



RESEARCH ARTICLE

Possible Association Between *Trichomonas Vaginalis* and Recurrent Urinary Tract Infections

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ABSTRACT

Recurrent urinary tract infections (rUTI) represent a primary health concern, significantly impacting the quality of life of affected individuals. *Trichomonas vaginalis* (TV) may be a predisposing factor for rUTI, as it can irritate the urethra and vagina, increasing the risk of bacteria entering the urinary tract and causing infection. This study aimed to investigate the correlation between TV concurrent infection and the occurrence of rUTI. A total of 100 mid-stream urine samples were collected from individuals with UTI; half had urinary tract infections (UTIs) for the first time, and the other half had a rUTI. After taking the sample and performing macroscopic and microscopic examinations, the sample is cultured on selective media and then identified by the VITEK 2 system. TV in urine samples was diagnosed by using nested PCR. Results: This study included 100 patients with UTIs aged between 18-50 years. The average age of the patients was 33.97 ± 7.72 . The percentage of females was 77% compared to 23% of males. Patients were from urban areas, 58% compared to 42% from rural areas. The current results showed that 17% of patients were positive for the diagnosis of TV, and 76.5% of rUTI were positive for TV. There is a genuine association between TV and rUTI. Individuals with UTIs are prone to have recurring episodes if they have a history of TV. TV is regarded as contributing factor for the recurrence of UTI.

INTRODUCTION

UTIs are inflammation of the urinary tract epithelium resulting from invasion by microorganisms [1]. It is an important and common disease affecting men and women of all ages [2]. UTIs are not the only problem, as recurrence of UTIs has become a major concern, as it was found that more than a quarter of women with a UTIs suffer from a recurrence of urinary tract infections within 6 months [3]. Recurrent UTI (rUTI) is the recurrence of a UTI at least twice within six months or thrice within one year [4]. It represents a major health problem that affects the quality of life of those infected and has a large economic burden in addition to the burden on health care resources [5]. Therefore, there is a need for real preventive plans and studies to reduce the recurrence of urinary tract infections. Bacteria are considered the primary cause of UTIs, especially Gram-negative bacteria, which are *Escherichia Coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae*, *Proteus mirabilis*, in addition to gram-positive bacteria that are no less important, such as *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*. [6]. In addition, there are less common secondary causes such as *Citrobacter* sp., *Enterobacter* sp., *Serratia* sp, *Candida* species and

Trichomonas vaginalis (TV) [7]. TV is the cause of the most prevalent nonviral STD in the world [8]. Vaginalitis is known to be caused by TV, in women, the infection often occurs in the cervix, vagina, and vulva, and the uterus is not affected [9]. In men, the infection occurs in the urethra, prostate, and seminal vesicles [10]. *Trichomonas* may cause serious side effects such as early placental membrane rupture, preterm delivery, infertility, pelvic inflammatory illness, and increased susceptibility to HIV transmission [11]. Roughly 50% of those infected with TV exhibit no symptoms, common indicators of infection in women experiencing symptoms include vaginal discharge, swelling or redness, inflammation of the cervix, irritation and inflammation of the vulvovaginal area, painful urination, itching, discomfort during sexual intercourse, and pain in the lower abdomen [12]. There are common factors between the TV parasite and rUTI in terms of symptoms, risk factors, gender, and age groups [13]. This calls for studies to be conducted to understand the possible relationship between them. This research aims to investigate the possible relationship between the TV parasite and the recurrence of UTI, in addition to studying the prevalence and epidemiology of TV in individuals with UTI.

MATERIALS AND METHODS

The clinical specimens for this study were collected from clean catch midstream urine samples in a sterile container. Of individuals suffering from recurrent urinary tract infection (rUTI) at Al-Qasim General Hospital and the Public Health Laboratory in Babylon during the period from October 1, 2023 to March 1, 2024. A total of 100 samples were collected from individuals who had a urinary tract infection (UTI), half of whom had a UTI for the first time and the other half had a rUTI, aged between 18 to 50 years. Before sample collection, all study participants received thorough instructions on collecting urine samples aseptically to prevent urethral contamination. Furthermore, the individuals provided informed consent. The research excluded patients with diabetes mellitus, renal abnormalities, infectious illnesses, those under 18 and above 50, and patients who did not provide informed permission. After taking the sample and performing macroscopic and microscopic examinations, the sample is cultured on blood agar, MacConkey agar, and mannitol salt agar, then identified by the VITEK 2 system. A TV in urine samples was diagnosed using nested PCR technology to ensure an accurate and more sensitive diagnosis.

