

A comparison of activity between Metronidazole and Fluconazole against *Trichomonas vaginalis* (in vitro)

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ABSTRACT

Objectives: 1-To detect *T.vaginalis* in women with vaginal discharge using wet mount and culture preparation 2-To determine the effect of the drug fluconazole *in vitro* on *T.vaginalis*, in comparison to metronidazole.

Subjects and methods: This study was conducted during the period from Nov.2007 to June 2008.The subjects enrolled in the study were 180 females attending out patients clinics of Al – Battol and Al – Salaam teaching hospitals in Mosul city. Two vaginal swabs from posterior vaginal fornix were taken from each subject. The first swab after mixed with normal saline was used for wet mount preparation and examined immediately. The second swab was placed in Bijou bottle containing 5 ml of Oxoid Trichomonas medium (CM161), and incubated at 37 °C and checked for the capsule 150 mg, metronidazole was prepared from flagyl tablet 200 mg. Both were cultured in CM161 medium where the number of the parasite was limited to about 19×10^4 organisms for each ml of the medium at zero time of culturing.

Results: From a total of 180 women. Twenty one cases (11.67%) were found positive for *T.vaginalis*. The highest incidence of infection was seen among (21 - 30) years age group comprising 47.6%; Pregnant women showed a lower infection rate 5 (23.8%) than non pregnant 16 (76.2%). Two drugs were tested *in vitro* against *T.vaginalis*. Metronidazole was statistically superior in its activity against *T.vaginalis* in comparison to fluconazole in all 4 days of experiment, and in all drug concentrations used with a statistically significant difference between the variables.

Conclusions: *T.vaginalis* is still prevalent in women with vaginal discharge where 11.7% are found positive for *T.vaginalis*. *In vitro* study on the effect of drugs against *T.vaginalis* in culture shows that metronidazole is superior in activity against *T.vaginalis* in comparison to fluconazole drug.

Key words: metronidazole, fluconazole, culture. *T.vaginalis*

INTRODUCTION

Trichomoniasis is a sexually transmitted infection caused by the protozoa *Trichomonas vaginalis*. Trichomonas infection is responsible for the most non viral sexually transmitted diseases worldwide [1]. Trichomoniasis is a significant cause of morbidity among all infected patients. Roughly 25% to 50% of the women in the U.S.A harbor the organism [2]. A n epidemiological study of Trichomoniasis among Iraqi women showed that the prevalence of infection among pregnant women was 3% while in non pregnant was 8% [3]. Other report indicated that 25% of examined Iraqi women had *T. vaginalis* infection which were diagnosed by the wet mount and culture methods [4]. In A study from Mousl 11.67% of women examined found positive for *T. vaginalis* [5]. Effective diagnosis and treatment of Trichomonas infection have been shown to eliminate symptoms. Conventional identification procedures for Trichomonas from vaginal swabs involve the isolation and subsequent identification of viable pathogens by wet mount microscopy or by cultures[6]. Although the microscopic examination of wet mount of vaginal secretions has been the main method of diagnosis of Trichomoniasis, however, microscopy has a relatively poor sensitivity(60%-

70%) when compared with culture [7]. *Trichomonas vaginalis* can be cultivated in a liquid media to allow small amount present in the original specimen like vaginal secretions to multiply and thus become easier to detect by microscopic examination. It is known that parasites do not form colonies on solid media in the same way as bacteria and fungi [8]. Metronidazole is highly effective in curing Trichomoniasis with cure rate >90 % and is regarded as the drug of choice [2,6], but there are known metronidazole-resistant strains of *T. vaginalis* [9]. This resistance is increasing in prevalence [9,10]. The CDC estimated that 2.5%-5% of *T. vaginalis* isolates are resistance to metronidazole and this resistance occurs widely throughout the U. S. Tinidazole has been successfully used for the treatment of metronidazole-resistant strains of *T. vaginalis* infection [9].

The aim of the present study was to determine *In vitro* the effect of drug fluconazole on *T. vaginalis* taken from infected women in comparison to metronidazole.

