

An *in vitro* study of zinc effect on *Trichomonas vaginalis* isolated from infected women

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Abstract: Background: The most common non-viral sexual transmission disease in humans, trichomoniasis, is caused by the protozoan parasite *Trichomonas vaginalis* (STD). Metronidazole is the most frequently recommended of the imidazole drugs used to treat trichomoniasis. In any case, these substances may result in undesired side effects and parasite resistance. As a result, this infection needs to be treated in a different way.

Aim: This study was designed to determine the relation between zinc intake and the *Trichomonas vaginalis* occurrence and also to find out the *in vitro* effect of zinc on *T. vaginalis* viability.

Methods: This study was carried out on 63 pregnant women whom attended (Azadi Teaching, Kirkuk General and Al-Nasr maternity) Hospitals from December 2021 till April 2022. Specimens of vaginal swabs and serum samples were obtained from all pregnant women. The swabs were first examined directly by normal saline on slide microscopically then inoculated onto Modified Diamond medium. Zinc levels and concentrations in the blood were assessed using serum. **Result:** *T. vaginalis* was isolated from pregnant women. The overall prevalence among 63 examined samples were positive by direct microscopically and culture with a rate of 5(8%) while 58 (92%) were negative. The 1.5mg/ml zinc concentration was the most effective on *T. vaginalis* trophozoites number than other concentration of zinc. **Conclusion:** Zinc is a very important anti- *T. vaginalis* and can be used it as dilution washing for treatment process.

Key words: Zinc, *Trichomonas vaginalis*, Effect, *In vitro*.

Introduction:

Normally, Lactobacillus predominates in the human vagina, helping to shield it from other possibly dangerous hazardous microbes and preventing their colonization [1].

A flagellated protozoan called *Trichomonas vaginalis* has five flagella, four of which are found at its anterior region. A thin, non-contractile costa supports the parasite's undulating membrane, which contains the fifth flagellum. The usual length and width of this parasite are 13 and 10 m, though its size and shape might vary. *Trichomonas vaginalis* has a straightforward life cycle that only involves the direct transmission of live trophozoites. It lacks cyst

stage and has trophozoite form, which distinguishes it from many protozoan parasites. Although it has a cosmopolitan distribution and has been found in every socioeconomic group and race, it lacks seasonal variations and has been found in every continent and region [2].

The protozoan flagellate *Trichomonas vaginalis* is the source of trichomoniasis, the most prevalent non-viral sexually transmitted infection. Although extremely common in sexually active women, it has long been disregarded among other potentially infected groups of people. *Trichomonas vaginalis* has received more attention recently due to research showing that it can negatively impact pregnancy and increase the risk of HIV infection. As a result, very sensitive diagnostic tests are now required [3].

The cause of trichomoniasis is the anaerobic parasite *Trichomonas vaginalis*. These parasite-infected pregnant women run the risk of having an unfavorable pregnancy outcome, such as infection following an abortion or hysterectomy, infertility, and increased propensity for malignant transformation in cervical tissues [4]. The most prevalent and treatable sexually transmissible infection, with high prevalence in women of childbearing age, is *Trichomonas vaginalis*. Uncertainty exists over the effects of infection and its treatment during pregnancy. [5]. Low birth weight and early birth have both been linked to *Trichomonas vaginalis* infections and poor reproductive consequences. *T. vaginalis* is a flagellate protozoan that is sometimes associated with poverty and is thought to be sexually transmissible. It has a history of being linked to unfavorable pregnancy outcomes, including premature membrane rupture, preterm delivery, low-birth-weight babies, infertility, and cytological abnormalities of the cervix [6].

In contrast to other STIs, the prevalence of STIs among sexually active women of all ages is shown to be similar across age groups. Transmission is virtually always sexual, and prevalence is highest in women who have had gonorrhoea in the past and have had several sex partners [7]. Preterm birth, an increased risk of post cesarean endometritis, and an increased risk of HIV transmission and infectivity are all associated with pregnancy [8,9,10].

