# Is trichomoniasis associated with adverse preganancy outcome?

Original Article Marwa M Hamouda<sup>1</sup>, Sara A Mohamed<sup>2</sup>, Suzan H Elgendy<sup>1</sup>, Nora E Saleh<sup>1</sup>, Wafaa M EL-Zayady<sup>1</sup> Departments of Medical Parasitology<sup>1</sup> and Obstetrics and Gynecology<sup>2</sup> Faculty of

Departments of Medical Parasitology<sup>1</sup> and Obstetrics and Gynecology<sup>2</sup>, Faculty of Medicine, Mansoura University, Mansoura, Egypt

## ABSTRACT

**Background:** Trichomoniasis is the most common curable sexually transmitted disease worldwide, suspected of increasing the risk for adverse pregnancy outcomes.

**Objective:** In this study, we aimed to determine influence of trichomoniasis on pregnancy outcome, detect the rate of trichomoniasis during pregnancy and to identify the risk factors of trichomoniasis.

**Patients and Methods:** Vaginal swabs were obtained from 200 pregnant females with a gestational age of 13 to 40 weeks calculated according to the last menstrual cycle. Clinical examination, vaginal discharge wet mount, Giemsa stain, and culture in modified Diamond's media were performed. Metronidazole treatment was given for all positive cases. Patients were advised to encourage their partners to receive the same treatment. Sociodemographic information was collected, and cases were divided into two groups, the study group (infected) and the control group (not infected). Pregnancy outcome (full term delivery, preterm labor, premature membrane rupture, low birth weight infants) was assessed according to the elapsed time from the first sonogram to the week of delivery.

**Results:** Twenty-four cases were positive for *T. vaginalis*. Positivity was mostly detected by culture (12%) followed by wet mount microscopy and/or Giemsa staining (8.5%). Premature rupture of membrane was detected in 17/24 (70%) of trichomoniasis positive and in 29/176 (16.5%) of trichomoniasis negative cases (P=0.0001). Preterm delivery was detected in 12/24 (50%) of positive cases and 29/176 (16.5%) of negative cases (P=0.0001). Low birth weight of infants was observed in 17/24 (70%) of positive cases and in 28/176 (15.9%) of negative cases (P=0.0001). Premature membrane rupture and preterm delivery were statistically significant in positive cases with bad compliance to metronidazole treatment (P=0.044) and (P=0.004), respectively. The high rate of infection was observed in age group of 26-35 years (P=0.017). In addition, isolation of the parasite was significantly (P>0.05) recorded in pregnant women presenting with vaginal discharge and history of previous infection.

**Conclusion:** Our results highlight the presence of preterm delivery and delivery of low birth weight infants in infected pregnant women presenting with vaginal discharge and a history of previous infection. Premature membrane rupture and preterm delivery were statistically significant in positive cases with bad compliance to metronidazole treatment. Therefore, pregnant women should be screened for trichomoniasis to reduce the incidence of preterm delivery and low birth weight.

Keywords: modified Diamond's media; pregnancy outcome; trichomoniasis; vaginal swab.

Received: 20 May, 2022; Accepted: 22 August, 2022.

Corresponding Author: Marwa M. Hamouda, Tel.: +20 1224142147, E-mail: drmarwahamouda@mans.edu.eg

Print ISSN: 1687-7942, Online ISSN: 2090-2646, Vol. 15, No. 2, August, 2022.

### **INTRODUCTION**

Trichomoniasis is a communicable disease caused by the anaerobic protozoan, *T. vaginalis*. With a global incidence of 276.4 million infections each year, the disease is the most prevalent curable sexually transmitted infection<sup>[1]</sup>. Previous studies in Egypt reported a prevelance of trichomoniasis of 8%<sup>[2]</sup>, 4%<sup>[3]</sup> and 27%<sup>[4]</sup>. In 25 to 50% of women, vaginal infection was asymptomatic<sup>[5,6]</sup>. On the other hand, symptomatic females present with vaginal scretions, dysuria and pruritus, vaginitis and cervicitis<sup>[5,7]</sup>. Many health problems have been associated with trichomoniasis, including cancer cervix, pelvic inflammatory disease, and infertility<sup>[8,9]</sup>. Premature rupture of membranes, preterm delivery, and low birth weight are major pregnancy risks<sup>[10,11]</sup>. Besides, *T. vaginalis* can produce destruction of the host epithelial cells by direct cell contact and subsequent release of cytotoxic substances. This may possibly lead to cytolysis, change in the host junctional complexes, and interruption of host tissues. These mechanisms were suggested for separation of human amniotic epithelial cell layer accounting for the adverse pregnancy outcome during infection<sup>[12]</sup>.