Nested PCR primers

The Nested PCR primers for the detection of *Trichomonas vaginalis* based on conserved region in the 18S ribosomal RNA gene were designed in this study using NCBI-Genbank database (GenBank: KM282382.1) and Primer3 plus. These primers was provided from (ScientificResercher.Co.Ltd, Iraq). As following in Table 1:

Table 1: Nested PCR primers

Primers	Sequence 5'-3'		Product size
Trichomonas vaginalis 18SrRNA gene PCR primer	F	AATCTATTTGGCGGCGACCA	696bp
	R	CTCGCCCTTGATCGACAGAA	
Trichomonas vaginalis 18SrRNA Nested PCR primer	F	GCCATTCGACTGAGTGACCT	539bp
	R	CCTTCGCCTCAGCTTCTCTC	

Statistical analysis: The statistical software SPSS version 26 and Microsoft Office Excel 2010 were used to analyze the current study's data. An independent sample t-test was used to examine the variance in means between the two groups, assuming the variable follows a normal distribution. The chi-square test was used to investigate the relationship between two categorical variables. Risk was

measured by estimating the odds ratio and calculating the 95% confidence interval. The threshold for statistical significance was set at a P value of less than 0.05, while the upper level of significance was set at 0.01 or less.

RESULTS

Frequency distribution of patients with UTI according to age

This study targeted a group of young people aged 18-50 (sexually active period) to see if TV, a sexually transmitted parasite, it associated with or a predisposing factor to increased recurrence of urinary tract infections. The mean age of patients with UTI in the current study was 33.97 ± 7.72 years and a wide range of affected ages from 18 to 50 years. On the other hand, the current study noted that the majority of patients were between 30-39 years old. As following in Table 2:

Table 2: Frequency distribution of patients with UTI according to age

n: number of cases; *SD*: standard deviation

Age (years)	NO (%)
< 30 years, <i>n</i> (%)	30 (30.0 %)
30-39 years, <i>n</i> (%)	41 (41.0 %)
> 40 years, <i>n</i> (%)	29 (29.0 %)
Mean \pm SD	33.97 ± 7.72
Range	18 -50

Frequency distribution of patients with UTI according to residency

The frequency distribution of patients with UTI according to residency is shown in Figure 1.

In the present study, most UTIs were form urban areas compared to rural areas, 58% versus 42 %.

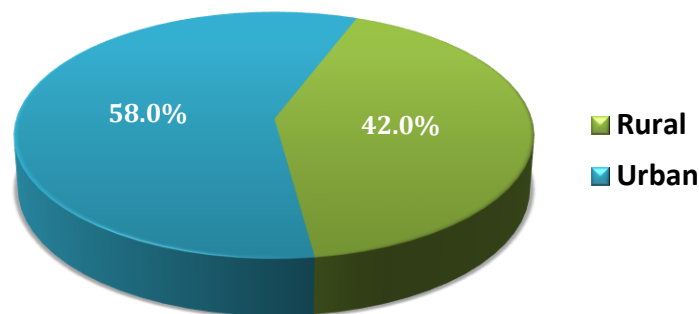


Figure 1: Frequency distribution of patients with UTI according to residency

Comparison of mean age and gender of patients with UTI according to residency

The overall average age of patients with UTIs was 33.97%; in rural areas, the average age was 33.50%, while in urban areas, it was 32.86%. The percentage of females with UTIs in urban areas was 84.5%, while the percentage of males was 15.5%. In rural areas, females constituted 66.7%, while males constituted 33.3%. As shown in Table 3.

Table 3: Comparison of mean age and gender of patients with UTI according to residency

Characteristic	Total <i>n</i> = 100	Urban <i>n</i> = 58	Rural <i>n</i> = 42	<i>P</i>
Age (years)				
Mean \pm SD	33.97 \pm 7.72	32,86 \pm 7.76	35.50 \pm 7.50	0.092
Range	18 -50	18-50	18-46	I NS
Gender				
Male, <i>n</i> (%)	23 (23.0 %)	9 (15.5 %)	14 (33.3 %)	0.037
Female, <i>n</i> (%)	77 (77.0 %)	49 (84.5 %)	28 (66.7 %)	C S

n: number of cases; **SD**: standard deviation **C**: chi-square test; **NS**: not significant at $p > 0.05$; **S**: significant at $p < 0.05$; **I**: independent samples *t*-test.

