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Article

Molecular Sequencing and Immunological Analysis of *Trichomonas vaginalis* in Women in Wasit province, Iraq

Baraa Abdulsalam Hraija*

Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq
*Correspondence: babdulsalam@uowasit.edu.iq

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Abstract: *Trichomonas vaginalis* is the most common parasitic sexually transmitted infection, which can lead to more severe reproductive health complications. Despite, *T. vaginalis* infection remains fairly diagnosed due to asymptomatic presentation or non-specific symptoms, necessitating robust diagnostic methodologies for accurate identification and treatment. Identifying the prevalence rate of human trichomoniasis in women of Wasit province (Iraq) using the rapid test and molecular PCR with sequencing and phylogenetic analysis of *T. vaginalis* isolates. Association of immunity to *T. vaginalis* infection through measurement the levels of cysteinyl leukotrienes (CysLTs), interleukin 8 (IL-8), and leukotriene B4 (LTB4) was aimed. Totally, 291 women having various socio-demographic factors and reproductive disorders were admitted to the private gynecological clinics in Wasit province (Iraq) during March-June (2025) and selected to the present study. All study women were subjected to sampling of vaginal swabs that processed and tested directly by the rapid test and then by molecular assay. Additionally, venous blood was drained, centrifuged, and the sera were utilized for measurement of immune markers. The findings of rapid test in vaginal swabs recorded that 3.78% of study women were having a positive reactivity to *T. vaginalis* antigen. Molecularly, 5.84% of study women were positively infected by *T. vaginalis* using the conventional PCR. Phylogenetic analysis of study *T. vaginalis* isolates identified a marked identity with the NCBI-BLAST *T. vaginalis* Indian *T. vaginalis* isolate (ID: JF513197.1). In comparison with the negatively individuals, the positively *T. vaginalis* infected women were reported a significant elevation in values of CysLTs (275.09 ± 17.09 pg/ml), IL-8 (130.83 ± 6.04 pg/ml), and LTB4 (1512.059 ± 90.73 pg/ml). This study indicates that rapid test and/or molecular PCR can provide important data about the prevalence of T. vaginals in Iraqi population of various socio-demographic aspects who appeared with or without clinical symptoms. Phylogenetically, this study showed that the local *T. vaginalis* isolates were markedly identical to the Indian *T. vaginalis* isolates; however, moreover sequencing and genetic information are greatly necessary as such data can be utilized in epidemiology of parasitic infection. Serological elevation of immune markers in positively infected women might further complicates the pathological landscape and enhance the inflammatory cascade with promoting the proliferation of pathogenic bacteria.

Keywords: Immunochromatography Assay, Phylogeny, Rapid Test, Sexual Transmitted Infection, Trichomoniasis

Introduction

Trichomonas vaginalis is anaerobic, flagellated protozoan parasite and the causative agent of a sexually transmitted disease called trichomoniasis that infects human in industrialized countries (Mirzadeh et al., 2021). The parasite, belongs to the Trichomonadida order in the Metamonada phylum of Eukaryota domain, was first described in 1836 by Alfred Francois Donné who observed it as a motile protozoa in vaginal or cervical secretions (Gharban, 2023; Eanzi and Al-Kaabi, 2024). In 1916, Hohne declared trichomoniasis as a clinical entity with a wide range of genitourinary symptoms in affected women which could lead to more severe reproductive health complications including adverse pregnancy outcomes, and increased susceptibility to other sexually transmitted diseases including human immunodeficiency virus (HIV) disease (Van Gerwen et al., 2023; Joy et al., 2025). Although, the intricate life cycle of parasite involves several distinct stages that are crucial for its survival, proliferation, and transmission, it exists solely as a trophozoite, meaning it lacks a cyst stage and replicates by binary fission with directly contributing to its high infectivity and rapid onset of symptoms (Matti, 2022; Murkute et al., 2025).

