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Changes in *Trichomonas vaginalis* IST1- gene after metronidazole therapy

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Abstract
Background: Virginal trichomoniasis is the most prevalent sexually transmitted illness with clinical manifestations vary from asymptomatic to symptomatic, including severe urethritis, vulvo-vaginitis, and cervicitis. Internal transcribed spacers (ITS1) are non-functional ribosomal RNA snippets that transcribed without further translation. This gene is critical in determining whether metronidazole should be used in treatment or not.
Aims: This research was intended for the first time in Iraq to discover the copy number changes in the *T. vaginalis* ITS1 gene after metronidazole using molecular qPCR.
Methods: fifty samples were obtained metronidazole sensitive group and resistant-group who were referred to the Gynecology Department of Al-Khansaa Teaching Hospital in Mosul, Iraq. All samples were examined wet mount and grown in 50 ml of TYM media. Metronidazole Inhibition percentage assay using Meingassner method. The ITS1 fragment was amplified using qPCR to study the changes in this fragment expression in both groups.
Results: The results revealed that the resistance samples showed significant lower inhibition zone in comparison to sensitive group. Significant increase in IST1 sequence copy number in to metronidazole-resistance group.
Conclusion: ITS1 copy number may serve as an indicator for identifying resistance, which might lead to the development of management and treatment techniques to limit its spread.

Keywords: *Trichomonas vaginalis*, metronidazole therapy, vulvo-vaginitis

Introduction
Virginal trichomoniasis is the most prevalent sexually transmitted illness that is not caused by a virus [1]. The clinical manifestations of the condition vary from asymptomatic to symptomatic, including severe urethritis, vulvo-vaginitis, and cervicitis [2], and this condition can lead to preterm or low-birth-weight child delivery and an increased chance of developing cervical cancer [3, 4]. Immuno compromised females are more susceptible to this type of infection [5].

One of the most concerning clinical problems associated with *T. vaginalis* infection is related to the ability of *T. vaginalis* to develop resistance against standard therapy [6]. Metronidazole is a broad-spectrum antibiotic used to treat a number of illnesses. It inhibits the development of certain bacteria and parasites [7]. It had limited effectiveness against viral infections [8] and was considered the gold standard therapy for *T. vaginalis*, previously known as a successful treatment with a cure rate of roughly 95% of the cases [9].

The first case of *T. vaginalis* resistant to metronidazole was described by Meri *et al.* in Finland in the early 60s [10]. Following that, many studies documented metronidazole resistance, and these works estimated the amount of resistance microorganisms at the time to be 2.5-5%10. Global metronidazole resistance cases range from 10–17% of infected individuals treated with metronidazole [11]. Some clinical isolates have shown declining susceptibility to metronidazole in different countries. Resistance to metronidazole can be defined as the inability of normal therapy to eradicate an infection [12].

Internal transcribed spacers (ITS1) are non-functional ribosomal RNA snippets that are transcribed without further translation in all eukaryotic species [13]. According to some biologists, this gene is critical in determining whether metronidazole should be used in treatment or not [14]. The development of advanced molecular technologies for detecting drug resistance may result in the detection of the resistant strain and increased therapeutic outcome. This research was intended for the first time in Iraq to discover the copy number changes in the *T. vaginalis* ITS1 gene after metronidazole using molecular qPCR.

Materials and Methods

Sampling

From January 2021 to March 2022, fifty samples were obtained from patients successfully treated (control sensitive group) with orally 250 mg metronidazole three times a day over a seven-day course and patients that had failed three previous rounds of treatment with metronidazole for trichomoniasis (resistant-group) who were referred to the Gynecology Department of Al-Khansaa Teaching Hospital in Mosul, Iraq. All samples were examined wet mount and grown in 50 ml of TYM media [15].

Metronidazole Inhibition percentage

Assay for metronidazole Inhibition percentage was carried out for each isolate subjected to an *in vitro* metronidazole test, as initially reported by Meingassner *et al*. [16] and modified by Narcisi and Secor [17]. For 48 hours under aerobic conditions, cells were cultured with escalating concentrations of metronidazole (200 mg/ml). Each experiment was repeated twice in duplicate for each isolate examined, and dimethyl sulfoxide was employed as a vehicle control.

Extraction of DNA

Centrifuged at 430 g for 20 minutes at 4 °C, cultures were pelleted and washed once with PBS (pH 7.4). Total nucleic acid was extracted according to the manufacturer's instructions using Cat. No. 10023 (Addbio, Korea). The ITS1 fragment was amplified using primers built on the ribosomal DNA (rDNA) gene of *T. vaginalis*. Forward primer sequence 5-ACA CCG CCC GTC GCT CCT AC-3 reverse primer: 5-AAT TTG CAT TCA AAG ATT AAC-3. Amplification via polymerase chain reaction using Promega master mix (Promega, USA).the final volume of 25µl/ well, the PCR reaction contain 200 ng DNA, 12.5µl master mixes 2.5 µl of each of the forward and reverse primers and 7.5 µl DNA/ RNA free water. Forty-five cycles of denaturation at 95oC for 2 minutes, annealing at 60oC for 30 seconds, and extension at 72 °C for 30 seconds were used in the qPCR reactions. The experiment was duplicated three times and the resulted data analyzed using Eco study software and presented as Fold change.

