Journal of the Egyptian Society of Parasitology, Vol. 51, No.1, April 2021
J. Egypt. Soc. Parasitol. (JESP), 51(1), 2021: 119 – 126
(Online: 2090-2549)

POTENTIAL ROLE OF *TRICHOMONAS VAGINALIS* IN WOMEN WITH PRIMARY AND SECONDARY INFERTILITY IN BENI-SUEF, EGYPT

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Abstract

Trichomoniasis plays a crucial role in causing some health problems such as ectopic pregnancy, undesirable outcomes of pregnancy and infertility.

This study evaluated the role of *T. vaginalis* in infertile women and assessed the relationship between trichomoniasis and female infertility in unexplained primary or secondary infertilities. A total of 90 female patients suffered from infertility were examined for trichomoniasis by Giemsa stained wet mount smears, and the Modified Diamond's medium culture as a diagnostic reference.

The results showed that *T. vaginalis* was 9/90 (10%) by culture method with significance with primary unexplained infertility.

Keywords: Egypt, *Trichomonas vaginalis*, Infertility, Modified Diamond's medium culture.

Introduction

Trichomonas vaginalis, which colonizes the genitourinary tract of men and women, is a sexually transmitted parasite with symptomatic or asymptomatic trichomoniasis (Mielczarek and Blaszkowska, 2016). The parasites adhere to the genitourinary tract squamous epithelial cells (Schalkwyk *et al,* 2015), in the reproductive period (18-49 years) of sexually active women (Sood *et al,* 2007). Apart from sexual intercourse transmission, infection occurred via the use of unsanitary public toilets (Leitsch, 2021). Trichomoniasis is asymptomatic in 35-85% of females and in 70-100% of males (Sutton *et al,* 2007).

In Egypt, trichomoniasis was reported by many authors (El-Sherbini *et al,* 2009; Kamal *et al,* 2015; Abdel-Magied *et al,* 2017; Elwakil *et al,* 2017). *T. vaginalis* infected females were presented with clinical symptoms ranged between asymptomatic to acute or chronic infections (Sherrard *et al,* 2011). They may complain of green or yellow vaginal discharge with offensive odor (McClelland, 2008) with itching, burning sensation in vaginal area, dysuria, dyspareunia, & pelvic pain (Secor *et al,* 2014). The severe cases inflammatory re-actions produced small hemorrhagic spots in vaginal membrane the "Strawberry Cervix", trichomoniasis pathognomonic sign (Hyun *et al,* 2010). In trichomoniasis infected men only 15-50% suffered from urethral irritation, urethral discharge, and/or dysuria (Kissinger *et al,* 2008).

Previously, trichomoniasis diagnosis was based on the clinical manifestations (Morsy *et al,* 1984), which often misdiagnosed infection due to confusion with other STDs infe ctions (Madhivanan *et al,* 2009). Thus, laboratory diagnosis was indicated to ensure the proper infection for early treatment (Paladine and Desai, 2018). The microscopic examination of fresh wet mount for trophozoites corkscrew motility was accepted (Huppert *et al*, 2007). However, culture was used as the gold standard for *T. vaginalis* diagnosis (Lecke *et al,* 2003), but often being unavailable in the developing countries public laboratories (Sood and Kapil, 2008). Other methods included real-time PCR assay, and an immunochromatographic test (Leli *et al,* 2016).

Trichomoniasis plays a role in some health problems such as infertility, ectopic pregnancy (Fichorova, 2009), and undesirable pregnancy outcomes (Oyeyemi *et al,* 2015), associated with an increase in susceptibility to HIV (Lemos and Garcia-Zapata, 2010), and cervical neoplasia (Afzan and Suresh, 2012).

The present study aimed to investigate the role of *T. vaginalis* in infertile women and to assess the relationship between *Trichomonas vaginalis* and female infertility, whether the cause of infertility was explained or unexplained amongst primary and secondary infertile women.