The global prevalence of *T. vaginalis* in women is approximately 5.3%, yet it can be as high as 14.6% in specific cohorts, underscoring its significant epidemiological burden (Li et al., 2025). Also, the adverse clinical manifestations and the potential for asymptomatic carriage highlight the necessity of understanding the varied diagnostic approaches including microscopy, culture and nucleic acid amplification tests to effectively manage this widespread infection (Azeez et al., 2024; Shankar et al., 2025). While, wet mount microscopy remains a commonly utilized point-of-care test, its sensitivity for *T. vaginalis* detection is notably low often ranging from 31% to 60%, limiting its utility in identifying asymptomatic or low-parasite-burden infections (Cardoso et al., 2024). Conversely, molecular assays such as nucleic acid amplification tests, offer superior sensitivity and specificity for *T. vaginalis* detection, enabling more precise diagnosis and guiding targeted therapeutic interventions (Gharban, 2023; Borges et al., 2024).

In Iraq, the earliest reports of *T. vaginalis* were from Al-Shabandar (1979) in Baghdad, Al-Mallan and Al-Janabi (1983) in Mosul, Kadir et al. (1988) in Erbil, Al-Saeed (1995) in Dohok, and Mahdi (1995) in Basra. Then, several studies have been conducted in Iraq (AL-Marjan and Sadeq, 2022; Al-Hasnawy and Rabee, 2023; Hansh, 2024) as well as in Wasit province (Rahi et al., 2014a, b; Rahi and Jaleel, 2022a, b) to estimate the incidence rate of *T. vaginalis* infection; however, data concerned to genotypic diversity remain underscoring in Iraq (Merdaw et al., 2018; Al-Rubaye and Alkhashab, 2022; Nasir et al., 2022), with complete lack of such information in Wasit province. Therefore, this study identifies the prevalence rate of human trichomoniasis in women of Wasit province (Iraq) using the rapid test and molecular PCR with sequencing and phylogenetic analysis of *T. vaginalis* isolates. Association of immunity to *T. vaginalis* infection through measurement the levels of cysteinyl leukotrienes (CysLTs), interleukin 8 (IL-8), and leukotriene B4 (LTB4) was aimed, also.

Materials and Methods

Ethical approval

Scientific Committee in College of Medicine (University of Wasit) was licensed this study.

Samples

Totally, 291 women having various socio-demographic factors and reproductive disorders were admitted to the private gynecological clinics in Wasit province (Iraq) during March-June (2025) and selected to the present study. All study women were subjected to sampling of vaginal swabs that processed and tested directly by the rapid test; and then, the samples were kept frozen at -20°C until be examined later by molecular assay. Additionally, 5ml of venous blood was drained from each study individual into free-anticoagulant glass-gel tube that centrifuged (5000rpm/5 min), and the obtained sera were kept into 1.5ml Eppendorf tubes and saved frozen (-20°C) until be tested serologically.

Rapid test

In the present study, Medical Rapid Diagnostic *Trichomonas vaginalis* Antigen Rapid Test Kit (Hangzhou Immuno Biotech, China) which based on the gold immunochromatography assay principle

was utilized. Following the manufacturer instructions, fresh vaginal swabs were tested directly, and the results were interpreted as positive, negative and invalid. The samples of invalid results were re-tested to indicate their positivity or negativity.

Molecular conventional PCR assaying

After thawing in water bath at 37°C, DNAs were extracted from the vaginal swabs using the gSYNCTM DNA extraction kit (Genaid, Taiwan), evaluated by the Nandrop spectrophotometer, and utilized to preparation of MasterMix tubes at a final volume of 25µl. Targeting 18S rRNA gene, one set of primers was designated to this study (BarF: 5'-AAC GATGCCGACAGGAGT TT-3' and BarR: 5'-CGTGCGTTGTTGACACACAT-3') based on NCBI-GenBank *T. vaginalis* isolates (https://www.ncbi.nlm.nih.gov/nuccore/KM603334.1). Post amplification in Thermal Cycler system following modified conditions (Table 1), electrophoresis of PCR products was performed in 1.5% agarose-gel for 90 minutes at 80 Am and 100 Volt and the positive samples for *T. vaginalis* isolates were visualized throughout the UV illuminator at an approximately 568 bp.

Table 1. Thermal cycler conditions for conventional PCR reaction.

Tool	Step	Temperature / Time	Cycle No.
Qualitative PCR	Initial denaturation	95°C / 5 min.	1
	Denaturation	95°C / 30 sec.	30
	Annealing	58°C / 30 sec.	
	Extension	72°C / 1 min.	
	Final extension	72°C / 5 min.	1

Sequencing and phylogeny

The DNAs of all positive *T. vaginalis* isolates were sequenced by the Macrogen Company (Korea), and the received data were submitted in the NCBI-GenBank database, got specific access numbers, and analysed phylogenetically throughout the MEGA software (version 11) and the NCBI-Viewer (version 1.26). Multiple sequence alignment, homology sequence identity, and phylogenetic tree analysis were done to indicate the close-relationship between the study local and NCBI-BLAST *T. vaginalis* isolates / strains.