Diagnosis of TV in patients with UTI enrolled in this study using specific primers

In order to overcome the issue of using microscopic diagnosis and use the accuracy of molecular methods that rely on amplifying nucleic acids, the polymerase chain reaction has emerged as the most reliable method for diagnosing and detecting microorganisms. The present results show that 17 (17.0%) of patients were positive of nested PCR in the diagnosis of TV parasite out of 100 patients with UTI, as shown in Figure (2, 3). Motile TV was found in 2 of them during a microscopic examination.

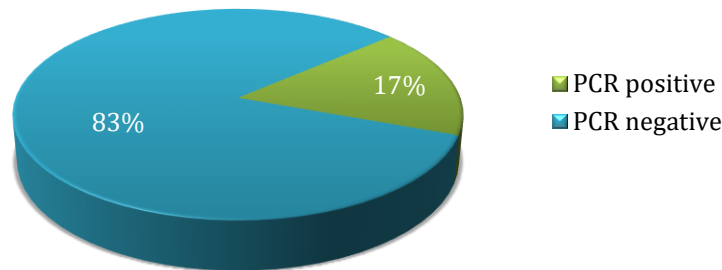
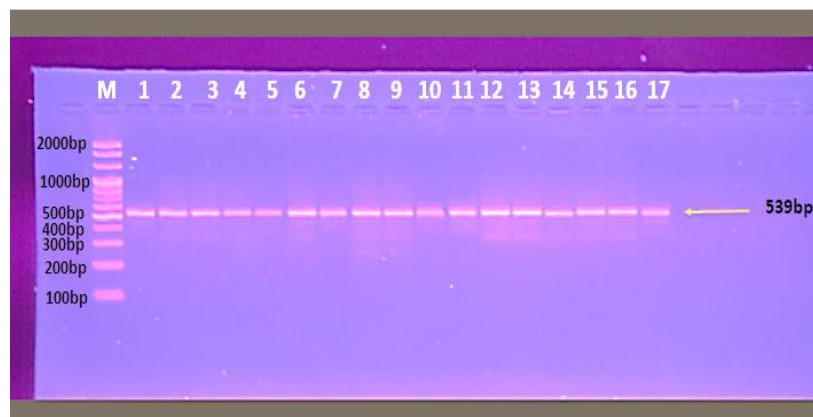
**Figure 2: Pie chart showing the positive detection rate of nested PCR in the diagnosis of TV parasite**

Figure 3 displays an agarose gel electrophoresis image illustrating the examination of the Nested PCR product of the 18S ribosomal RNA gene in *Trichomonas vaginalis*. The DNA used for this

investigation was isolated from urine samples. The marker M (2000-100bp) and lanes 1-17 exhibited only positive Nested PCR with a PCR product 539bp.

Frequency of mean age, gender, and residency distribution according to results of nested PCR examination for TV parasite

The average age of the patients whose results appeared positive for the TV test was 37.70%, while the average age of those whose results appeared negative for the TV test was 32.20%. The percentage of males whose results appeared positive for the TV test was 17.6%, while the percentage of females was 82.4%.

As for those who showed negative results for the TV test, they were 75.8% for females and 24.1% for males. In urban areas, the percentage of people infected with TV was 41.2%, while in rural areas, it was 58.1. As shown in Table 4.

Table 4: Comparison of mean age, gender and residency distribution according to results of nested PCR examination for TV parasite

Characteristic	Total <i>n</i> = 100	Positive <i>n</i> = 17	Negative <i>n</i> = 83	<i>p</i>
Age (years)				
Mean ±SD	33.97 ±7.72	37.70 ±5.70	32.20 ±7.89	0.028
Range	18 -50	25-46	18-50	I S
Gender				
Male, <i>n</i> (%)	23 (23.0 %)	3 (17.6 %)	20 (24.1 %)	0.656 C NS
Female, <i>n</i> (%)	77 (77.0 %)	14 (82.4 %)	63 (75.9 %)	
Residency				
Urban, <i>n</i> (%)	58 (58.0 %)	7 (41.2 %)	51 (61.4 %)	0.123 C NS
Rural, <i>n</i> (%)	42 (42.0 %)	10 (58.8 %)	32 (38.6 %)	

n: number of cases, **SD**: standard deviation; **C**: chi-square test; **NS**: not significant at $p > 0.05$; **S**: significant at $p < 0.05$. **I**: independent samples *t*-test.