Materials and Methods

This cross-sectional study was carried out on married female patients at the childbearing period who attended the Department of Obstetrics and Gynecology, Faculty of Medicine, from November 2019 to October 2020. A total of 90 females suffered from primary or secondary infertility were included. They aged between 20-35 years, with asymptomatic or complained of vaginal discharge with or without any other manifestations suggestive of trichomoniasis as itching, dysuria, and/or dyspareunia. They were categorized into females without or with explained infertility causes (ovarian, tubo-peritoneal, or male factor). The exclusion criteria included the pregnant females with a history of immunosuppressive drugs or antibiotics administration or menopause ones.

Medical sheets were filled out on each one, whom was subjected to gynecological examinations. For trichomoniasis diagnosis, wet mount Giemsa stained smears microscopy and modified Diamond's medium cultures were done. For each patient, a non-lubricated sterile speculum was introduced and 2 sterile swab sticks collected the vaginal secretion samples from the vaginal posterior fornix. The vaginal swabs were divided into 2 parts; first one was mixed with 2ml sterile phosphate-buffered saline (PBS) in a tube at pH 7.2 for wet mount staining and examination. Second one was cultured into the modified Diamond medium (Gelbart *et al*, 1990).

All samples were immediately transported to Department of Parasitology for examined. For wet mount microscopy a drop of PBS solution was added and immediately careful examined for the trophozoites motility under x100 & x 400 objectives. Diamond medium cultures were incubated at 35-37°C, and microscopically examined within 48 to72hrs for the trophozoites motility daily and up to one week to exclude the infection.

Statistical analysis: Data were analyzed by statistical package of social science (SPSS) software version 25 for windows 10. Simple descriptive analysis was in numbers and percentages of qualitative data and arithmetic means as a central tendency measurement. T-test was used to compare between two groups and Chi square test to compare between more than two groups. Binary logistic regression analysis detected different female infertility risk factors as age, and occupation of trichomoniasis patients. *P*-value < 0.05 was considered significant.

Results

Positive *T. vaginalis* was 9/90(10%) by the Diamond culture method. The efficacy of direct wet mount smear and Giemsa stain were both 66.6% as compared to reference index (Modified Diamond culture).

Patients ages, and residence did not significantly correlate with infections (P=0.177). But, the occupation showed a significant difference (P=0.005). Vaginal discharge and dysuria showed significance difference, but itching and dyspareunia did not show significant difference.

Trichomoniasis was detected in 8(88.9%) with primary infertility, and 1(11.1%) with secondary infertility, with significance difference, but 6(66.7%) and 3(33.3%) were positive patients with unexplained and explained infertility, respectively with significant difference. As to infertility, male factor showed a significance (P=0.031). Association between primary/secondary infertility with or without explained infertility showed significant difference between infection and unexplained infertility (*P*<0.001). Multivariable binary logistic regression predicted risk factors for unexplained infertility in patients after adjusting age and occupation. *T. vaginalis* was the cause for unexplained infertility (P=0.049), increased probability 4 times (OR 95% CI was 4.01, 1.01 to 18.55). Details were given in tables (1 to 6) & figure (1).

Table 1: Positive and negative women using different tests in compared to Modified Diamond test.

		Culture				Total	
		Positive	Percent	Negative	Percent	Count	Percent
Direct wet mount	Positive	6	66.7%	0	0.0%	6	6.7%
	Negative	3	33.3%	81	100%	84	93.3%
Total		9	100%	81	100%	90	100%
Giemsa staining	Positive	6	66.7%	0	0.0%	6	6.7%
	Negative	3	33.3%	81	100%	84	93.3%
Total		9	100%	81	100%	90	100%

Table 2: Validity of direct wet mount and Giemsa staining compared to Modified Diamond culture.