MATERIAL AND METHODS

Subjects

The study was carried out during the period from November 2007 to June 2008 at Al- Batool and Al -Salaam Teaching Hospitals in Mosul City. From 180 female attending Out Patient Clinics of these hospitals complaining from vaginal discharge.

Materials

Swab

Sterile cotton tipped swabs were used to take vaginal discharge.

Oxoid Trichomonas Medium (CM161)

Composition : Liver infusion (25.0 gm), Sodium chloride (6.5 gm), Agar (1.0 gm), Dextrose (5.0 gm) and Distilled water (1000 ml). This medium was used in the present study for culturing *T. vaginalis* parasite

Drugs

Two drugs were used in this study:

-Fluconazole capsule of 150 mg. (Brawn Pharma Limited, India).

-Metronidazole (Flagyl) tablet of 200 mg. (Holland Medicine company, Netherlands)

Methods

Vaginal swabs examination

Two vaginal swabs were taken from each patient. The patient was put in lithotomy position and a non-lubricated sterile bivalve speculum was used to expose the cervix and vagina and vaginal discharge was collected using two sterile cotton tipped applicator and simultaneously introduced into the posterior vaginal fornix [4]. The first swab was placed in tube containing 2 ml of normal saline for wet mount examination [4]. The wet mount were examined microscopically immediately after taking the swab.

The swab about (5 drops) was placed under a sterile condition on Bijou bottle containing 5 ml of (CM 161) culture [11]. Then incubated at 37°C and checked for the presence of parasite after 24hr, 48 hr, until 7 days.

Oxoid Trichomonas Medium (CM161)

The medium prepared by dissolving 37.5 gm of powder of the mentioned substances in a sterile flask, then the mixture brought to boiling in order to dissolve the powder completely, the pH was adjusted to 6.4 by using pH meter (electronic), then the medium was sterilized by an autoclave at 121°C for about 15 minutes then it was left to cool, 80ml of inactivated horse serum was added to the medium, the inactivation of the serum was made by putting container of horse serum in a water bath at 56°C for 30 minutes.

After that and under a septic technique the addition of the antibiotics was done, these antibiotics were penicillin G (1000 000 I.U/L) and streptomycin (500 000 I.U/L) in addition to antifungal agent as nystatin (0.1 gm/L).

The medium then poured in Bijou bottle under a septic condition (5 ml in each of these sterile bottles) and stored at 4 °C in the refrigerator till used [11,12].

Drugs

Fluconazole concentration was prepared from fluconazole capsule 150 mg , Metronidazole was prepared from Flagyl tablet of 200 mg.

T. vaginalis was cultured in liquid medium (Oxoid *Trichomonas* CM161), the number of the parasite in each tube was limited by about 19×10^4 organisms for each ml of the medium at zero time of culturing. Three different concentrations of fluconazole were prepared and three tubes for each concentration 0.025, 0.05 and 0.1 mg/ml of the media according to the metronidazole concentration that give complete inhibition of the parasite [11]. Another three tubes of three different concentration of metronidazole were also prepared as a control.

The number of the parasite was followed up every 24hr. The number of the parasite was calculated by using hemocytometer, the mean of the number in the 3 tubes was counted, then multiplied by 10^4 which represent thickness of the slide.

Statistical analysis

Statistical formula was used to detect variances among parameters in the study at probability 0.05 and 0.01[13].

RESULTS

Evaluation of the effect of fluconazole drug on *T. vaginalis* (*In vitro*) in comparisons to metronidazole

Data which represent the association between drugs (metronidazole and Fluconazole), their concentration and the time of incubation starting from day 0 which represent the starting point of the experiment to day 4 in all concentrations tested (0.1, 0.05 and 0.025), are shown in (Table 1).

Metronidazole was statistically superior in its activity against *T. vaginalis* in comparison to fluconazole. For metronidazole no parasite could be detected by day 2 at the concentration of 0.1 mg/ml and by day 3 at the concentration of 0.05 mg/ml and by day 4 at all concentrations used. Although a gradual reduction of the parasite seen by using fluconazole with all concentrations used in all 4 days of experiments. However, fluconazole showed its highest activity (no parasite detected) only at day 4 of the *in vitro* experiments by using concentration of 0.1mg/ml.