In general, there is a higher risk of ectopic pregnancy, pelvic inflammatory disease (PID), tubal infertility, and cervical cancer. After an incubation period of up to 28 days following sexual intercourse, symptoms appear. Micro-ulcerations and epithelial damage are brought on by *Trichomonas vaginalis*. Only approximately half of all infected women exhibit symptoms. The most typical indications and symptoms are an excessive amount of yellow or green vaginal discharge, vulvar itching, vaginal/vulvar erythema, vaginal odor, and occasionally a red, erythematous cervix, also known as a "strawberry cervix"[10]. Neonatal infection with symptoms is uncommon. Vaginal discharge in newborns is described, and exposure to maternal trichomoniasis is most likely the cause of transmission [11].

Zn²⁺ is crucial to the parasites' metabolic processes. The hydrogenosome, an organelle involved in the metabolism of pyruvate, experiences interference with its operation. In the pathogenic protozoan *Tritrichomonas fetus*, the hydrogenosome is the primary location of the early Zn²⁺ impact [12]. In fact, research on zinc has shown that it has antibacterial properties against a variety of microorganisms, including bacteria, viruses, chlamydia, and fungus [13]. *Trichomonas vaginalis*, which is sensitive to relatively low concentrations of Zn²⁺ chloride and sulfate, is similarly impacted by this antimicrobial effect. The minimal trichomonocidal concentration (MTC) for both is 1.6 mM [14].

The estimated global prevalence of trichomoniasis in 2016 was 0.6% in men and 5.3% in women. It's possible that zinc, which is present in prostatic fluid, contributes to the reduced prevalence of infection in men. *Trichomonas vaginalis* is reported to be inhibited *in vivo* by zinc's anti-trichomonal activity, which is known to occur *in vitro*. The male lower urinary tract's known antimicrobial defense against infections is zinc. *Trichomonas*

vaginalis infection may be prevented in part by the oxidative nature of the male genital tract and high zinc concentration (2.3-15.3 mM) in human prostatic secretions. It is suggested that zinc is toxic to the trophozoite and inhibits several pathogenic factors in the parasite [14].

Aim of study:

This study was designed to determine the relation between zinc intake and the *Trichomonas vaginalis* occurrence and also to find out the *in vitro* effect of zinc on *T. vaginalis* viability.

Methodology:

Agreement consideration:

Ethical issue were obtained from Kirkuk Health Directorate after providing scientific seminar in the medical laboratory techniques department with complete filling information formula which were sent for interviewing and completing with scientific communities in Kirkuk Health Directorate.

Study design:

This is a cross-sectional study was done in Kirkuk city in three main hospitals.

Study subjects and sampling collection:

All samples (63) taken by a sterile cotton-tipped stick and vaginal swabs were used to transport a sample of discharge to the laboratory. A speculum was utilized to help insert the swabs into the vagina. All samples were prone to direct microscopy examination, Then culture on Modified Diamond medium. Serum zinc level was measured to each pregnant.

Study setting and period:

The study was done in three main hospitals (Azadi Teaching, Kirkuk General, Al-Nasr)

The period study was from December 2021 to April 2022 .

Direct Microscopy examination:

The specimens should be studied as soon as possible after collection since *T. vaginalis* is fragile in conditions outside the body. Trichomonads were visible on saline wet mount examination, and the vaginal swabs were present [15]. A high pH in the vaginal fluid (>4.5) is a sign of trichomoniasis. In order to observe the combination under a light microscope for motile *Trichomonas*, a drop of the mixture was placed on a clean, grease-free microscope slide, covered with a coverslip, and observation was performed. Confirmation was then made using a 40 objective lens [16].(figure1)



Figure1: *Trichomonas vaginalis* identification vaginal swab microscopy;

Culture Technique:

Vaginal swabs were taken from the transport media from the positive subject and utilized to inoculate *Trichomonas* on the medium (Diamond medium). The cultures were then cultured at 37°C for a total of 5 days while being monitored daily for motility under a microscope [16].

Diamond medium Component and preparation:

Diamond medium is used for the selective detection and cultivation of *Trichomonas species*

The medium was purchased commercially from stores selling scientific materials from Baghdad the PH (at 25⁰C) 6.0 ± 0.2. , the media was partially modified.

