

<https://doi.org/10.33472/AFJBS.6.6.2024.61-73>



African Journal of Biological Sciences



Microscopic and molecular identification of *Trichomonas vaginalis* in the northern Parts of Basrah Province

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Article History
Volume 6, Issue 6, Feb 2024
Received: 01 Mar 2024
Accepted : 08 Mar 2024
doi:10.33472/AFJBS.6.6.2024.61-73

Abstract

The current study aims to study and diagnose *Trichomonosis* among women in the northern regions of Basra Governorate (Medina District, Qurna District, Imam al-Sadiq District, Bani Mansour, Al-Huwair, Al-Khass, Al-Sharsh, Al-Deir) using the polymerase chain reaction (PCR) method and comparing it to the microscopy method. Classic and explain the relationship between infection and diseases of the female reproductive system and the demographic nature of patients. In this study, 100 samples were collected during the period that extended from August 2022 to February 2023, each of vaginal secretions, urine, and blood serum, from exclusively married women, whose ages ranged between 16-50 years, visiting outpatient clinics in the northern areas of Basra Governorate, and who suffer from gynecological and sewage infections. Urinary tract or more severe symptoms such as uterine enlargement or cervical ulcers. The results of microscopic examination of urine and vaginal secretion samples showed that the infection rate with the *T. vaginalis* parasite was 4%, while the infection rate according to molecular examination of vaginal secretion samples was only 18%. The results of the distribution of the infection rate according to age groups showed that the highest rate of infection with the parasite was recorded at 41.7 in female patients whose ages ranged from 31-40 years, and the lowest infection rate was recorded at 8.2% in female patients whose ages ranged from 16-30 years, while the infection rate reached 20%. In the age group 41-50 years. The results also showed that the highest infection rate was in patients who suffered from combined clinical symptoms (vaginal discharge, itching, and dysuria) (44.44%), and the lowest rate (11.11%) was in patients who suffered from a clinical symptom related to vaginal discharge only. The results of testing the effect of education level on infection with the *T. vaginalis* parasite indicated that the highest infection rate was observed in uneducated patients, reaching 66.7%, compared to educated patients (33.3%). Infection with the *T. vaginalis* parasite and the color of vaginal secretions have a significant correlation, with patients whose secretions tend to be white, followed by, at a lower level, vaginal secretions that tend to be yellow, and finally the lowest percentage of secretions that tend to be green. The percentages were as follows: 44.44%, 33.33%. 22.22%. The study noted a significant correlation between infection with the *T. vaginalis* parasite and the odor of vaginal secretions, with a percentage of 77.78% of patients suffering from an unpleasant odor in vaginal secretions. And 22.22% for affected women who do not suffer from the presence of odor. Also, the highest percentage of infection with the *T. vaginalis* parasite was in infected patients who used contraceptives (66.67%) compared to patients (33.33%) who did not use contraceptives. The highest infection rate was (66.67%) in patients who used birth control pills compared to patients who used IUDs as contraceptives (33.3%).

Keywords: *Trichomonas vaginalis*, Basrah, PCR

Introduction

Trichomonas vaginalis is a protozoan parasite that causes *Trichomoniasis*, a sexually transmitted disease (STD). The annual Prevalence (%) is estimated at 276.4 million globally [7]. The parasite that causes the disease lives in the human reproductive system, in the vagina and urethra of women and in the prostate and seminal vesicles of men [10]. It is transmitted primarily through sexual intercourse. It is associated with various health complications such as pelvic inflammatory disease (PID), pregnancy complications, cervical cancer, prostatitis, infertility, and HIV acquisition. It also causes complications during pregnancy and may affect newborns as well. In addition to the fact that it leads to uterine and prostate cancer[12]. Yellow-green foamy vaginal discharge with a strong unpleasant odor, soreness, itching and irritation of the genital area are the main symptoms in women. *T. vaginalis* attaches to vaginal epithelial cells, destroys cells and tissues, and further multiplies the risk of HIV infection [3] . Routine clinical diagnosis is usually based on microscopic observation of mobile parasites in wet preparation. Soon. There is no study that focuses solely on *T. vaginalis* infection in women in the northern regions of Basra Governorate, so the study aimed to determine the prevalence of the *T. vaginalis* parasite in the northern regions of Basra Governorate using phenotypic and molecular tests, and comparing the incidence of infection with the demographic nature of the samples.