Personal non-commercial use only. PUJ copyright © 2022. All rights reserved

Diagnosis of trichomoniasis is difficult since its symptoms resemble those of other sexually transmitted infections, and it needs a test with high sensitivity to confirm the infection. The diagnosis is made by traditional direct wet mount microscopy, staining, or culture technique. Other diagnostic methods including immunological detection or nucleic acid-based assays showed higher sensitivity and specificity<sup>[13]</sup>. Ghallab *et al.*<sup>[4]</sup> reported that nested PCR (nPCR) was highly significant in diagnosis of trichomonisasis in multiattribute analysis than wet mount, Giemsa staining and culture. The result of the previous study confirmed that nPCR is a powerful diagnostic tool for batch sample diagnosis and in strain typing to explain the nature and extent of genetic diversity of *T. vaginalis*<sup>[4]</sup>.

The epidemiology of trichomoniasis can be influenced by several risk factors such as age, residence, socioeconomic level, education, marital status and the method of contraception used, the presence and the character of vaginal discharge, the drug used, and the history of any other sexually transmitted infections<sup>[14]</sup>. Despite its high prevalence substantial accompanying morbidity and and associated HIV acquisition<sup>[15]</sup>, *T. vaginalis* received relatively little public health attention. Therefore, we aimed to determine the influence of trichomoniasis on pregnancy outcome and to detect the rate and risk factor of trichomoniasis in pregnant women. Our study used conventional direct wet mount microscopic examination, Giemsa stain, and Diamond's medium culture.

#### SUBJECTS AND METHODS

This observational cohort prospective study aimed to determine influence of trichomoniasis on pregnancy outcome. The study was conducted at the Medical Parasitology Department, Faculty of Medicine, Mansoura University, during the period from May 2020 and April 2021.

**Study design:** Vaginal swabs were collected from pregnant women for parasitological examination using wet mount, Giemsa stain, and culture in modified Diamond's media. Accordingly, cases were divided into two groups, those pregnant with trichomoniasis (infected study) and pregnant without trichomoniasis (control non-infected). All positive cases for *T. vaginalis* and their partners received metronidazole treatment. Pregnancy outcome was assessed to evaluate influence of trichomoniasis.

**Target population and participants selection:** The present study enrolled pregnant women attending the Obstetrics and Gynecology Outpatient Clinic at Mansoura University Hospital. Participants were randomly chosen from non-complicated pregnant women of the second and third trimesters (13 to

40 weeks gestional age), with or without vaginal discharge, who attended for a routine antenatal appointment at the hospital and aging between 18 to 45 years old (inclusion criteria). The last menstrual cycle date was recorded to calculate gestational age, that was validated by ultrasonography. A standardized questionnaire was used to collect sociodemographic data and obstetric history from all participants. Complicated pregnancies with placenta previa, multiple pregnancies, hydramnios, congenital malformations, intrauterine growth retardation, other medical issues such as insulin-dependent diabetic mellitus, chronic hypertension, maternal heart illness, and pre-eclampsia were ruled out of the study as exclusion criteria.

Accordingly, two hundred pregnant women were enrolled in the study aging between 18 to 45 years old and a gestational age of 13 to 40 weeks, with or without complaints of vaginal discharge. Based on the results of culture examination for *T. vaginalis* infection, pregnant women were divided into two groups; pregnancy with trichomoniasis and pregnancy without trichomoniasis.

**Clinical data and samples collected:** All study participants were subjected to the following:

- 1. A full medical questionnaire fulfilling the demographic data, menstruation history, genitourinary complaints and obstetric history.
- 2. Clinical examination: Each participant was examined physically for the routine medical parameters, i.e, gestional age, infant parameters according to gestional age, patient blood pressure.
- 3. Two vaginal specimens (cotton swabs) were collected from the posterior fornix of the vagina regardless of the presenc or absence of complaints. One sample was used for microscopic slide examination either as wet mount and/or Giemsa stained, and the other was used for cultivation in modified Diamond medium (MDM).

Wet mount smear preparation: The swab was immediately inoculated in a tube containing 3 ml sterile PBS (pH 7.2). Prior to removal, the swab was placed against the tube's side, and the tube was vortexed for 30 sec to express as much fluid as possible<sup>[16]</sup>. Using a sterile pasteur pipette, a drop of the vortexed fluid was placed on a clean glass slide, covered with a clear coverslide and examined microscopically for motile *T. vaginalis* trophozoites in 100x or 400x magnifications. Each slide was thoroughly examined for 2-3 min. The parasite was identified by its oval or pyriform shape (10-20  $\mu$ ) and characteristic twitching motility<sup>[17]</sup>.