Table 1: *In vitro* association time between time, drug and concentration and *T. vaginalis*

Time/day	Drug	Concentrations of drugs (mg./ml of the media)		
		0.100	0.05	0.025
		Mean of the No. of parasites		
d ₀	Metronidazole	18.667 a	18.667 a	18.333 a
	Fluconazole	19.000 a	18.667 a	19.667 a
d ₁	Metronidazole	0.667 ghi	3.333 d	6.000 c
	Fluconazole	3.667 d	6.000 c	7.667 b
d ₂	Metronidazole	0.000 h	0.333 gh	1.667 ef
	Fluconazole	1.333 efg	2.000 e	3.333 d
d ₃	Metronidazole	0.000 h	0.000 h	0.667 fgh
	Fluconazole	0.333 gh	0.667 fgh	1.333 efg
d ₄	Metronidazole	0.000 h	0.000 h	0.000 h
	Fluconazole	0.000 h	0.333 gh	0.667 fgh
Mean of concentration		4.367 c	5.000 b	5.933 a

* Number of *T. vaginalis* was limited by approximately 19×10^4 .

* d₀ = the time of beginning of the experiment (zero time).

* The means that carry different verbs in each case are statistically different at $P \leq 0.05$ according to Duncan Test.

A gradual reduction in the number of parasites was noted from the time of beginning of the experiment (d0) to (day 4). Metronidazole showed superior action against *T. vaginalis* than fluconazole which was statistically significant in all 4 days of experiment. (Table 2)

Table 2: The relation between the mean number of *T. vaginalis* and th tim and the drug *in vitro*

Time	drug	mean of No. of <i>T. vaginalis</i>
d ₀	Metronidazole	18.556 a*
	Fluconazole	19.111 a
d ₁	Metronidazole	3.333 c
	Fluconazole	5.778 b
d ₂	Metronidazole	0.667 ef
	Fluconazole	2.222 d
d ₃	Metronidazole	0.222 ef
	Fluconazole	0.778 e
d ₄	Metronidazole	0.000 f
	Fluconazole	0.333 ef

*The means that carry different verbs in each case are statistically different at $P \leq 0.05$ according to Duncan Test.

By using different concentrations of both drugs , Metronidazole showed a superior activity against *T. vaginalis* which was statistically significant. The high concentration indicates a higher activity of the drug (Table 3)

Table 3: The relation between the mean number of *T. vaginalis* and types of drugs and their concentrations *in vitro*

drug	Concentration (mg/ml of culture media)		
	0.100	0.05	0.025
mean of No. of parasite			
Metronidazole	3.867 e	4.467 d	5.333 bc*
Fluconazole	4.867 cd	5.533 b	6.533 a

*The means that carry different verbs in each case are statistically different at $P \leq 0.05$ according to Duncan Test.

DISCUSSION

The 5-nitroimidazole group of drug include antiprotozoal effective agents which can be used in the treatment of trichomoniasis [14]. This group contains different drugs as metronidazole, tinidazole ,ornidazole and nimorazole [14,15].The mechanism of action is not well understood, however, it has been documented that the 5-nitro group when reduced to a nitro radical within *Trichomonas vaginalis* is responsible for the antiprotozoal activity[14,16]. These agents may bind to the DNA of the parasite and inhibit protein synthesis causing cell death[17]. 5-nitroimidazole group is usually available with recommended doses [15]. The standard treatment for trichomniasis is a single 2 gm oral dose of metronidazole which is effective but has more side effects, the alternative regime; 500 mg twice daily for 7 days [17].The second regime is better because of the side effect with large doses. In addition, patients treated over 7 days protected for this period from immediate re infection whereas this protection is not reliable with a single does unless the sexual partner are treated simultaneously [14].The benefits of single dose of metronidazole or tinidazole are better patient's compliance, lower total dose and possible decrease of subsequent candidial vaginitis [15]. Metronidazole can also be taken intravenously [16].The cure rate of oral or intravenous metronidazole about 80-95% [15,18]. Treatment of sexual partner at the same time may increase the cure rate to more than 95% [15]. If there is treatment failure, retreat with 500 mg twice daily for 7 days [19]. Because metronidazole is most commonly used to treat *T.vaginalis* infection, potential management problems include hypersensitivity and drug resistance can occur [17].