Materials and Methods

100 samples were collected for both vaginal swabs and urines from women visiting private clinics in the northern areas of Basrah Governorate who suffer from gynecological infections and urinary tract infections or more severe symptoms such as uterine hyperplasia or cervical ulcer. Their ages ranged from 16-50 years, and the samples were distributed into three age groups (30≤, 31-40, 41-50). Ten-milliliter samples were collected by a sterile test tube. The vaginal swabs were collected by cotton swabs and two drops of saline solution were placed with them, and they were divided into two parts, one part dedicated to direct microscopic examination and the other part to save for molecular diagnosis, demographic data were recorded age, location, occupation, marital status, chronic diseases, reason for visiting the doctor and the clinical symptoms diagnosed by the doctor in the sample collection form.

Microscopic diagnostics

The vaginal swabs were examined directly using optical electron microscopy after being placed on a glass slide with a drop of saline solution and covered with a glass cap and examined under a regular optical microscope at 40x and 100x lenses. Expulsion of the adrenaline at 2000x speed for three minutes by centrifuge. A drop of precipitate was placed on the glass slide, covered with a glass cap and examined under a standard optical microscope under the 40x lens and then 100x.

Molecular Diagnosis

DNA Extraction

The parasite's DNA was extracted from swabs preserved in normal saline and from some samples that gave a monofilament result during microscopic examination using the Genomic

DNA extraction kit from Junaid Company, according to the company's instructions. Nanodrop device was used to measure the concentration of deoxyrib nucleic acid at a wavelength of 260 nm and measurement of purity at a wavelength of 260/280.

Nested Polymerase Chain Reaction (nPCR)

The parasite's DNA was amplified in two steps using two different primers.

The first step was done by amplifying a conserved region of the gene using an outer primer consisting of TvOp-FWD , forward primer consisting of 27 b p 5-GTGAAAATCTCATGGGGTATTA ACTT-3 and the reverse primer TvOp-REV consisting of 29 b p 5-GTTTTATTTTTTCACTGGAAAATAACGCTT-3. It was mixed by 2\µl each with the rest of the reaction components GoTaq Green Master Mix (Promega) by 25/µl, Nuclease-free water by 11/µl, DNA by 10\µl. Distribute the mixture in tubes, PCR, 50\µl for each tube, the mixture was added by 45 µl and the extracted DNA by 5 \ µl and mixed by Micropipette well and then to the nPCR device according to the protocol for the first reaction. Pre-denaturation denaturation at 94C for two minutes, one cycle. It is followed by Denaturation at 94 degrees C for 45 seconds, then Annealing at c62 for one minute, then Extension at c72 for a minute, 30 cycles. And the last step is the final extension at a temperature of c72 for 10 minutes, in one cycle.

The second step was to amplify the inner segment of the target gene using a TvIp initiator primer consisting of the TvIp-FWD forward primer consisting of 17 bp 5-AACATCCCCAACATCTT-3. and the TvIp-REV reverse primer consisting of 18 bp 5CCATTCTTTTAGACCTT-3. The conditions for the second nPCR reaction mixture mentioned previously were applied except for 5 µl that was taken from the first nPCR. The nPCR products were electrophoresed using 1% (w/v) agarose gel and visualized using electrophoresis system.

Results

Diagnosis of *T. vaginalis* parasite

During the current study period, which extended from August 2022 to February 2023, 100-140 samples were collected. 100 samples each of vaginal secretions and urine from married women visiting outpatient clinics in the northern areas of Basra Governorate who suffer from gynecological infections, urinary tract infections, or more severe symptoms such as uterine enlargement or cervical ulcers.

Microscopic diagnosis

Microscopic diagnosis was based on observing the vegetative stage of the *T. vaginalis* parasite in samples of vaginal secretions or urine under a light microscope at 40 magnification (Figure 1).