Frequency of number of recurrent UTI according to results of nested PCR examination for TV parasite

The study included 100 patients with UTI, 50 (50%) of whom had first-time infection and 50 (50%) had rUTI. The results showed that 17% of the total tested positive for the TV test. The rate of patients with rUTI developing TV was 76.5%. At the same time, the rate of patients with a one-time UTI developing TV was 23.5%. As shown in Table 5.

Table 5: Comparison of number of recurrent UTI according to results of nested PCR examination for TV parasite

Characteristic	Total <i>n</i> = 100	Positive <i>n</i> = 17	Negative <i>n</i> = 83	<i>p</i>
Number of recurrent UTI				
One Time, <i>n</i> (%)	50 (50.0 %)	4 (23.5 %)	46 (55.5 %)	0.017 C S
Recurrent, <i>n</i> (%)	50 (50.0 %)	13 (76.5 %)	37 (44.5 %)	

n: number of cases; **SD**: standard deviation; **C**: chi-square test; **S**: significant at $p < 0.05$

DISCUSSION

A total of 100 patients in the current study had a UTI, 50% of whom had a UTI for the first time, and the other 50% had a rUTI. Females accounted for the highest percentage of UTI cases, 77%, compared to males, 23%. Many studies have reached results similar to the current study [14–16]. The reason is due to the anatomical structure of the urethra in females, in addition to the hormonal fluctuations that occur during the menstrual cycle.

In the present study, most UTIs were from urban area compared to rural areas, 58% versus 42 %, respectively, due to the difference in lifestyle in urban areas and the high population density compared to rural areas. However, some studies that showed different results [17,18], stating that the disease was more widespread in rural areas than urban areas. The cause might be decline in living circumstances and limited access to medical care.

The results show that the overall average age of individuals with UTI is 33.97%. In urban areas, the average age of infected people was 32.86 years, while in rural areas it was 33.5 years. Although the average age is slightly higher in rural areas, there is no statistically significant difference between the average age of patients with UTI in rural and urban areas. Females accounted for 84.5% of UTI cases in urban areas while 15.5% were male. In rural areas, the proportion of UTI cases among women was 66.7%, whilst males accounted for 33.3% of the cases.

There are statistically significant differences between the sexes in patients suffering from UTI ($p = 0.037$), and the proportion of males and females differs statistically between rural and urban areas, where women were more susceptible to UTI for the reasons mentioned previously, In addition, behavioral factors and differences in hygiene practices and toileting habits between the sexes can influence the risk of UTI [19].

The current study shows that there is a statistically significant difference ($p = 0.028$) in the average age between patients who were *T. vaginalis* positive (37.7 years) and those who were TV negative (33.2 years), which means that this parasite can infect the elderly more than young people. The results of this study were supported by some previous studies [12,20].

The decreased occurrence of TV in younger age cohorts may be partly attributed to variations in susceptibility and immunological response to infection [11]. Or may be the reason for the discovery of cases of persistent, untreated of TV infection in older age groups, since most TV cases are asymptomatic [12,38].

Although TV is a sexually transmitted disease (STD) and its prevalence between the sexes is supposed to be in similar proportions, according to the data of the current study [with no statistical significance] the infection rate in females (82.4%) was higher than in males (17.6 %). The reason may be that the symptoms in men are mild and spontaneous recovery may occur, and this has been confirmed by some studies [21]. Therefore, the difference in prevalence between men and women may be biological [22]. In addition, it may be the presence of zinc in the prostate fluid in men. This is the reason for the low infection rate because, as it is known, zinc acts as an anti-TV in vitro [23,36].

Although TV is found in all ethnic communities and social classes, its prevalence varies between demographic groups and regions. According to the current study's data, TV was found in rural areas at a rate of 58.8%, while in urban areas it was 41.2%.

Some studies supported the current study regarding prevalence by place of residence [24,25] and reported that the highest frequency was in rural areas. At the same time, many other studies in Iraq recorded a higher incidence in urban areas than in rural areas [26–28].

The reason for this disparity is the difference in educational level between rural and urban communities, as many studies in Iraq [28,29], showed that the infection rate decreases with

advancement in the educational level. It found that the infection rate in illiterate women was higher than that of educated women, the reason may be due to awareness health, especially sexual awareness [30].