Items	Wet mount	Giemsa stain
P-value	0.001*	0.001*
Sensitivity	66.67(29.9 - 92.5)	66.67(29.9 - 92.5)
Specificity	100(95.5 – 100)	100(95.5 – 100)
Positive predictive value (PPV)	100(85-100)	100(85-100)
Negative predictive value (NPV)	96.4(91.5 - 98.6)	96.4(91.5 - 98.6)
Kappa	0.935	0.935
Overall agreement	96.7%	96.7%

*: Significant ($p< 0.05$).

Table 3: *T. vaginalis* positive and negative cases as to socio-demographic and environmental characters.

Variants	Positive culture N=9(%)	Negative culture N=81 (%)	*P*-value
Age (years)	Mean: 31.1±4.5	Mean: 28.9±4.4	0.177
Residence: Rural	7 (77.8%)	48 (59.3%)	0.280
Urban	2 (22.2%)	33 (40.7%)	
Occupation: Not working	4 (44.4%)	68 (84%)	0.005*
Working	5 (55.6%)	13 (16%)	

*: Significant ($p< 0.05$)

Table 4: Relationship of *T. vaginalis* positive and negative cases with complaints

Complain	Cases	Positive culture N=9(%)	Negative culture	*P*-value
Discharge	17	6 (66.7%)	11 (13.6%)	0.001*
Itching	15	2 (22.2%)	13 (16.05%)	0.933
Dysuria	13	4 (44.4%)	9 (11.1%)	0.007*
Dyspareunia	19	1 (11.1%)	18 (22.2%)	0.765

*: Significant ($p< 0.05$)

Table 5: *T. vaginalis* positive & negative cases, primary/secondary infertility and unexplained/explained infertility.

Items	Positive culture N=9(%)	Negative culture	*P*-value
Infertility: Primary	8 (88.9%)	28 (34.6%)	0.002*
Secondary	1 (11.1%)	53 (65.4%)	
Infertility: Unexplained	6 (66.7%)	25 (30.9%)	0.041*
Explained	3 (33.3%)	56 (69.1%)	
Infertility cause: Ovarian	0 (0.0%)	25 (30.9%)	0.031*
: Tubo-peritoneal	1 (11.1%)	20(24.7%)	
: Male factor	2 (22.2%)	11 (13.6%)	

*: Significant ($p< 0.05$)

Table 6: Association between primary/secondary infertility with unexplained/explained infertility.

Infertility	Unexplained infertility N=31(%)	Explained infertility N=59 (%)	Total N=90(%)	*P*-value
Primary	25 (80.6%)	11 (18.6%)	36 (40%)	<0.001*
Secondary	6(19.4%)	48(81.4%)	54(60%)	

*: Significant ($p< 0.05$)

Discussion

Generally, trichomoniasis influenced the fertility of humans (Brunham *et al,* 2015). In men, the infection increased the seminal fluid viscosity, semen agglutination, and particles debris percentage which in turn decreesed the sperms motility and quality (Benchimol *et al,* 2008; Al-Marsomy and Hassan, 2020). In women, trichomoniasis infection caused pelvic inflammatory disease involving inflammation of ovaries, fallopian tubes, endometrium, and pelvic peritoneum ending in tissue damage (Ruggeri *et al,* 2016; Chayachinda and Rekhawasin, 2017). Moreover, the *T. vaginalis* phagocytosed the sperm cells, and their by-products causing infertility in the infected husband and his wife (Benchimol and De Souza, 1995; Benchimol *et al,* 2008). Tsevat *et al.* (2017) in USA reported that apart from *Chlamydia trachomatis* and

Neisseria gonorrhoeae involved in reproductive tract morbidities including tubal factor infertility and pelvic inflammatory disease, other sexually transmitted organisms, such as *Mycoplasma genitalium, Trichomonas vaginalis,* and others within the vaginal microbiome, might be important factors involved in the infertility pathogenesis. They concluded that improved clinical diseases to prevent infection ascending would prevent the persistent burden of infertility. Küng *et al.* (2019) in Austria reported that *T. vaginalis,* the causative agent of the commonest sexually transmitted disease worldwide, was associated with severe complications, including the infertility, preterm labor, cancer and increasing risk of HIV transmission.