**Giemsa-stained slide preparation:** A drop from the prepared vortexed fluid was applied on a slide and allowed to dry in air. The slide was then fixed in methanol for one minute before staining for 20 min with 20% Giemsa stain (diluted 1:20 with distilled water). To remove any residual stain, the slide was cleared with normal tap water then allowed to dry. Trophozoites with characteristic features and extended flagellae were detected on the stained preparation by microscopic magnification at 1000x<sup>[18]</sup>.

**Cultivation on MDM:** The second swab was promptly placed into a culture tube containing 10 ml MDM, that was prepared according to the manufacturer's instructions<sup>[19]</sup>. The prepared medium was sterilized by autoclaving at 121°C for 15 min after the pH was corrected to 6.0 with HCL or NaOH. To suppress bacterial and fungal growth, 0.5 ml of the antibiotics mixture (20.000 I.U./ml Penicillin G, 20 mg/ml Streptomycin sulfate, 40g/ml Fluconazole) were added to cooled one ml sterile inactivated bovine serum. Culture tubes were preheated to 37°C for 15 min before vaginal swabs were inserted into the media. Following inoculation the cultures were examined microscopically daily for one week for the presence of *T. vaginalis* trophozoites .

**Metronidazole treatment:** According to microscopic and/or culture results, all positive participants were prescribed treatment which was also recommended for their husbands. Metronidazole treatment was provided, at a dose of 500 mg twice a d for 7 d<sup>[20]</sup>.

**Recording and assessment of pregnancy outcome:** All participants were followed up during their regular visits to record their pregnancy outcome. Gestational age was determined from the last menstrual period and was confirmed by ultrasonography. The duration of gestation at delivery was detected by calculating the elapsed time from the first visit sonogram to delivery. Preterm birth was defined as delivery at less than 37 completed weeks (259 d) of gestation<sup>[21]</sup>.

**Statistical analysis:** The data was examined using SPSS 15 for Windows. The frequency distribution process was used to calculate the number of cases and percentages for qualitative variables, while the mean and standard deviation (SD) or the median and range were used for quantitative data. The Chi-square test was used to determine whether category variables are related. If the Chi-square assumptions are broken, Fisher's exact test was employed. To compare the means of two groups, the independent-samples t-test was employed. A one-way analysis of variance (ANOVA) was employed to compare the means of more than two groups. Statistical significance was considered when *P* value was less than 0.05.

**Ethical considerations:** The Research Ethics Committee of the Faculty of Medicine, Mansoura University, Egypt, approved the study protocol (IRB code: R.19.01.391). Written consents were obtained from all participants after describing the aim of the study and the examination procedures. Trichomoniasis-positive cases were prescribed treatment after describing the nature of infection and after obstetrician approval.

#### RESULTS

**Detection rate of** *T. vaginalis* **infection by different diagnostic methods:** Out of 200 pregnant women who participated in our study, infection with *T. vaginalis* was detected in 17/200 (8.5%) through microscopic vaginal swabs examination using wet mount smear or permanently-stained samples. Meanwhile, cultivation of samples on MDM diagnosed trichomoniasis in 24/200 (12%) cases.

Accuracy of wet mount and Giemsa staining test in relation to MDM: Comparing the parasitological trichomoniasis by microscopic detection of examination and culture results, all microscopically positive samples (17/24; 70.8%) were confirmed positive by cultivation. However, 7/24 (29.2%) microscopically negative samples proved positive on cultivation. Thus considering the MDM as the gold standard test for detecting trichomoniasis, microscopic examination of vaginal swabs (wet mount smears and/or giemsa-stained slides) revealed a sensitivity of 70.8% and specificity of 100%. The lower sensitivity for microscopic examination in relation to culture results (Table 1) was statistically significant (*P*>0.001).

**Sociodemographic data among studied cases:** Based on the results of the gold standard MDM, the participants were divided into pregnancy with trichomoniasis group (24/200;12%)) and pregnancy without trichomoniasis group (176/200;88%). According to age distribution of participants, the positive trichominiasis group showed higher prevalence of infection (58.3%) in the age range of 26-35 y than other ages groups , with a statistically significant difference (*P*=0.017) (Table 2). The residence of the participated women in rural or urban area was nearly equally distributed without any significant difference between

Table 1. Accuracy of wet mount and Giemsa stining test in relation to modified Diamond's culture.

