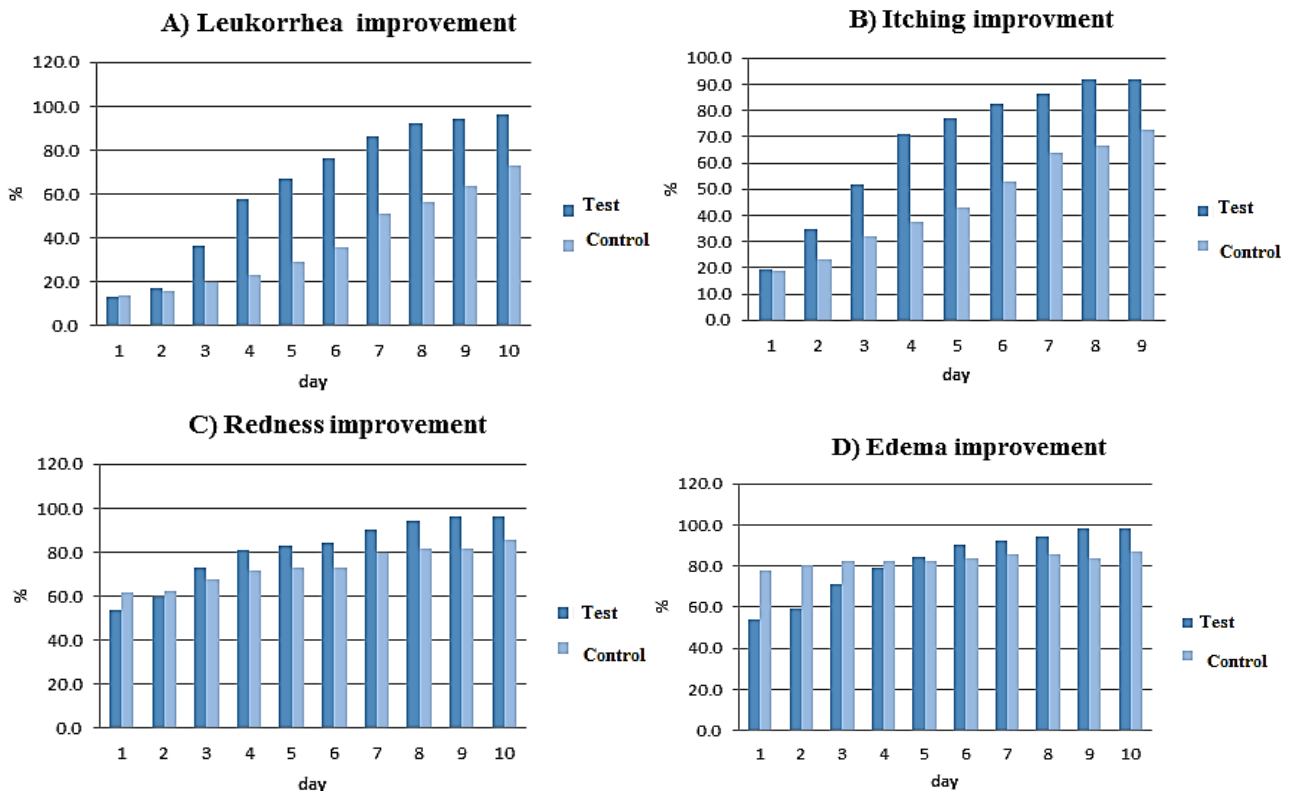


Figure 2: Percentage of self-reported symptoms (A: leucorrhoea, B: itching, C: redness, D: edema) during the days of follow-up in the test and control groups

For example, Nasralhi and colleagues (35) reported the significant antifungal activity of silver nanoparticles against two types of fungi, *Candida albicans* and *Saccharomyces cerevisiae*, so that the electron microscope analyzes showed that this compound exerts its antifungal activity by disrupting the membrane potential and forming pores in the cytoplasmic membrane of the mentioned fungi. In another study, Carrizales and colleagues (36) also investigated the *in vitro* activities of two groups of antimicrobial agents alone and in combination with AgNPs against ten resistant clinical isolates (against several drugs) as well as their cytotoxicity and showed that infections caused by multidrug-resistant microorganisms can be treated using a synergistic combination of antimicrobial drugs and AgNPs.