Results

The results of this work revealed that the resistance samples showed significant lower inhibition zone in comparison to sensitive group (p<0.001) as shown in Figure 1.

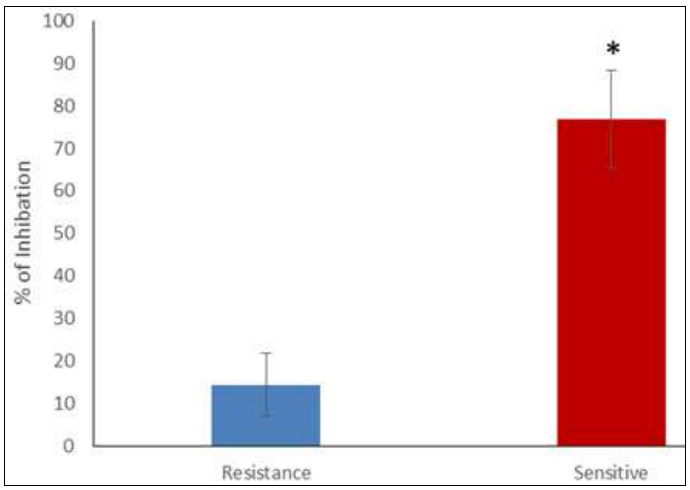

Fig 1: Percentage of inhibition after treatment with metronidazole treatment for both sensitive and resistance samples.* mean significant at p<0.01.

The results of this work revealed that the resistance samples showed significant increase in IST1 sequence copy number in comparison to metronidazole-sensitive group as shown in Figure 2.

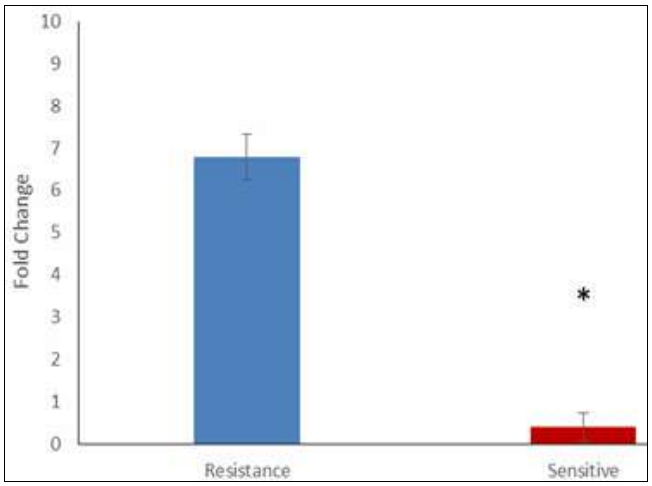

Fig 2: Fold changes in IST1 sequence in both metronidazole sensitive and resistant strains. * mean significant at p<0.01.

Discussion

This research was carried out to study the copy number changes in the ITS1 gene of *T. vaginalis* isolated from patients who had sensitive and resistant strains. Although there has been a link between metronidazole resistance and ITS1 in clinical isolates of *T. vaginalis* studied before [18], this is the first study that indicated that ITS1 copy number changes between the metronidazole sensitive and metronidazole resistant groups.

Previous research has shown a link between ITS1 mutations and medication resistance, but not the copy number, and this work may provide a clue to explain the mechanism by which *T. vaginalis* develops metronidazole resistance after treatment failure. The internal transcribed spacer (ITS) was chosen as a potential gene for determining metronidazole sensitivity in *T. vaginalis* as this gene sequence is conserved across similar species and because little is known about the genetic diversity and medication resistance characteristics of this parasite [19].

Changing copy number and specific mutations at the genetic level can significantly contribute to the drug resistance phenomena [20]. Saghaug demonstrated *in vitro* resistance to metronidazole of 15-50% [21]. Upcroft *et al*. discovered 17.4 percent metronidazole resistance using PCR in another study [22]. Because our methodologies were different from those used in the previous research, our findings differed.

The results of this work show that the changes in the ITS1 copy number may serve as an indicator for identifying resistance, which might lead to the development of management and treatment techniques to limit its spread. Clearly, further research with bigger samples from diverse endemic locations is required to reconcile this paradox.

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Author's Contribution

Not available

Conflict of Interest

Not available

Financial Support

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