<b>Diagnosis of</b> T. vaginalis		Cu	Culture (gold standard)		Accuracy measures	
		Positive	Negative	Total	Variable	%
Wet mount and Giemsa staining	Positive Negative	17 7	0 176	17 183	Sensitivity – Specificity PPV	70.8 100 100
	Total	24	176	200		
Stunning		AUC = 0.82; 95%CI (	0.72-0.89); <i>P</i> = 0.00	1*	NPV 96.2	

**AUC:** Area under curve; **95% CI:** Confidence interval 95%; **PPV:** Positive predictive value; **NPV:** Negative predictive value; **\*:** Significant (*P* > 0.05).

the positive and negative trichomoniasis participants. Also the occupation and educational level showed nonsignificant difference between the participants (Table 2).

There was a significant difference between pregnancy with and without trichomoniasis as regards the presence of vaginal discharge (P=0.012), as 15/24 (62.5%) of the former cases complained of vaginal discharge versus 63/176 (35.8%) of the latter group. History of previous genitourinary infection was higher in 15/24 (62.5%) trichomoniasis positives, than in

Table 2. S	Sociodemographic	data among studied	groups.

49/176 (27.8%) trichomoniasis negatives respectively, with significant difference (*P*=0.001) (Table 2).

**Descriptive data of positive trichomoniasis cases:** Good compliance to metronidazole treatment was observed in 13/24 (54.2%) of the trichomoniasis-positive cases group. Partner treatment was applied in 16/24 (66.7%) of them. More than two antinatal care visits were attended by 79.2% of cases in their 2nd trimester. More than one culture positive were found in 8 (33.3%) cases who received more than one course of metronidazole treatment. A single course of

Risk factors –		Trichomoniasis negative	Trichomoniasis posative	Statistical analysis
		No. (%)	No. (%)	P value
	18-25	58 (34.9)	6 (25.0)	
Age <sup>@</sup>	26-35	49 (29.5)	14 (58.3)	0.017*
	36-45	59 (35.6)	4 (16.7)	
D! -!	Urban	92 (52.3)	12 (50.0)	0.8
Residence	Rural	84 (47.7)	12 (50.0)	
Education	Illiterate	23 (13.1)	1 (4.2)	
	Primary	36 (20.5)	7 (29.2)	0.2
	Vocational	72 (40.9)	13 (54.1)	0.2
	Higher	45 (25.5)	3 (12.5)	
Occupation	Housewife	81 (46.0)	13 (54.2)	0.4
	Working	95 (54.0)	11 (45.8)	
Vaginal Absent	Absent	113 (64.2)	9 (37.5)	0.012*
discharge	Present	63 (35.8)	15 (62.5)	
Previous	Negative	127 (72.2)	9 (37.5)	0.004*
infections Posit	Positive	49 (28.8)	15 (62.5)	0.001*
Т	otal	176 (100)	24 (100)	

<sup>®</sup>: Ten cases of trichomoniasis negative cases declined to state their age (Total = 166). #: Chi-square test; \*: Significant (*P* > 0.05).

metronidazole treatment failed to clear the infection in 8 cases (33.3%) who required repeated course(s) to be culture negative (Table 3).

**Pregnancy outcome among studied groups:** There was significant difference as regard the detected adverse pregnancy outcomes between the trichomoniasis-positive and the trichomoniasisnegative groups. Premature rupture of membranes occurred significantly (P=0.0001) in 17/24 (70.8%) of the former and in 29/176 (16.5%) of the latter. Consequently, preterm delivery occurred in 12/24 (50%) of the former and in 29/176 (16.5%) of the latter group indicating significant difference (P=0.0001). Low birth weight infant also occurred significantly (P=0.0001) in 17/24 (70.8%) of the former and in 28/176 (15.9%) of the latter group (Table 4).

**Metronidazole treatment compliance in relation to pregnancy outcome:** On correlation between metronidazole treatment compliance and the adverse pregnancy outcome among the trichomoniasis positive participants, premature rupture of membrane and preterm delivery were statistically significantly higher in patients with poor metronidazole compliance than

Table 3. Descriptive data of positive trichomonaisis cases	s.
--	----

Characte	ristics	No. (%)
Metronidazole	Positive	13 (54.2)
good compliance	Negative	11 (45.8)
Partner	Positive	16 (66.7)
treatment	Negative	8 (33.3)
Numbers of visits	1-2 visits > 2 visits	5 (20.8) 19 (79.2)
Numbers of	One test	16 (66.7)
tests positive	> one test	8 (33.3)
Numbers of treatment course	None One course > one course	3 (12.5) 13 (54.2) 8 (33.3)
Time of the	2 <sup>nd</sup> trimester	19 (79.2)
first test	3 <sup>rd</sup> trimester	5 (20.8)

patients successfully treated (72.7% vs 30.8%; *P*= 0.044 for the premature rupture of membrans, 81.8% vs 23.1%; P: 0.004 for preterm delivery) respectively (Table 5). There was no statistically significant effect of metronidazole treatment compliance and low birth weight infant (Table 5).