The social and economic level also played a role in the spread of TV, as some studies reported that the spread of TV was higher in societies with a low economic levels [25]. The reason may be due to the deterioration of living conditions and the lack of visits to hospitals and health centers, as well as the lack of awareness, poor health conditions, and, most importantly, difficulty accessing treatment. The absence of a statistically significant disparity between urban and rural residence is intriguing and warrants more exploration in more extensive research.

Although TV is considered one of the most common treatable STDs worldwide, it is difficult to assess the incidence of TV due to the high incidence of infections are asymptomatic. Moreover, it is not a reportable disease, so there is not much surveillance data on TV.

A survey conducted by Hansh [31] in Iraq in 2024 reported on the rate of TV spread in the Iraqi governorates, where the governorates of Maysan and Tikrit witnessed the highest infection rates, 75.%, 62% respectively, while the lowest infection rate was in the Basra and Sulaimania governorates 1.6% and 1.66%, respectively. The researcher attributed the reason for this Disparity to the difference in geographical location, duration of study, sample size, method of diagnosis, and the nature of social traditions prevailing in society.

There was a substantial statistical disparity in the incidence of TV infections between those with a first UTI and those who had a recurrent UTI. Therefore, the prevalence rate TV of in people with frequent urinary tract infections is higher than in other people. Four primary hypotheses have been proposed to explain why individuals with TV are more likely to get recurring UTIs. First, These individuals have a deficiency in their local immunity due to TV infection, rendering them more vulnerable to peripheral colonization [32,35]. The second hypothesis is that TV serves as a vector for the spread of urinary tract pathogen due to its ability to absorb *mycoplasma*, *Neisseria gonorrhoea*, and other bacteria and viruses.

Furthermore, TV can induce irritation and inflammation in the tissue, this occurs when the parasite attaches itself to the mucous membrane of the cervix, urethra, and vagina, and releases cysteine proteinase, which acts as a cell separation agent, causing the shedding of epithelial cells and compromising the structural integrity and defense barrier of the genitourinary system, As a result, the host becomes more vulnerable to UTI [33]. The last one, the TV parasite, may disrupt the natural bacterial equilibrium in the vagina, this alteration in bacterial equilibrium might also heighten the susceptibility to UTIs [34]. One study, which was somewhat similar to the current study, it was conducted by Chang et al. 2016 in Taiwan, which reported no difference in the recurrence rate of UTI for people with and without TV infection [13,37].

The difference in the results of these two studies is due to several reasons, including the nature of the study and the type of variables. His study included 65 women with an average age of 57.4 ± 14.3 years, and the follow-up period was four months. Immunochromatographic strips were used to diagnose TV infection, which had a prevalence rate of 16.9%. During the follow-up period, eight women had a recurrence of UTIs. However, there was no significant difference in the risk of recurrence between women who had or did not have TV. The primary cause for this is that, due to ethical considerations, all women who tested positive for TV were provided with therapy. It is possible that the local defense barrier was restored following the treatment for TV, resulting in a comparable recurrence rate in both groups.

Furthermore, the data of the present research varied from previous studies in terms of sample size, kind of variable, and age. As far as we know, this research is the first in Iraq to show that the occurrence of TV among individuals with urinary tract infections is 17%, which is two to five times

more than its occurrence in other populations. The increase in UTI cases indicates that individuals with TV are susceptible to getting rUTIs. However, if TV is treated and the urogenital defense barrier is restored, the risk of rUTIs will be reduced. Hence, in order to enhance and diminish the occurrence of rUTI, testing for TV should be taken into account, and more research is required to explore the possible advantages of treating rUTI in such instances.

CONCLUSION

According to this research, females are more vulnerable to urinary tract infections. Moreover, the metropolitan regions accounted for the bulk of urinary tract infections.

The present investigation found no disparity in the ages of individuals with urinary tract infections between rural and urban regions. Older individuals are more susceptible to the TV parasite compared to younger individuals. Furthermore, there is no significant correlation between the proliferation of television among men and females. There is no discernible disparity in the distribution of TV between rural and urban regions, as shown by statistical analysis. There is a genuine correlation between UTIs and recurrent urinary tract infections, as persons with UTIs are susceptible to recurring episodes of urinary tract infections. Thus, television is regarded as a contributing factor to the recurrence of urinary tract infections.

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CONFLICT OF INTEREST

No conflict of interest is present in the current study.

ETHICS APPROVAL

The required ethical clearance was received from the hospital's ethics committee, along with the permission of the patients. Prior to sample collection, all patients were thoroughly informed and given the option to grant their permission for the purposes of testing and publishing of findings.

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