Also, there is differences in the prevalence of *Candida vaginitis* that has been linked to risk factors such as high sexual activity, use of contraceptives, poor hygiene and antibiotics/ drug abuse as is common in the younger, more sexually active age-groups (37). So that, some studies have shown that the prevalence of *Candida* increased significantly in women below 35 years. For example, Chiaka

Mbakwem-Aniebo showed that the age group 20-29 years had the greatest prevalence of *Candida* species (37). Another similar study by Emeribe (38) reported the prevalence of *Candida albicans* to be 6.5% out of 200 individuals predominantly aged 20 to 30 years. In contrast, mean age of all of our patients was 37.57 and was not different between two groups, statically. Similarly, Alo et al. study (39) observed a higher prevalence in the 36 to 40 age-group while the age-group 20 – 25 years had the lowest prevalence.

In addition, Xianling Zeng (40) study showed that occupation, and educational level posed almost no effect on the occurrence of candidiasis, but the results of our study showed more occurrence of candidiasis infection in working people. Moreover, there was no statistically significant difference between the two groups in terms of other individual-social characteristics, including marital status, level of education, and occupation of the patient.

In the present study, after the intervention, the rate of all symptoms decreased in both group, but the post-intervention rate of leucorrhoea, itching and redness was smaller in test group compared to control. Moreover, post-intervention edema rate was

not statistically different between two study arms. In agreement with our study, the results of Meneses study (41) also suggest that the silver nanoparticles in the starch showed different antifungal susceptibility patterns than clotrimazole. So that, clotrimazole as azole-type antimycotic drug interferes with the biosynthesis of ergosterol, which is an important component of the fungal cell membrane.

Furthermore, studies showed that in most situations, it will take an average of 10 to 14 days for the candidiasis infection to clear up completely. For instance, the overall average duration of treatment for candidemia in Rola Husni study (42) was 14 days. Unlike other studies, the median time on treatment for our patients was 4-7 days and statistically was fewer in test group (4 days) compared to control group (7 days). Moreover, satisfaction rate in test group was statistically higher than the patients allocated to control but only one person in the test group and one person in the control group expressed dissatisfaction with the use of the drugs due to increased burning, itching, skin redness and inflammation.

One of the strengths of the present study is the homogeneity of the study groups (in terms of intervening variables including age, education level, etc.) and the elimination of some of them by considering the inclusion and exclusion criteria, random assignment of control and test groups.

Moreover, since none of the drugs showed any side effects, the total adherence for both groups was > 90% throughout the study and the 102 subjects completing the study reported taking all of the prescribed medication. Because of limitations of our study including the type of the study (case-control study), inability to blind study because of the interventional nature of the study, low sample size, and other possible factors, further studies should be performed in larger multivariable cohorts and controlled trials to express more precise evidence in the future and to generalize this idea to a larger population.

Conclusion

Considering the positive potential of Nivasha spray 15ppm in the treatment of candidiasis, it can be suggested as a suitable drug for the treatment of candidiasis.

Conflict of Interests

Authors declare no conflict of interests.

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References

1. Vrablik J, Masata J, Jedlickova A, Hajickova M. Prospective study the prevalence of different candida strains and their sensitivity to different antimycotic treatment in women with vulvovaginal candidiasis. *Ceska Gynekologie*. 2007;72(1):27-32.
2. Kent HL. Epidemiology of vaginitis. *American journal of obstetrics and gynecology*. 1991;165(4):1168-76.
3. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences*. 2011;108(supplement_1):4680-7.
4. Sobel JD. Bacterial vaginosis. *Annual review of medicine*. 2000;51:349.
5. Aali B, Tohidi A. Prevalence of candida vaginitis among symptomatic patients in kerman. *Journal of Inflammatory Diseases*. 2000;4(1):42-8.
6. Turovskiy Y, Sutyak Noll K, Chikindas ML. The aetiology of bacterial vaginosis. *Journal of applied microbiology*. 2011;110(5):1105-28.
7. Schumann JA, Plasner S. Trichomoniasis. *StatPearls [Internet]: StatPearls Publishing*; 2021.
8. Akbarzadeh M, Bonyadpoure B, Pacshir K, Mohagheghzadeh A. Causes and clinical symptoms of vaginal candidiasis in patients referring to selective clinics of Shiraz University of Medical Sciences (2009). *Journal of Arak University of Medical Sciences*. 2010;13(3):12-20.
9. Vacheva-Dobrevski R, Kovachev S, Nacheva A, Stoev S, Vasilev N. Comparative study of itraconazole and fluconazole therapy in vaginal candidosis. *Akusherstvo i Ginekologija*. 2004;43(1):20-3.
10. Mashburn J. Vaginal infections update. *Journal of midwifery & women's health*. 2012;57(6):629-34.
11. Ece G, Samlioglu P, Akkoçlu G, Atalay S, Kose S. The evaluation of the distribution of yeast like fungi 'Candida Species' at a tertiary care center in western Turkey. *International Journal of Medical Sciences*. 2012;9(7):617.
12. Mohammed HR, Musa JA, Mhawish MZ, Hussein FJ, Kareem ZH. COMPARATIVE STUDY OF CANDIDA ALBICANS IN IRAQ. *World Bulletin of Public Health*. 2022;9:142-8.