Immunology

According to manufacturer instructions (SunLong Biotech, China) of quantitative ELISAs' kits, the concentrations of CysLTs (Cat. No: SL0576Hu), IL-8 (Cat. No: SL1004Hu), and LTB4 (Cat. No: SL1068Hu) were measured in the sera of study population. For each immune marker, the kit's contents and the sera were prepared, processed, and the absorbance was read by the ELISA Reader at an optical density (OD) of 450nm. Then, the ODs and concentrations of Standards in addition to ODs of the samples were plotted on the Standard Curve to calculate the concentration of immune marker tested samples.

Statistical analysis

The t-test in GraphPad Prism software was applied to detect significant differences between the values of positively and negatively infected individuals at $p<0.05$ with calculation of 95%confidence interval (95%CI), (Al-Graibawi et al., 2021).

Results

The findings of rapid test recorded that 11 of 291 (3.78%) women were shown a positive reactivity to *T. vaginalis* antigen in vaginal swabs (Figure 1).

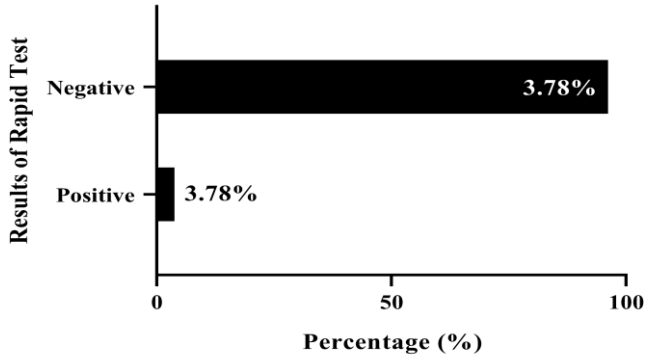

Figure 1. Total results for testing 291 vaginal swabs by rapid test

Molecularly, 5.84% (19/291) of study women were positively infected by *T. vaginalis* using the conventional PCR (Figures 2, 3).

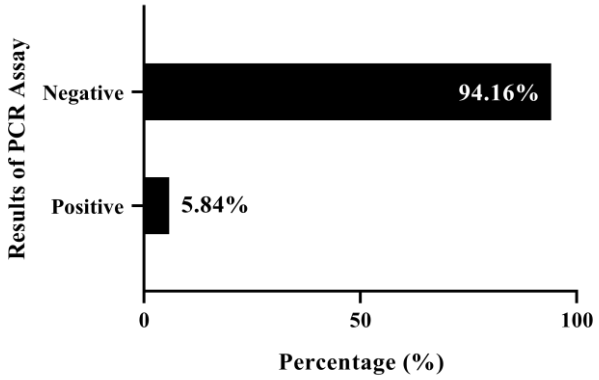

Figure 2. Total molecular results for testing 291 vaginal swabs by PCR assay

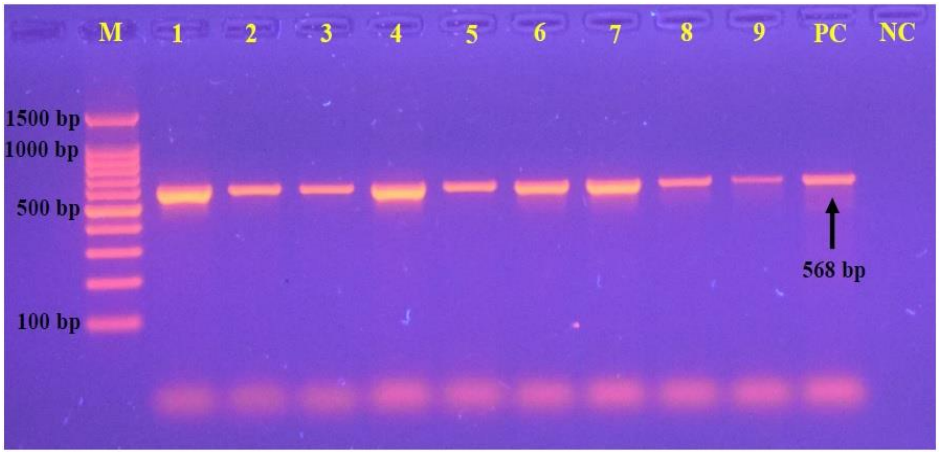

Figure 3. Agarose gel electrophoresis for PCR products

Lane (M): Ladder marker (100-1500bp)
Lane (PC): Positive Control
Lane (NC): Negative control
Lanes (1-9): Positive samples of *T. vaginalis* isolates at approximately 568bp