Figure (1) shows *T. vaginalis* in vaginal secretions at a 40 magnification.

Molecular diagnosis

Nested polymerase chain reaction (nPCR) assay was used to detect deoxyribonucleic acid of the *T. vaginalis* parasite in vaginal secretion samples using specialized primers to amplify the 18S rRNA gene. The amplified band, with a size of 290 base pairs, indicates the presence of the *T. vaginalis* parasite in vaginal secretion samples (Figure 2).

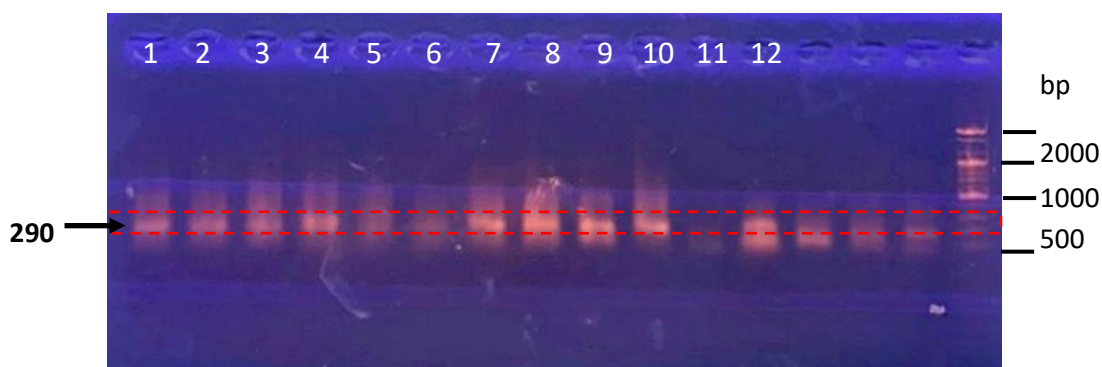


Figure (2) shows the detection of DNA from the *T. vaginalis* parasite in vaginal secretion samples. Column 1-12 represent the samples in which the parasite's DNA is present.

Table (1) Percentage of infection with the *T. vaginalis* parasite, distributed according to diagnostic methods.

Diagnosis	Type of sample	Samples examined	Infected samples	Prevalence (%)
Microscopic	Urine	100	1	4
	Vaginal secretions	100	3	
Molecular	Vaginal secretions	100	18	18
Statistical analysis		Chi-square value: 10: 01		p = 0.0016

Demographic factors

The percentage of infection with the *T. vaginalis* parasite, distributed according to age groups. The results of the distribution of the infection rate according to age groups showed that the highest rate of infection with the parasite was recorded in visits whose ages ranged from 31-40 years, and the lowest infection rate was recorded at 8.2% in visits whose ages ranged from 16-30 years (Table 2). While the infection rate reached 20% in the age group 41-50 years. In addition, the results of the statistical analysis showed that there were significant differences between age and the rate of infection with the *T. vaginalis* parasite.

Table (2) Percentage of infection with the *T. vaginalis* parasite, distributed according to age groups.

Age group	Samples examined	Infected samples	Prevalence (%)
16 – 30	61	5	8.2
31 – 40	24	10	41.7
41 – 50	15	3	20
Total	100	18	18
Statistical analysis	Chi-square value: 31.75		p = 0.0001

Infection with the *T. vaginalis* parasite, distributed according to clinical symptoms

The results of the clinical study showed that the highest rate of parasite infection (44.44%) was observed in patients suffering from combined clinical symptoms (vaginal discharge, itching, and dysuria), and the lowest rate of infection (11.11%) was observed in patients suffering from a clinical symptom related to vaginal discharge only. In addition, the results of the statistical analysis showed that the infection rate increases gradually with an increase in the number of clinical factors combined (Figure 3).