In the present study, 90 females complained of infertility, but *T. vaginalis* was detected in 9 of them (10%). This more or less agreed with Abou-Kamar *et al.* (2017) and Hamdy and Hamdy (2018) who reported rates of 13% & 8% in El-Mansoura and Beni-Suef Cities respectively, by using latex agglutination test and culture. But, El Gayar *et al.* (2016) and Hegazy *et al.* (2020) reported higher prevalence of 37.7% & 22.5% respectively in Ismailia and Menoufia Governorates using culture and PCR techniques. Arbabi *et al.* (2018) reported that the overall prevalence rate of *T. vaginalis* infection in the Iranian Population ranged between minimally 0.4% and maximally 42%, and they added that *T. vaginalis* as an important disease associated with human immunodeficiency virus (HIV). Al-Ardi (2021) found a high rate of trichomoniasis (31.7%) among women in Al-Hamza City, Iraq. The worldwide prevalence of trichomoniasis ranged between 0.9% and 80% (Valadkhani *et al,* 2008; Javanbakht *et al,* 2013). Also, Siracusano *et al.* (2014) reported that *T. vaginalis* as a parasitic disease was endemic in many countries, especially in the developing ones, where the infertility was a major burden, depending on the socioeconomic conditions, educational level, and healthcare quality.

In the present study, the direct wet mount smear and Giemsa stained smear detected six cases, compared to nine by Modified Diamond culture, the trichomoniasis gold index diagnosis. The sensitivity and specificity of both were 66.67% & 100%, and 66.67% & 100% respectively, compared to culture. Hamdy and Hamdy (2018) found that the wet mount and Giemsa stained smear gave sensitivities of 16.7%, & 50% and specificities of 100%, & 100% respectively. Hegazy *et al*. (2020) found that 30/200(15%), and 26/200 (13%) positive cases were detected by Giemsa stained smear, and wet mount respectively, with sensitivities of 67%, & 58% and specificities of 100%, & 93.5% respectively compared to Diamond reference index.

In the present study, among the trichomoniasis in infertile patients, which ages ranged between 20 and 35years, infection was more at the 31 years, but without significant difference (P=0.177). Mahmoud *et al*. (2015) recorded high infection rates among age groups 21-30 & 31-40 years, without significant positive cases above 40 years (P=0.627). Hamdy and Hamdy (2018) found that the positivity was high in the age group 31-40 and none case was after the age of 40 years, without significant difference (*P*=0.2).

In the present study, *T. vaginalis* infection was more common among women living in rural areas than urban ones, without significant difference (P=0.28). This agreed with Dahab *et al*. (2012) in Sudan who reported that trichomoniasis were more common in rural areas than urban ones, with significant difference. Al-Ardi (2021) in Iraq reported a higher rate of infection in rural than in urban areas. Nevertheless, Ton *et al.* (2015) in Vietnam, and Hamdy and Hamdy (2018) in Beni-Suef found that the trichomoniasis was significantly common among females in the urban areas.

In the present study, a significant association was between working patients and infection (P=0.005), compared to housewives. The working women use the public toilets at the working places. This agreed with Crucitti *et al.* (2011) who reported non-sexual tric-

homoniasis transmission. Amadi and Nwagbo (2013) in Nigeria found that *T. vaginalis* was more among employees than housewives. But, Nouraddin and Alsakee (2015) in Iraq found more trichomoniasis cases were among housewives than working ones without significant differences.

In the present study, the presence of vaginal discharge and dysuria was significantly association with infection, while itching and dyspareunia did not. Hussein *et al*. (2015) in Egypt found that dyspareunia and dysuria were the commonest symptoms among positive women, followed by vaginal discharge, and itching, without significant difference except for dyspareunia and dysuria. But, Mahmoud *et al*. (2015) reported no significant symptomatic difference among trichomoniasis females (P=0.129). Hamdy and Hamdy (2018) found a significant association between trichomoniasis and dyspareunia (P<0.001) and dysuria (P=0.003), and none with discharge and itching.