Diamond medium prepared by 5.6 grams in 88 milliliters of distilled water. Heat is sometimes required to completely dissolve a material. To sterilize, distribute in 90-ml bottles, then autoclave at 10 lbs pressure for 20 minutes at 115⁰C, then cool to 50⁰C and aseptically add the following (per 90 ml of medium) [10ml of sterile in Human serum, 0.5 of sterile penicillin(1,000 U/ml) and 0.5ml of streptomycin(150 µg/ml)solutions, 1ml of sterile Nystatine solution 50000IU and 1ml of 20% glucose]as anti-microbials and antifungals, mix thoroughly[17].

The vaginal swab in normal saline solution put 20µL to the 10 ml of modified diamond medium tube then incubated within the dark and in anaerobic condition at 37⁰C for 24 hours.

The number of *Trichomonas* trophozoite in 10 μ L was 8 \times 10³ calculated using hematochamber.

Effect of Zinc sulfate component on *T vaginalis* in vitro:

Prepare microtiter plate to put in it 250 μ L from diamond medium culture that contain of *Trichomonas* with 20 μ L of zinc solution then incubated it at 37 °C for 24 hours , 48 hours and 72 hours to include the rate of effected of zinc on *Trichomonas* growth on hematocyte chamber.

In order to conduct an experiment to test the effectiveness of zinc, a microtiter plate was used to put 250 μ L of the medium in each weel and add 20 μ L of the culture parasite and then zinc was added after diluting it with the following concentrations (0.25, 0.75 and 1.5) mg / ml in each weel and it was incubated for three days at 37 °C and then the parasite number was read daily for three days. For the control group that be used without adding any substance to the negative control group, and 0.75mg/ml of metronidazole was added as the positive control group. Each experiment was conducted for each concentration three iterations. (Figur2)

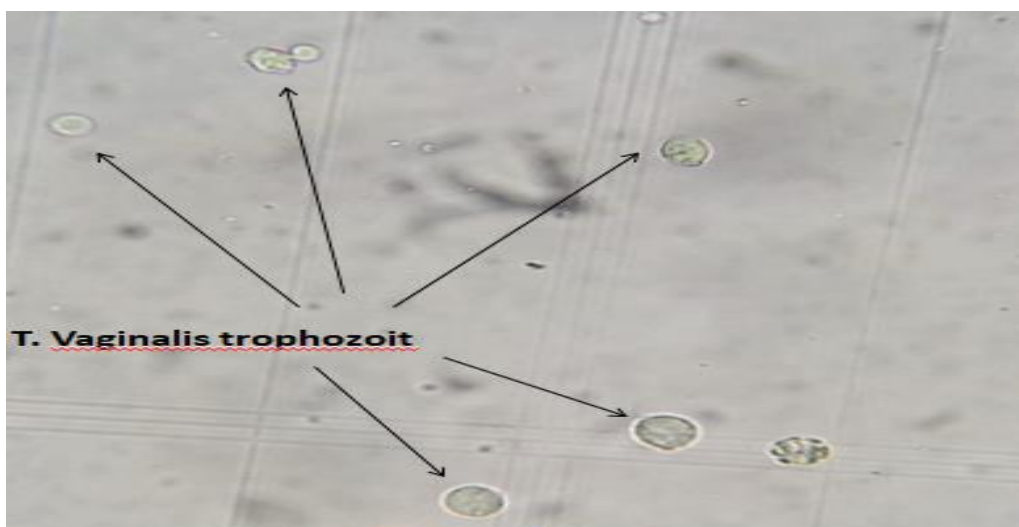


Figure2: *Trichomonas vaginalis* identification on hamatochamber calculator:

The effect and inhibition ratio of the additives were measured using the following mathematical laws below:

- The percentage of effect = (No, of parasite in treatment group/no. of parasite on control group) \times 100
- Inhibition rate = (No, of parasite in control group – No. of parasite on treatment group) / No. of parasite on control group \times 100 [18].

Estimation of zinc level in serum:

The zinc level was estimated in serum samples using spectrophotometer instrument Germany which depending on the rate of absorption and the proportional concentration of zinc in the sample to do this. All procedure was done following the instruction of the kit 50 μ L of serum was added to 1ml of Reagent after mixing well; leave it for 5 minutes at a temperature of 37⁰C or for 10 minutes at 25 degrees. The absorbance read was recorded at the wavelength 560 nm [19].