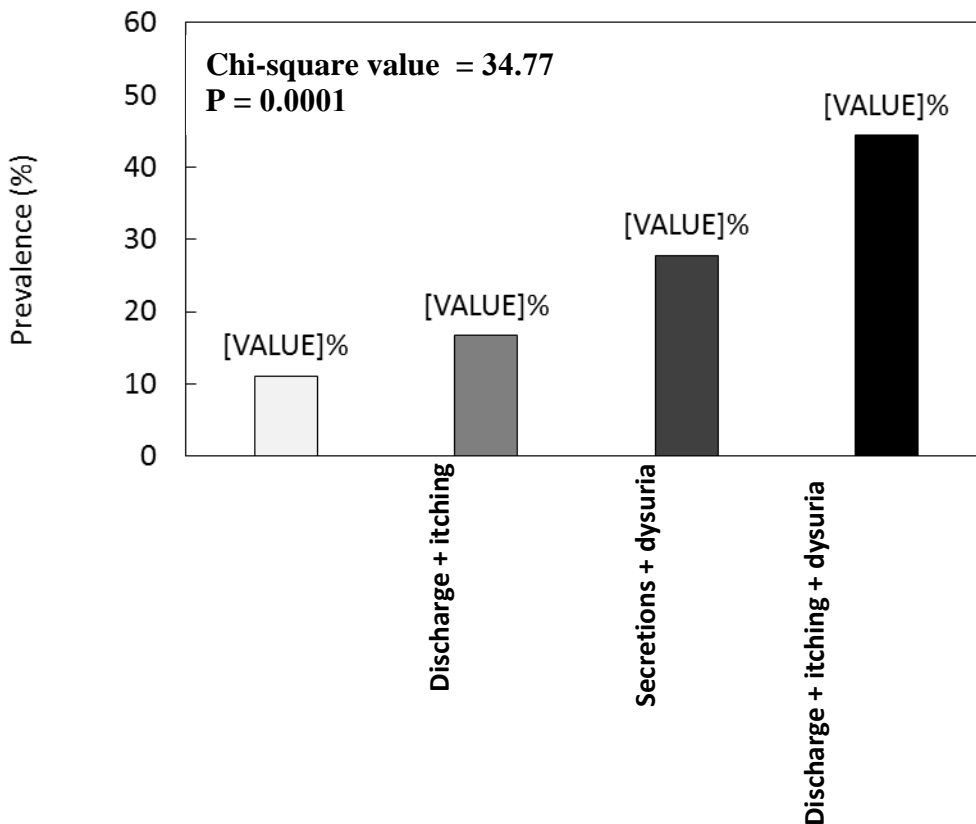


Figure (3) shows the relationship between the nature of secret symptoms and infection with the *T. vaginalis* parasite.

Infection with the *T. vaginalis* parasite, distributed according to the educational level

The results of the test on the effect of education on infection with the *T. vaginalis* parasite showed that the highest infection rate was observed in uneducated female visitors, reaching 66.7% compared to educated female visitors (33.3%). The results of the statistical analysis indicated that there was a significant difference between the rate of infection with the *T. vaginalis* parasite and the educational level of outpatient clinic visits in the areas north of Basra Governorate (Figure 4).

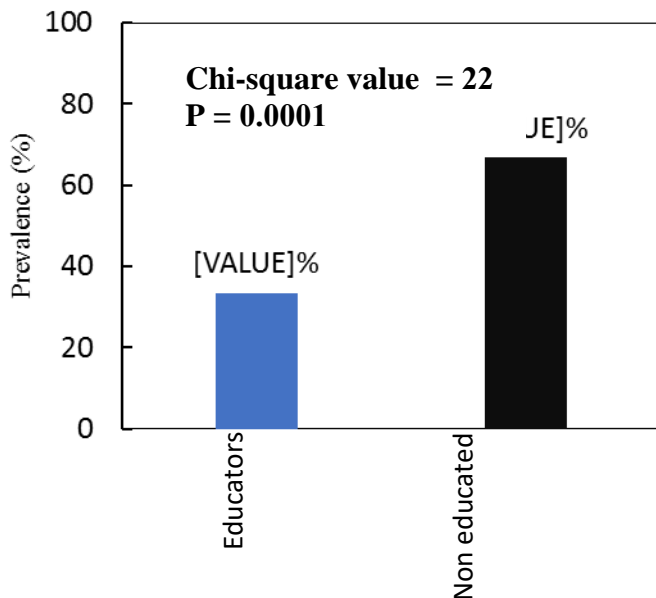


Figure (4) shows the relationship of learning to infection with the *T. vaginalis* parasite.