13. Hosseini SS, Ghaemi E, Niknejad F, Joshaghani H, Rabiee M, Azadi F, et al. Investigation of the relationship between different *Candida* species and some risk factors affecting the incidence of Vulvovaginal Candidiasis. *The Iranian Journal of Obstetrics, Gynecol Infert.* 2019;22(7):13-22.
14. Sasani E, Rafat Z, Ashrafi K, Salimi Y, Zandi M, Soltani S, Hashemi F, Hashemi SJ. Vulvovaginal candidiasis in Iran: A systematic review and meta-analysis on the epidemiology, clinical manifestations, demographic characteristics, risk factors, etiologic agents and laboratory diagnosis. *Microbial pathogenesis.* 2021;154:104802.
15. Sobel JD, Suprapaneni S. *Candida parapsilosis* vaginal infection—a new site of azole drug resistance. *Current Infectious Disease Reports.* 2018;20:1-4.
16. Parveen SD. An approach to etiology, diagnosis and management of different types of candidiasis. *Journal of Yeast and Fungal Research.* 2013;4(6):63-74.
17. Saturnino A, Sisenando H, Pereira AR, Vale A, Pires LM, de Araújo JT, et al. vulvovaginitis: Vaginal pH changes and associated microflora. *Acta Cirurgica Brasileira.* 2005;20:266-9.
18. Bonyadpour B, Akbarzadeh M, Mohagheghzadeh AA. Characterization of *Candida* species isolated from cases of vulvovaginitis in women referring to selected gynecological clinics. *Journal of Midwifery and Reproductive Health.* 2016;4(4):741-7.
19. Hainer BL, Gibson MV. Vaginitis: diagnosis and treatment. *American family physician.* 2011;83(7):807-15.
20. Arastehfar A, Kargar ML, Mohammadi SR, Roudbary M, Ghods N, Haghighi L, Daneshnia F, Tavakoli M, Jafarzadeh J, Hedayati MT, Wang H. A high rate of recurrent vulvovaginal candidiasis and therapeutic failure of azole derivatives among Iranian women. *Frontiers in microbiology.* 2021;12:655069.
21. Shakuntala C, Kaur KP. SHORT ANOVAGINAL DISTANCE, A RISK FACTOR FOR RECURRENT VAGINITIS. *INDIAN JOURNAL OF SEXUALLY TRANSMITTED DISEASES.* 2005;26(1):33.
22. Rahimzadeh-Torabi L, Doudi M, Naghsh N, Golshani Z. Comparing the antifungal effects of gold and silver nanoparticles isolated from patients with vulvovaginal candidiasis in-vitro. *KAUMS Journal (FEYZ).* 2016;20(4):331-9.
23. Sánchez-Moreno P, De Vicente J, Nardecchia S, Marchal JA, Boulaiz H. Thermo-sensitive nanomaterials: recent advance in synthesis and biomedical applications. *Nanomaterials.* 2018;8(11):935.
24. Barkat MA, Beg S, Naim M, Pottoo FH, Singh SP, Ahmad FJ. Current progress in synthesis, characterization and applications of silver nanoparticles: precepts and prospects. *Recent Patents on Anti-Infective Drug Discovery.* 2018;13(1):53-69.
25. Mohammed AE, Al-Qahtani A, Al-Mutairi A, Al-Shamri B, Aabed K. Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. *Nanomaterials.* 2018;8(6):382.
26. Jafarzadeh M, Salouti M, Shokri R. Antifungal Activity of Silver Nanoparticles, Copper Nanoparticles, Their Combination and with Amphotericin B against *Candida glabrata* In vitro and In vivo. *Journal of Arak University of Medical Sciences.* 2017;20(6):12-21.
27. Lotfali E, Toreyhi H, Sharabiani KM, Fattahi A, Soheili A, Ghasemi R, et al. Comparison of antifungal properties of gold, silver, and selenium nanoparticles against amphotericin b-resistant *Candida glabrata* clinical isolates. *Avicenna Journal of Medical Biotechnology.* 2021;13(1):47.
28. Dodangeh N, Kheirkhah M, Abolghasemi J, Mojab F, Farshad F. Comparison of the effects of glycyrrhiza glabra vaginal cream and clotrimazole on the symptoms of fungal vulvovaginitis. *Iran Journal of Nursing.* 2019;32(117):24-34.
29. Boroumand Z, Golmakani N, Mazloun SR, Dadgar S, Golmohamadzadeh S. The effect of spray silver nanoparticles (Nivasha) on intensity of cesarean wound pain; A randomized clinical trial. *The Iranian Journal of Obstetrics, Gynecology and Infertility.* 2018;21(9):83-92.
30. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MdP, Acosta-Torres LS, et al. Nano based drug delivery systems: recent developments and future prospects. *Journal of nanobiotechnology.* 2018;16(1):1-33.
31. Panáček A, Kolář M, Večeřová R, Pruček R, Soukupová J, Kryštof V, et al. Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials.* 2009;30(31):6333-40.
32. Perween N, Khan H, Fatima N. Silver nanoparticles: an upcoming therapeutic agent for the resistant *Candida* infections. *J Microbiol Exp.* 2019;7:49-54.
33. Kim K-J, Sung WS, Suh BK, Moon S-K, Choi J-S, Kim JG, et al. Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. *Biometals.* 2009;22(2):235-42.
34. Jia D, Sun W. Silver nanoparticles offer a synergistic effect with fluconazole against fluconazole-resistant *Candida albicans* by abrogating drug efflux pumps and increasing endogenous ROS. *Infection, Genetics and Evolution.* 2021;93:104937.
35. Nasrollahi A, Pourshamsian K, Mansourkiaee P. Antifungal activity of silver nanoparticles on some of fungi. 2011:233-239.