#### PARASITOLOGISTS UNITED JOURNAL

Table 4 December of outcome among atudied and

Pregnancy outcome		Trichomoniasis negative	Trichomoniasis posative	Statistical analysis <sup>#</sup>
		No. (%)	No. (%)	P value
Pregnancy rupture	Negative	147 (83.5)	7 (29.2)	0.0001*
membrane	Positive	29 (16.5)	17 (70.8)	
Preterm labor	Negative	147 (83.5)	12 (50.0)	0.0001*
	Positive	29 (16.5)	12 (50.0)	0.0001*
Full term delivery	Negative	36 (20.5) <sup>@</sup>	12 (50.0)	0.004*
	Positive	140 (79.5)	12 (50.0)	0.001*
Low-birth weight	Negative	148 (84.1)	7 (29.2)	0.0001*
	Positive	28 (15.9)	17 (70.8)	0.0001*

#: Chi-square test; \*: Significant (P > 0.05); @: 7 cases showed post-term delivery among the control group.

Table 5. Metronidazole	treatment compliance	in relation to pregnancy outcome.

		Metronidazo	Statistical analysis	
Pregnancy outcome		Negative	Positive	— Statistical analysis
		No. (%)	No. (%)	P value
Pregnancy rupture	Negative	3 (27.3)	9 (69.2)	0.044*
membrane	Positive	8 (72.7)	4 (30.8)	0.044*
D ( 11	Negative	147 (83.5)	10 (76.9)	0.004*
Preterm labor	Positive	29 (16.5)	3 (23.1)	0.004*
Full term delivery	Negative	36 (20.5) <sup>@</sup>	3 (23.1)	0.004*
	Positive	140 (79.5)	10 (76.9)	0.004*
	Negative	148 (84.1)	3 (23.1)	0.476
Low-birth weight	Positive	28 (15.9)	10 (76.9)	0.476

#: Chi-square test; \*: Significant (**P** > 0.05).

#### DISCUSSION

Trichomoniasis is the most prevelant non-viral sexually transmitted human infection globally, affecting all ages, races, and socioeconomic classes. Prevalence percentages of T. vaginalis vary widely worldwide, ranging from 0.9% to 80%<sup>[22]</sup>. Several studies in Egypt reported prevelances between 5% and 91.3%<sup>[2]</sup>. Despite being mostly an asymptomatic sexually transmitted infection, several sequels and complications can occur during pregnancy including premature rupture of membranes, premature delivery, and low birth weight infants. Therefore, exploring the rate and the sequel of this infection with pregnancy was the aim of this study. Trichomoniasis was found to occur in 12% of women attending ordinary prenatal care fullfilling the inclsion criteria, i,e. pregnant women aging between 18 to 45 y old and a gestational age of 13 to 40 w, with or without complaints of vaginal discharge. The occurrence of atypical vaginal discharge and a previous history of infection were both risk factors for infection in this population. A recent study of infection in pregnant women in Egypt similarly recorded a prevalence of 11.7%<sup>[17]</sup>. In addition, pregnant women from Durban had a comparable prevalence rate of 10% of trichomoniasis<sup>[23]</sup>. Prevelances in pregnant women in other countries ranged between 17% and 20% in Africa, 16% and 53% in the United States and 0.8% in Asia<sup>[24]</sup>.

The present study detected premature rupture of membranes, preterm births and low birth weight of infants in 70.8%, 50% and 70.8% of infected women, respectively. Other studies<sup>[17,25]</sup> recorded a link between vaginal trichomoniasis, premature birth, and low birth weight babies. Preterm birth and consequent low birth weight infants were 30% more probable in pregnant women infected with *T. vaginalis* than in non-infected women. This link which was also validated in a metaanalysis research<sup>[26]</sup> supported our findings. Moreover, our study observed the effect of metronidazole treatment on trichomoniasis by comparing the pregnancy outcome in infected pregnant females who had completed a metronidazole 7-days treatment course to those who didn't comply to the course. Interestingely, we found significant decrease in the percentages of premature rupture of membranes and preterm delivery in the former group when compared to the second group who didn't complete the treatment course. Another report on pregnancy outcome in treated cases recorded full term birth in 14/35 (40%) and preterm birth in 19/35 (54.28%)<sup>[17]</sup>. Leitich et al.<sup>[27]</sup> advised treating high risk patients who had previous history of preterm delivery, with oral metronidazole as it may reduce the incidence of preterm birth. These findings are in contrast to previously reported results by Ajiji et al.[28] in a systematic review and metanalysis. The reviewers rejected the relationship between metronidazole treatment during pregnancy and protection from preterm delivery, stillbirth, low birth weight infant, and caesarian delivery. Okun et al.<sup>[29]</sup> claimed no benefit resulted from the use of metronidazole and other antibiotic therapy in the treatment of bacterial vaginosis or *T. vaginalis* during pregnancy to lower the risk of preterm birth. In confirmation Morency and Bujold<sup>[30]</sup> observed that metronidazole treatment of bacterial vaginosis and T. vaginalis should be avoided during the second trimester of pregnancy in high risk women with previous history of preterm delivery since it was associated with higher risk of preterm birth. Later in a systematic review and metanalysis, Sheehy et al.<sup>[31]</sup> controversially considered that treating trichomoniasis and bacterial vaginosis during pregnancy with metronidazole not only was efficient and displayed no teratogenic hazard, but also decreased the incidence of preterm birth when incorporated with other antibiotics.