Infection with the parasite *T. vaginalis* is distributed according to the color of vaginal secretions

The results of a study on the relationship between infection with the *T. vaginalis* parasite and the color of vaginal secretions showed that there is a significant correlation between them, as the highest rate of infection for female reviews is in vaginal secretions that tend to be white in color, at 44.44%, followed by, at a lower level, in vaginal secretions that tend to be yellow in color, at 33.33% (Figure 5). While the lowest infection rate was observed in secretions that tend to be green in color, reaching 22.22%.

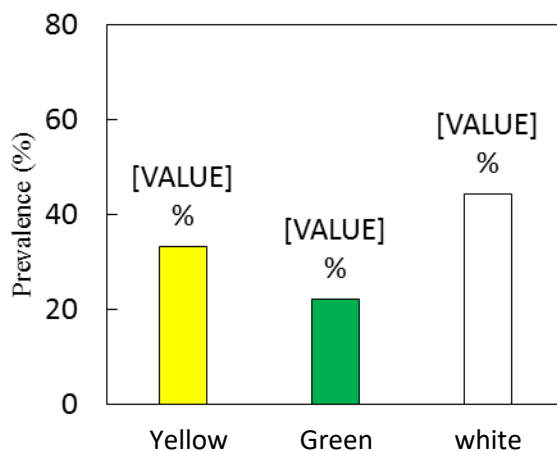


Figure (5) The relationship between the color of vaginal secretions and infection with the *T. vaginalis* parasite.

Infection with the parasite *T. vaginalis* is distributed according to the smell of vaginal secretions

It was noted in the current study that there is a significant correlation between infection with the *T. vaginalis* parasite and the odor of vaginal secretions, as the percentage of infected women who suffer from an unpleasant odor in vaginal secretions reached 77.78% (Figure 6). While the percentage of infected women who do not suffer from an unpleasant odor in vaginal secretions was 22.22%.

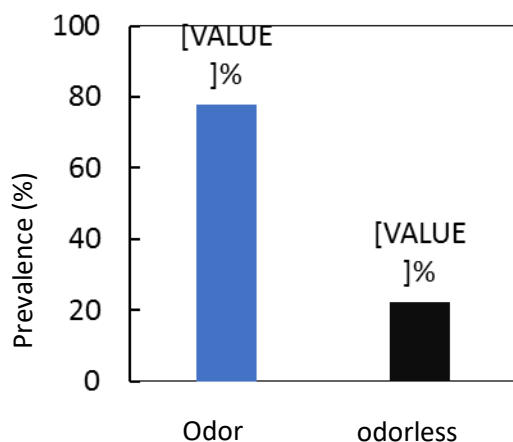


Figure (6) The relationship between infection with the *T. vaginalis* parasite and the odor of vaginal secretions.

Infection with *T. vaginalis* parasite, distributed according to contraceptives

The results of the current study showed that the highest rate of infection with the *Trichomonas vaginalis* parasite is in infected women who use contraceptives (66.67%) and the lowest in infected women who do not use contraceptives (33.33%). The results of the statistical analysis also confirmed that there is a significant difference between infection with the parasite and use of contraceptives (Figure 7). In addition, the highest infection rate (66.67%) was among infected women who used birth control pills compared to infected women who used the IUD as a contraceptive (33.3%) (Figure 8).

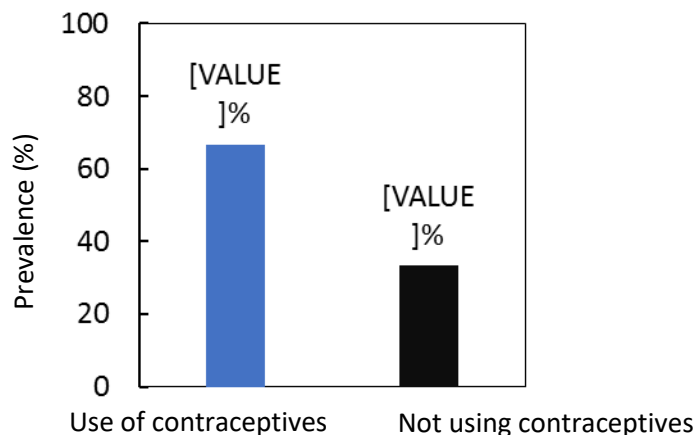


Figure (7) shows infection with the *T. vaginalis* parasite and the use or non-use of contraceptives.