36. Lopez-Carrizales M, Velasco KI, Castillo C, Flores A, Magaña M, Martinez-Castanon GA, et al. In vitro synergism of silver nanoparticles with antibiotics as an alternative treatment in multiresistant uropathogens. *Antibiotics*. 2018;7(2):50.
37. Mbakwem-Aniebo C, Osadebe AU, Athanasonny E, Okonko IO. Prevalence of *Candida* spp. and age-related disparities amongst women presenting with vaginitis at the Obstetrics and Gynaecology (O&G) Clinic in a Tertiary hospital in Port Harcourt, Nigeria. *African Health Sciences*. 2020;20(1):51-8.
38. Emeribe AU, Nasir IA, Onyia J, Ifunanya AL. Prevalence of vulvovaginal candidiasis among nonpregnant women attending a tertiary health care facility in Abuja, Nigeria. *Research and Reports in Tropical Medicine*. 2015;6:37-42.
39. Alo M, Anyim C, Onyebuchi A, Okonkwo E. Prevalence of asymptomatic co-infection of candidiasis and vaginal trichomoniasis among pregnant women in Abakaliki, South-Eastern Nigeria. *Prevalence*. 2012;2(7):87-91.
40. Zeng X, Zhang Y, Zhang T, Xue Y, Xu H, An R. Risk factors of vulvovaginal candidiasis among women of reproductive age in Xi'an: a cross-sectional study. *BioMed research international*. 2018;2018.
41. Meneses ML, Recalde M, Martin PL, Pardo AG. Antifungal activity of silver nanoparticles and clotrimazole against *Candida* spp. *Brazilian Journal of Pharmaceutical Sciences*. 2022;58.
42. Husni R, Chrabieh R, Dib RW, Vazquez J, Guimaraes T, Fernández A, et al. Timing for step-down therapy of candidemia in non-neutropenic patients: an international multi-center study. *Mediterranean journal of hematology and infectious diseases*. 2021;13(1).

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