Two other studies recorded increased risk of pretem delivery in pregnant women who received metronidazole for less than three days<sup>[21,32]</sup>. In one of the reports treatment was by 2 g administered as a single dose and then repeated 48 h apart<sup>[21]</sup>; and in the other report 400 mg/d was administered for two days<sup>[32]</sup>.

In the present study, trichomoniasis was more common in the age group of 26-35 y, followed by the age group 18-25 y. This finding was consistent with other previous studies<sup>[17,33]</sup>, confirming that trichomoniasis is commonly associated with female patients at childbearing ages, since these age periods represent the period of sexual activity<sup>[34]</sup>. Sociodemographic data concerning residency, education level, and occupation did not differ between our study groups. Our results do not conform with a report of Kamal et al.[17] on females living in rural areas of Minia governorate in Egypt. The researchers recorded a prevelance 11.7% of trichomoniasis and attributed this to poor socioeconomic conditions and restricted education to primary education levels which were considered as asignificant risk factors for infection. Another report by Ibrahim et al.<sup>[35]</sup> revealed that trichomoniasis was more common among women living in rural regions than in urban areas, but there was no statistically significant difference. A risk factor for trichomoniasis was the presence of abnormal vaginal discharge along with a previous history of genitourinary infection. The bivariate analysis of reports on presence of vaginal discharge revealed a significant correlation with the infection (P=0.012). Other investigations had reached the same conclusion<sup>[23,35]</sup>.

Previously the Diamond modified broth culture was advocated as the standard gold test<sup>[17,36]</sup>. In our study, all 24 pregnant females presenting clinically as trichomoniasis were proved positive with Diamond culture (100%). Accordingly, our results recorded

by the wet mount and Giemsa staining tests, had a sensitivity and specificity of 70.8% and 100 %. respectively for both examination methods. Hamdy and Hamdy<sup>[2]</sup> declared that the wet mount and Giemsa stained smear had sensitivities of 16.7% and 50%, respectively, and specificities of 100% and 100%. When compared to the Diamond reference index, Hegazy et al.<sup>[37]</sup> showed that Giemsa stained smear and wet mount detected 30/200 (15%) and 26/200 (13%) positive cases, respectively, with sensitivities of 67%, 58%, and specificities of 100%, and 93.5% respectively. Ibrahim *et al.*<sup>[35]</sup> also reported that direct wet mount smear and Giemsa stained smear detected six cases, compared to nine by MDM with sensitivity and specificity for both of 66.67% and 100% respectively as compared to the Diamond culture. Other innovative studies are required to investigate the validity of adminsteration of a high dose for a short time to elevate the risk of preterm delivery among women with past history of preterm delivery compared to a small dose for a longer time<sup>[28]</sup>. It is essential to investigate and treat patients with trichomoniasis before pregnancy to prevent the bad sequel of trichomoniasis on the outcome of pregnancy and to reduce the probability of metronidazole treatment during pregnancy due to its duobtfoul safety during pregnancy.

In conclusion, the asymptomatic sexually transmitted T. vaginalis is a silent danger that may affect pregnant women causing unfavourable consequances. Thus repeated examination using the favourable culture MDM is preferred specially if vaginal discharge or previous infections were reported as risk factors. The association with premature ruptue of membranes and premature deliveries are higher in infected than noninfected cases. Treatment with metronidazole during pregnancy is controversial and needs further studies evaluating the benefit and the hazards of its administration on the pregnancy outcome and validating the optimum dose and its duration of administration. Therefore, further study is recommended using different treatment regimens on pregnant women with or without high risk factor (women with previous history of preterm delivery).