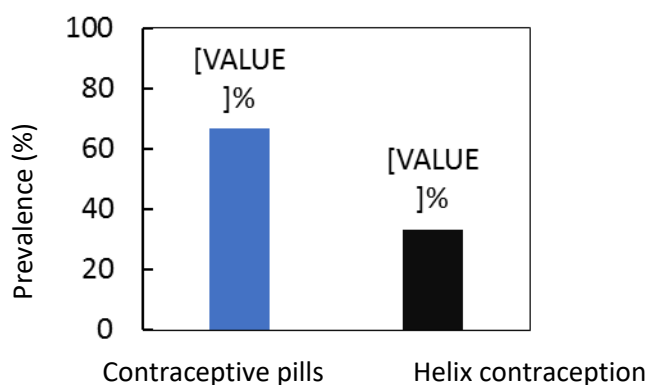


Figure (8) shows the relationship between infection with the *T. vaginalis* parasite and the type of contraceptives.

Discussion

Data on the prevalence of sexually transmitted infections (STI) of *T. vaginalis* in Iraq are very limited because most studies use conventional diagnostic methods [3]. The current study describes the prevalence of *T. vaginalis* among women with alarming symptoms in the northern areas of Basra Governorate in southern Iraq by means of two diagnostic methods, polymerase chain reaction technique and direct microscopy method.

The study estimated a low prevalence of the parasite *T. vaginalis* by conventional microscopic detection (4%). This percentage is small when compared with the results of microscopic diagnosis of both vaginal diuresis and secretions in other research. Also when compared with

molecular diagnosis in this study (18%) and in other studies using polymerase chain reaction technique, that showed that urine samples have a 90% and 100% sensitivity to secretions to detect the parasite that confirms the infection of the urinary tract by 90% and 100% genital tract by the parasite during infection. The reason for this is that the polymerase chain reaction (PCR) technique is able to detect *T. vaginalis* in a single cell in a urine sample or secretions, so it is recommended to use the PCR assay to detect microorganisms in the urine [9]. In another local study, the total percentage of microscopically diagnosed infections in secretions was more than twice the percentage obtained during the current study. This is a prevalence rate at the level of Baqubah city compared to the low rate achieved by the current study conducted on areas of northern Basra Governorate. The higher prevalence of infection compared to the current study may be due to the adoption of women in the study who had exclusively vaginitis [2], while the current study evaluated *Trichomoniosis* in women who attended regular health check-ups. The results of molecular diagnosis showed a higher and more accurate prevalence rate. Which confirms the specialization and sensitivity of molecular methods in the processes of detecting the parasite. This is a high percentage compared to a study of the Shiraz University of Medical Sciences in southern Iran among 534 women whose DNA of the *Trichomonas vaginalis* parasite was detected in (4.86%) [3]. Routine screening for *Trichomoniosis* using nPCR is very reliable and specific, as nPCR showed the highest rank in diagnostic performance [11],[6],[5],[4]. PCR is also shown as a highly sensitive and specific method (100% and 90.9%) for the detection of samples that fail to detect *T. vaginalis* in direct microscopy of urine and vaginal secretions. The reason for this is that PCR has the advantage of needing only DNA, from either viable or non-viable organisms, as only one sample has a negative result when examined directly by microscope but positive with PCR [9]. Recent advances in testing for primary sexually transmitted parasites have increased the chances of diagnosing and treating this important sexually transmitted infection.