Resistance against metronidazole is frequently reported and cross resistance among the family of 5-nitroimdazle drug is also common [20]. Metronidazole resistance was estimated to occur in about 5% of all clinical cases of trichomoniasi [10,14,21]. The problem of metronidazole resistance can be overcome by increasing the dose of metronidazole to 2-4 gm /day for 3-5 days [17,22]. If the treatment failure persist after increasing the dose of metronidazole, then tinidazole can be used as metronidazole doses [15,22].

For treatment of pregnant patient, it has been stated that metronidazole is safe on all trimesters of pregnancy and it can be given as 2 gm single dose for treatment of symptomatic patients [23]. But this dose is not recommended in treatment of asymptomatic patients [17]. It is better to avoid sex until both partners are cured [16]. But no need for sex restriction if both of them are treated at the same time [24].

In vitro* activity of fluconazole drug against clinical isolates of *Trichomonas vaginalis

This is one of the leading trials to study the action of fluconazole against *T. vaginalis in vitro*. The results of this study provide evidence that fluconazole is also active against *T. vaginalis*. However, the data showed the superiority of metronidazole in all concentration used (0.1, 0.05, 0.025 mg/ml of the media), furthermore by using the other variable (i.e, time, starting from day 0 to day 4) a similar results were obtained indicating the superiority of metronidazole to fluconazole tested by the reduction in the in the number of parasites recorded after each day of the experiment. In a related study by Delmas *et al* (2002) [32] investigated the antitrichomonal activities of a variety of benzothiazole. Metonidazole showed a higher activity against *T. vaginalis* which is in parallel to the present study.

Other studies tested the effect of other antifungal azole group of drug on *T. vaginalis* which showed the *in vitro* effectiveness of Ketoconazole on *T. vaginalis* [11].

A study cited a cure rate of 90% if topical clotrimazole combined with oral tinidazole in case of metronidazole resistance [25].

There were two reasons behind the choice of fluconazole in the present study, firstly, to overcome the superimposed candida infection [26]. Secondly, because it is cited that fluconazole has excellent *in vivo* and *in vitro* activities against other protozoan parasite with less side effects in comparisons to other available antiprotozoal therapeutic agents [27,28].

In addition, fluconazole has been tried in the treatment of leishmaniasis caused by *Leishmania major*, 80 patients received a six-week course of oral fluconazole 200 mg. daily, of whom 63 had complete healing of lesions after 3 months [29].

Other chemotherapeutic agents which may be used in treatment of trichomoniasis as Hamycin which is an aromatic polyene related to amphotericin B. It can introduce cell death in *T. vaginalis* and other eukaryotic cells. This drug is currently used in India as topical treatment for trichomoniasis, unfortunately reported side effects indicated hamycin toxicity which may limit its future use [14]. Other drug investigated for antitrichomonal activities as sulfimidazole [30]. These chemo-therapeutic agents have all shown some promise in the treatment of trichomoniasis, but research either in the preliminary stages or are not being systematically pursued at this time.

The results of this study show that a comparison of different drugs for *T. vaginalis* infection is of great usefulness to evaluate the action of these drugs. Our present research is focused and restricted to the treatment of trichomoniasis *in vitro*. Trichomoniasis is still regarded as the most common sexually transmitted diseases worldwide according to WHO, with approximately 174 millions new cases per year [31]. The future direction for this research showed extend to study other drugs like Ketoconazole to be tested both in *in vitro* and *in vivo* for better understanding of the treatment of this sexually transmitted disease.

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