The result calculated by formula below:

$$\frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} = \text{Result} \times \text{Concentration (200)}$$

Normal value = (70 – 115) µg/dl

Statistical analysis:

Number and percentage was used for calculation of the diseased and control patients. Chi-seqer test was used to determine with the relation between the studied variable and P-value <005 regarded significant.

Result:

According to our result, *Trichomonas vaginalis* was isolated from pregnant women. The overall prevalence among 63 examined samples were positive by direct microscopically and culture with a rate of 5(8%) While 58 (92%) were negative as in TABLE 1

TABLE 1: Number of *T. vaginalis* in study sample:

No. of examined samples	No. of positive samples	No. negative samples
63	5(8%)	58 (92%)

Table 2 presents that the trophozoite numbers were reduced using zinc concentration specially at 1.5mg/ml concentration the number of trophozoit was 7.5×10^3 in comparison to control and 0.25mg/ml concentration was 17.5×10^3 and this is similar at period time for 48 hours and 72 hours and according to control positive group was 16.2×10^3 the zinc at 1.5mg/ml concentration was be more effect on the trophozoit numbers.

The inhibition of the growth was defect according to concentrations, where we note that the proportion of zinc added at a concentration of 1.5mg/ml has a greater impact on the effectiveness of the parasite number to reduce by 32.2 % compared to the effect of metronidazole treatment with (69.5%) and this is during incubation for 24 hours and the extent of inhibition is (68.0%) and this is an indication that zinc is an important element for the inhibition of the parasite, and the same at period for 48 hours and 72 hour.

Table 2: Frequency effect of zinc on *Trichomonas vaginalis* trophozoites after the period time

Period time effect	Concentrations (mg\ml)	No. of <i>T.vaginalis</i> trophozoites X103 with inhibition rates		
		Trophozoite no.	Effect %	Inhibition %
24 hours	0.25	17.5	75.1	25.0
	0.75	12.5	53.6	46.4
	1.5	7.5	32.2	68.0
	Positive Control (metronidazole)	16.2	69.5	30.5
	Negative control (without treatment)	23.3		
48 hours	0.25	13.3	69.0	30.7
	0.75	10.0	52.0	48.0
	1.5	4.2	22.0	78.1
	Positive Control (metronidazole)	12.5	65.1	35.0
	Negative control (without treatment)	19.2		
72 hours	0.25	3.3	25.0	75.2
	0.75	1.7	13.0	87.2

	1.5	8.3	63.0	94.0
	Positive Control (metronidazole)	5.8	44.0	56.4
	Negative control (without treatment)	13.3		

According to the formula listed above.

As Table 3 presents that the percentage of zinc concentration measurement in the serum of women with *Trichomonas vaginalis* was not differ compared to the normal values of zinc in the body and with control group indicating that there is no any effect of zinc concentration on the severity of *T. vaginalis* among the normal values between (70 -115) µg /dl.

Table3: Serum zinc level in *T. vaginalis* infected women:

Cohort	No. of patient (%)	Level of zinc serum (µg/dl)
Control group	58 (92%)	56 – 115
Patients <i>T. vaginalis</i> group	5 (8%)	81 – 100
P-value	0.000****	
Normal value of zinc	70 – 115 µg/dl	

**** high significant.

Discussion:

Trichomoniasis has long been considered a disease of women. *Trichomonas vaginalis* has been identified from almost all genitourinary tissues and is a genitourinary tract site specific pathogen. However, asymptomatic trichomoniasis affects a lot of female patients. Vaginal discharge, reported in more than 50% of cases when symptoms do appear, is the most typical presenting complaint among women with *Trichomonas vaginalis*, followed by pruritus or dysuria [20].

The fast environmental changes appear to affect *T. vaginalis* (e.g., temperature, microflora, pH, iron, polyamines, zinc, host immune responses, and other unknown factors) [21]

Positive index cases were determined using wet mount microscopy and culture, and the study's goal was to explore concordant infection in the spouses of women with trichomoniasis.

Table 2 show that in our study concluded that zinc material would be decrees the growth of *Trichomonas vaginalis* that reach to zero in 3rd day incubation this material was the more effective to kill the *T. vaginalis* more than the drug of metronidazole, about the zinc material that found research that show zinc is important to be anti-parasite in seminal fluid that rich of zinc, Laura Isabel Vazquez-Carrillo, et al., evaluate the effects of Zn²⁺ on the cytotoxicity and protein profile of *Trichomonas vaginalis*, as well as the morphology of the organism and the proteinases involved in its interaction with DU-145 prostatic cells. Particularly interesting were the effects of Zn²⁺ on the protein and transcript levels of TvCP65 and the identification of differentially expressed proteins [21]. The combination of metronidazole with other drugs has been extensively tested for the treatment of *T. vaginalis* [22].