This review summarizes the tests currently available, and highlights their characteristics, performance, advantages and limitations to be adhered to. Including DNA amplification tests, which will certainly significantly improve the quality of diagnosis of *Trichomoniosis*, especially in women. In light of the expanded list of testing options now available [13], *Trichomoniosis* is better identified and controlled, which will ultimately make it possible to reduce the adverse reproductive consequences associated with *T. vaginalis* infection [8].

The current study also showed a positive association of *Trichomonas vaginalis* infection with demographic nature, as the incidence was highest in the age group (13 - 40 years), followed by the age group (16 - 30 years), which may be due to an increase in sexual activity, or the high infection rate may be due to Younger age leads to an increase in the number of younger age group patients attending the clinic. Finally, the age group (41-50). In some studies, age groups from 41 to 50 years have been found to have a significantly high prevalence of *T. vaginalis* infection that is not limited to specific age groups [3]. The reason for the decrease or loss of the incidence of infection in menopause may be attributed to sexual inactivity, which is represented

by a decrease in the percentage of glycogen, estrogen, and the natural pH of the vagina, and these factors do not encourage the growth and reproduction of the parasite [13], as well as the difference in the number of samples taken in each age group, which affects the The rates of parasite presence vary (15) and may be due to the accumulation of untreated infections with age. A local study conducted in Basra Governorate on men showed lower rates than the current study for both microscopic and molecular diagnosis[9]. The study also showed that the highest percentage of patients affected by reviews were uneducated. And also from the bad economic reality. The reason may be due to the lack of regular examination and irregular treatment, which explains the urgent need to reduce class, health and social disparities. The higher prevalence of infection in other studies compared to our results could also be attributed to the target population, or to women with vaginitis or with high-risk behaviours, whereas Trichomoniosis in the current study was assessed in women who attended a regular health check-up. How samples are collected and diagnostic techniques are also effective factors that could be other reasons for this difference [4]. The reason may also be an obsession with personal hygiene and the use of lotions that change the normal flora. A population study was also found in the United States of America, which used a polymerase chain reaction test and achieved rates of 2.8% among adolescents, and this percentage is lower than the results of the current study [11.]

The results of different studies varied depending on the type of study population. Some studies indicate that asymptomatic cases represent 25% to 50% of cases. According to studies, 10% to 50% of females are asymptomatic.[14] Common symptoms include vaginal discharge, vulvar itching, dysuria, or unpleasant odor. In comparison with these results, our study revealed a higher proportion of asymptomatic cases, as 44% of female patients were infected with Trichomoniosis with white vaginal discharge, itching, and dysuria. The reason for the white color of the secretions may be due to ulcers and ruptures occurring in the epithelial cells of the genital areas as a result of the parasite's attachment and feeding on carbohydrates, fats, and red blood cells [15].

Other symptoms in females, such as the amount of discharge, color, vaginal itching, dysuria, dyspareunia, and lower abdominal pain, were compared with those of Trichomonas vaginalis patients, and there was a significant difference between these same symptoms. We have found that the smell of the secretion is described as "unpleasant" in the majority of patients with Trichomoniosis, as a distinct fishy odor was observed in more than 77% of patients with the infection. The reason may be due to a change in the pH of the vaginal flora or as a result of a change in the levels of lactobacilli due to the infection, which causes the organisms to grow. A pathogenic microflora that causes damage to the vaginal flora and thus produces unpleasant odors

Patients who use different contraceptive methods are the most in the study population. The current study and local studies have also proven the effect of artificial contraceptives used by

women on the incidence of Trichomoniosis, which is considered a risk factor for infection as one of the variables that causes a hormonal imbalance or disorder in Normal vaginal flora [1].

Note that the clinical signs and symptoms of *Trichomonas vaginalis* infection in women and men are not sensitive or specific enough to be used in diagnosis. Therefore, positive identification of the organism is required. With these symptoms. Hygiene education, sterilization and good use of toilets are very important factors in preventing *Toxoplasma vaginalis* infection. The emergence of infection in the current study was exclusively among married women, and the reason responsible for this is that infection occurs directly through sexual contact with infected women, and there is no illicit relationship. The prevalence of *T. vaginalis* infection reported locally has varied depending on reviews of women and the diagnostic techniques used. Classic [10].

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