**Author contribution:** Hamouda MM suggested the topic, designed and wrote the methodology, collected and interpreted the data, collected the literature, drafted the work and approved the final form. Mohamed SA performed the clinical examination, collected the data, revised the work critically for important intellectual content and approved the final version to be published. Elgendy SH helped in laboratory investigations and statistical analysis, revised and approved the final version to be published. Saleh NE helped in drafting the work, edited and revised it critically for important intellectual content. El-Zayady WM helped in literature search and data analysis, reviewed and approved the final version.

**Conflict of interest:** The authors have no conflict of interest.

**Funding statement**: No funding was received for conducting this study.

#### REFERENCES

- Salakos E, Korb D, Morin C, Sibony O. A case of nontreated *Trichomonas vaginalis* infection and severe preterm labor with positive obstetrical outcome. J Gynecol Obstet Hum Reprod 2018; 47(4):171-173.
- Hamdy DA, Hamdy HG. Prevalence, sociodemographic factors and clinical criteria of *Trichomonas vaginalis* infection among symptomatic women in beni-suef governorate, Egypt. J Egypt Soc Parasitol 2018; 48(1):109-117.
- Mohamed B, Elleboudy N, Hussein H, Khalifa K, Azab M. Genotyping of *Trichomonas vaginalis* isolates from Egypt. PUJ 2019; 12(3):209-220.
- 4. Ghallab MMI, Alaa D, Morsy SM. Multiattribute analysis of *Trichomonas vaginalis* diagnostics and its correlation with clinical complaints and contraceptive methods in a symptomatic Egyptian cohort. Infect Dis Obstet Gynecol 2021; 2021:5525095.
- Naidoo S, Wand H. Prevalence and incidence of *Trichomonas vaginalis* infections in women participating in a clinical trial in durban, south africa. Sex Transm Infect 2013; 89(6):519-522.
- 6. Bouchemal K, Bories C, Loiseau PM. Strategies for prevention and treatment of *Trichomonas vaginalis* infections. Clin Microbiol Rev 2017; 30(3):811-825.
- Kusdian G, Gould SB. The biology of *Trichomonas* vaginalis in the light of urogenital tract infection. Mol Biochem Parasitol 2014; 198(2):92-99.
- Swygard H, Sena AC, Hobbs MM, Cohen MS. Trichomoniasis: Clinical manifestations, diagnosis and management. Sex Transm Infect 2004; 80(2): 91-95.
- Fichorova RN. Impact of *T. vaginalis* infection on innate immune responses and reproductive outcome. J Reprod Immunol 2009; 83(1-2):185-189.
- Sherrard J, Ison C, Moody J, Wainwright E, Wilson J, Sullivan A. United kingdom national guideline on the management of *Trichomonas vaginalis* 2014. Int J STD AIDS 2014; 25(8):541-549.
- 11. Oyeyemi OT, Fadipe O, Oyeyemi IT. *Trichomonas vaginalis* infection in nigerian pregnant women and risk factors associated with sexually transmitted infections. Int J STD AIDS 2016; 27(13):1187-1193.
- 12. Benchimol M, Pereira-Neves A, De Souza W. Pathogenesis of *Trichomonas vaginalis* in humans. In: Singh SK, ED. Human emerging and re-emerging infections: Viral and parasitic infections, 1<sup>st</sup> edition, John Wiley & Sons Inc., 2015; 423-439.
- 13. Divakaruni AK, Mahabir B, Orrett FA, Rao AS, Srikanth A, Chattu VK, *et al.*, Prevalence, clinical features, and diagnosis of *Trichomonas vaginalis* among female sti clinic attendees in trinidad. J Family Med Prim Care 2018; 7(5):1054-1057.