In the current study, trichomoniasis gave significant association with primary unexplained infertility, and the male factor was the only agent of infertility, with significance (P=0.031), while the ovarian factor in women was not detected. This agreed with Kaya *et al.* (2015) in Turkey who reported that *T. vaginalis* was positive in 4/22(18%) infertility women, three patients with trichomoniasis as unexplained infertility, and a patient due to male factor infertility. They added that the infection was not detected in women with tubal factor infertility, which may be due to immunological changes, production of cytokine and reactive nitrogen. Midlej and Benchimol (2010) recommended that *T. vaginalis* adhesion affected the sperms motility and caused phagocytosis, lysis, and digestion of sperm cells. Lucena *et al.* (2014) reported that the trichomoniasis in the semen influenced the effectiveness of sperm and capability to fertilize the woman egg.

In the present study, Metronidazole 500mg was given orally twice a day for 7 days, and to abstain from sex until they and their partners treated. El-Sherbini *et al.* (2010) in Egypt proved the anti-trichomoniasis activity of from *Pomegranate granatum* (in-vitro) and *Comphora molmol* (in-vivo) extracts. CDC (2015) reported that the nitroimidazo-les are the only class anti-microbial effective drug for trichomoniasis. Sheehy *et al.* (2015) in Canada found that metronidazole treated bacterial vaginosis and trichomoniasis during pregnancy, without teratogen risk. Bouchemal *et al.* (2017) in France reported that metronidazole (Flagyl®) developed in 1959 and approved in 1960s for trichomoniasis treatment, as being cheap, effective, and generally well tolerated, with common side effects, such as mild gastrointestinal disturbances. But, Dan and Sobel (2007) reported failure to cure trichomoniasis in three women who showed nitroimidazole resistance. Raja *et al.* (2016) in India found that tinidazole® at lower dose gave a good efficacy than metronidazole in long-term cure rates in preventing relapses with better side effect profile.

In the present study, the multivariable binary logistic regression analysis showed that trichomoniasis increased the unexplained infertility probability for four times (OR 95%, CI was 4.01, 1.01 to 18.55). This agreed with Al-Marsomy and Hassan (2020) they reported that prompt diagnosis and treatment of trichomoniasis helped in improving the implantation rate in the patients with unexplained infertility, as *T. vaginalis* pathogenicity caused inadequate conditions in the female reproductive tract, which delayed the pregnancy. Wiringa *et al.* (2020) in USA isolated *T. vaginalis* always from the women vagina with pelvic inflammatory disease in the PEACH cohort. They added that although no significant, prospective associations between trichomoniasis and sequelae were novel, but under-scored was the need for more investigations into whether *T. vaginalis* played etiological role in adverse reproductive or gynaecological outcomes. Al-Ardi (2021) found a high trichomoniasis rate (11.6%) among infertile women, and incriminated *T. vaginalis* as the main cause of infertility.

Tsang *et al.* (2019) in USA suggested the role for *T. vaginalis* in the development of clinically significant the prostate cancer, but they did not support the hypothesis that trichomoniasis serostatus was associated with mortality among the prostate cancer patients. Besides, Saleh *et al.* (2021) in Egypt reported that chronic *T. vaginalis* infection was associated with prostate cancer, and added that it did not seem that the trichomoniasis aggravated the cancer status.

Conclusion

Generally speaking, *T. vaginalis* (trichomoniasis) is one of the most prevalent sexually transmitted diseases (STDs) among both couples, also nonsexual transmission occurred.

The Metronidazole® proved to be effective in treating trichomoniasis infection. The data indicated that *T. vaginalis* must be screened in infertile women, as an etiological agent.

Studies on the pathophysiology of the female infertility due to the chronic trichomoniasis are ongoing and will be published in due time elsewhere.

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Explanation of figure

Fig. 1: *Trichomonas vaginalis* in Modified Diamond culture × 40.