Approximately 300 enzymes use Zn²⁺ as a catalytic element. [23]. The male microenvironment depends heavily on this metal, which is a necessary nutrient and important to many biological processes. DNA-binding proteins, which have Zn²⁺ fingers, are just one example of the many proteins that contain Zn²⁺ as both a structural and catalytic component. Men's trichomoniasis may be resolved in part by the Zn²⁺ levels detected in the prostate [24, 12]. Krieger and Rein noted that *Trichomonas vaginalis* is killed by typical human prostatic fluid Zn²⁺ concentrations in

vitro, which may prevent or treat trichomoniasis in the majority of affected men. There is no discernible difference between *Trichomonas vaginalis* survival in canine prostatic fluid and survival in Zn²⁺ with same caption content, according to studies [12].

A study of Krieger J.N., *et al.*, show that Zinc salts at quantities comparable to those in a typical man's prostatic fluid quickly killed trichomonads. The zinc sensitivity of 15 clinical isolates of *Trichomonas vaginalis* varied somewhat (minimal inhibitory concentration, 0.8-6.4 mM). Small variations in the kinetics of zinc killing of various trichomonad strains were revealed by the time-kill method. *Trichomonas vaginalis* substrains that were generally zinc-resistant may be chosen from a population of the bacteria that was zinc-sensitive. After several iterations of substrains in growth media devoid of additional zinc, zinc resistance proved a stable property. The natural history of *Trichomonas vaginalis* infection in males may be significantly influenced by variations in the zinc sensitivity of infecting *Trichomonas vaginalis* strains or in the zinc content of host prostatic secretions [13].

Trichomonas vaginalis has long been thought to be sequestered in the prostate. It is believed that organisms from this reservoir are shed during sexual activity and repeatedly infect the female genitourinary tract. Concentration levels of zinc detected in uninfected prostatic secretions suppress bacteria that are known to cause prostatitis. The current investigation shows that zinc has a similar inhibitory effect on *Trichomonas vaginalis* proliferation. This finding would indicate that the prostate is not likely to be a reservoir for latent infection by these pathogens in otherwise normal persons [25]

Women from KwaZulu-Natal, South Africa, were the subjects of study by Abbai *et al.* and Naidoo *et al.*, which revealed that women under the age of 25 had a greater chance of contracting STIs.[26, 27]. Behavioral and biological variables contributed to the higher infection risk in younger women [26]. Additionally, Abbai *et al.* discovered that women who have had a STI in the past are at significantly higher risk of contracting another infection. A lower degree of education was one of the additional risk factors for contracting an illness [26, 28].

Table 3 shows that serum zinc of Trichomonal patient in comparison with control patient not has changing the p-value are highly significant. A research from Thuy Nguyen Dac Luong *et al.*, to determining the connection between the clinical traits of patients with genital warts and the serum zinc level. There was a case-control investigation. Clinical diagnosis was used to identify genital warts, and the number of affected locations or the spread of lesions were used to gauge the disease's severity. Atomic absorption spectrophotometry was used to determine the serum zinc content. The study had a total of 78 patients with genital warts and 78 healthy volunteers. The genital wart group's mean serum zinc value was lower than the control group's (81:83 13:99 vs. 86:66 17:58 g/dL), but this difference was not statistically significant ($P > 0.05$). Patients with more than one damaged site, a spread larger than 2 cm², or ten or more lesions had substantially lower mean serum zinc concentrations than the control group ($P < 0.05$). The findings revealed that patients' low serum zinc levels may be linked to severe genital warts [29].

Our study concluded that zinc material would decrease the growth of *Trichomonas vaginalis* that reach to zero in 3rd day incubation this material was the more effective to kill the *T. vaginalis* more than the drug of metronidazole, about the zinc material that found research that show zinc is important to be antiparasite and concluded Zinc is a very important anti-microorganism and can be used it as dilution washing for treatment process.

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