- 14. Ton Nu PA, Nguyen VQ, Cao NT, Dessi D, Rappelli P, Fiori PL. Prevalence of *Trichomonas vaginalis* infection in symptomatic and asymptomatic women in central vietnam. J Infect Dev Ctries 2015; 9(6):655-660.
- 15. Torok MR, Miller WC, Hobbs MM, Macdonald PD, Leone PA, Schwebke JR, *et al.*, The association between oral contraceptives, depot-medroxyprogesterone acetate, and trichomoniasis. Sex Transm Dis 2009; 36(6):336-340.
- 16. Mahmoud A, Sherif NA, Abdella R, El-Genedy AR, El Kateb AY, Askalani AN. Prevalence of *Trichomonas vaginalis* infection among Egyptian women using culture and latex agglutination: Cross-sectional study. BMC Womens Health 2015; 15:7.
- 17. Kamal AM, Ahmed AK, Mowafy NME, Shawki HE, Sanad AS, Hassan EE. Incidence of antenatal trichomoniasis and evaluation of its role as a cause of preterm birth in pregnant women referring to Minia university hospital, Egypt. Iran J Parasitol 2018; 13(1):58-66.
- 18. Radonjic IV, Dzamic AM, Mitrovic SM, Arsic Arsenijevic VS, Popadic DM, Kranjcic Zec IF. Diagnosis of *Trichomonas vaginalis* infection: The sensitivities and specificities of microscopy, culture and pcr assay. Eur J Obstet Gynecol Reprod Biol 2006; 126(1):116-120.
- Gelbart SM, Thomason JL, Osypowski PJ, James JA, Hamilton PR. Comparison of Diamond's medium modified and Kupferberg medium for detection of *Trichomonas vaginalis*. J Clin Microbiol 1989; 27(5): 1095-1096.
- 20. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010; 59(RR-12):1-110.
- 21. Klebanoff MA, Carey JC, Hauth JC, Hillier SL, Nugent RP, Thom EA, *et al.*, Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. N Engl J Med 2001; 345(7):487-493.
- 22. Chetty R, Mabaso N, Abbai N. Genotypic variation in *Trichomonas vaginalis* detected in south african pregnant women. Infect Dis Obstet Gynecol 2020; 2020: 1687427.
- 23. Dessai F, Nyirenda M, Sebitloane M, Abbai N. Diagnostic evaluation of the BD Affirm VPIII assay as a pointof-care test for the diagnosis of bacterial vaginosis, trichomoniasis and candidiasis. Int J STD AIDS 2020; 31(4): 303-311.
- 24. Muzny CA, Van Gerwen OT, Kissinger P. Updates in Trichomonas treatment including persistent infection and 5-nitroimidazole hypersensitivity. Curr Opin Infect Dis 2020; 33(1):73-77.
- 25. Rasti S, Mitra B, Gholamabbas M, Moniri R. Complications of trichomoniasis on the pregnant women. Jundishapur J Microbiol 2011; 4(1):31-6.
- 26. Silver BJ, Guy RJ, Kaldor JM, Jamil MS, Rumbold AR. *Trichomonas vaginalis* as a cause of perinatal morbidity: A systematic review and meta-analysis. Sex Transm Dis 2014; 41(6):369-376.
- 27. Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor

for preterm delivery: A meta-analysis. Am J Obstet Gynecol 2003; 189(1):139-147.

- 28. Ajiji P, Uzunali A, Ripoche E, Vittaz E, Vial T, Maison P. Investigating the efficacy and safety of metronidazole during pregnancy: A systematic review and metaanalysis. Eur J Obstet Gynecol Reprod Biol X 2021; 11:100128.
- 29. Okun N, Gronau KA, Hannah ME. Antibiotics for bacterial vaginosis or *Trichomonas vaginalis* in pregnancy: A systematic review. Obstet Gynecol 2005; 105(4):857-868.
- 30. Morency AM, Bujold E. The effect of second-trimester antibiotic therapy on the rate of preterm birth. J Obstet Gynaecol Can 2007; 29(1):35-44.
- 31. Sheehy O, Santos F, Ferreira E, Berard A. The use of metronidazole during pregnancy: A review of evidence. Curr Drug Saf 2015; 10(2):170-179.
- Odendaal HJ, Popov I, Schoeman J, Smith M, Grove D. Preterm labour: Is bacterial vaginosis involved? S Afr Med J 2002; 92(3):231-234.
- 33. Mabaso N, Naicker C, Nyirenda M, Abbai N. Prevalence and risk factors for *Trichomonas vaginalis* infection in

pregnant women in south africa. Int J STD AIDS 2020; 31(4):351-358.

- 34. Mabaso N, Abbai NS. A review on *Trichomonas vaginalis* infections in women from africa. S Afr J Infect Dis 2021; 36(1):254.
- 35. Ibrahim SS, Ismail MAM, El-Askary HM, Khalil EM, Khalil DM, Raafat AM. Potential role of *Trichomonas vaginalis* in women with primary and secondary infertility in Beni-Suef, Egypt. J Egypt Soc Parasitol 2021; 51:119-126.
- 36. Domeika M, Zhurauskaya L, Savicheva A, Frigo N, Sokolovskiy E, Hallen A, *et al.*, Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. J Eur Acad Dermatol Venereol 2010; 24(10):1125-1134.
- 37. Hegazy A, Kersh WME, Moharm IM, Ammar AI, Hemida AS, Atia AF. Immunological and cytopathological assessment of *Trichomonas vaginalis* infection in asymptomatic and symptomatic females at menoufia governorate, Egypt. Int J Curr Microbiol 2020; 9:686-705.