Sequencing data of all positive *T. vaginalis* isolates were submitted in the NCBI-GenBank database, named (Iraq-Hasbraa isolate 1, Iraq-Hasbraa isolate 2, Iraq-Hasbraa isolate 3, Iraq-Hasbraa isolate 4, Iraq-Hasbraa isolate 5, Iraq-Hasbraa isolate 6, Iraq-Hasbraa isolate 7, Iraq-Hasbraa isolate 8, Iraq-Hasbraa isolate 9, Iraq-Hasbraa isolate 10, Iraq-Hasbraa isolate 11, Iraq-Hasbraa isolate 12, Iraq-

Hasbraa isolate 13, Iraq-Hasbraa isolate 14, Iraq-Hasbraa isolate 15, Iraq-Hasbraa isolate 16, and Iraq-Hasbraa isolate 17) and got respectively specific access numbers (PV948764.1, PV948765.1, PV948766.1, PV948767.1, PV948768.1, PV948769.1, PV948770.1, PV948771.1, PV948772.1, PV948773.1, PV948774.1, PV948775.1, PV948776.1, PV948777.1, PV948778.1, PV948779.1, and PV948780.1). Phylogenetic analysis revealed that all positive *T. vaginalis* isolates have ranges of similarity (*) at 99-99.65% and mutation / changes at 0.003-0.02% with the NCBI-BLAST *T. vaginalis* isolates / strains in particular Indian *T. vaginalis* isolate (ID: JF513197.1), (Table 2, Figures 4-7).

Table 2. Homology sequence identity (%) of study and global NCBI-GenBank *T. vaginalis* isolates

Local isolate		**NCBI-BLAST isolate**				**Identity**
Name	**Access. No.**	**Isolate**	**Source**	**Country**	**Access. No.**	**(%)**
Iraq-Hasbraa 1	PV948764.1	SA2	Vagina	India	JF513197.1	99.36
Iraq-Hasbraa 2	PV948765.1	SA2	Vagina	India	JF513197.1	99.14
Iraq-Hasbraa 3	PV948766.1	SA2	Vagina	India	JF513197.1	99.09
Iraq-Hasbraa 4	PV948767.1	SA2	Vagina	India	JF513197.1	99.31
Iraq-Hasbraa 5	PV948768.1	SA2	Vagina	India	JF513197.1	99.30
Iraq-Hasbraa 6	PV948769.1	SA2	Vagina	India	JF513197.1	99.22
Iraq-Hasbraa 7	PV948770.1	SA2	Vagina	India	JF513197.1	99.12
Iraq-Hasbraa 8	PV948771.1	SA2	Vagina	India	JF513197.1	99.29
Iraq-Hasbraa 9	PV948772.1	SA2	Vagina	India	JF513197.1	99.37
Iraq-Hasbraa 10	PV948773.1	SA2	Vagina	India	JF513197.1	99.37
Iraq-Hasbraa 11	PV948774.1	SA2	Vagina	India	JF513197.1	99.42
Iraq-Hasbraa 12	PV948775.1	SA2	Vagina	India	JF513197.1	99.46
Iraq-Hasbraa 13	PV948776.1	SA2	Vagina	India	JF513197.1	99.51
Iraq-Hasbraa 14	PV948777.1	SA2	Vagina	India	JF513197.1	99.58
Iraq-Hasbraa 15	PV948778.1	SA2	Vagina	India	JF513197.1	99.64
Iraq-Hasbraa 16	PV948779.1	SA2	Vagina	India	JF513197.1	99.65
Iraq-Hasbraa 17	PV948780.1	SA2	Vagina	India	JF513197.1	99

Figure 4. MSA of study and NCBI-GenBank *T. vaginalis* isolates / strains by the MEGA software

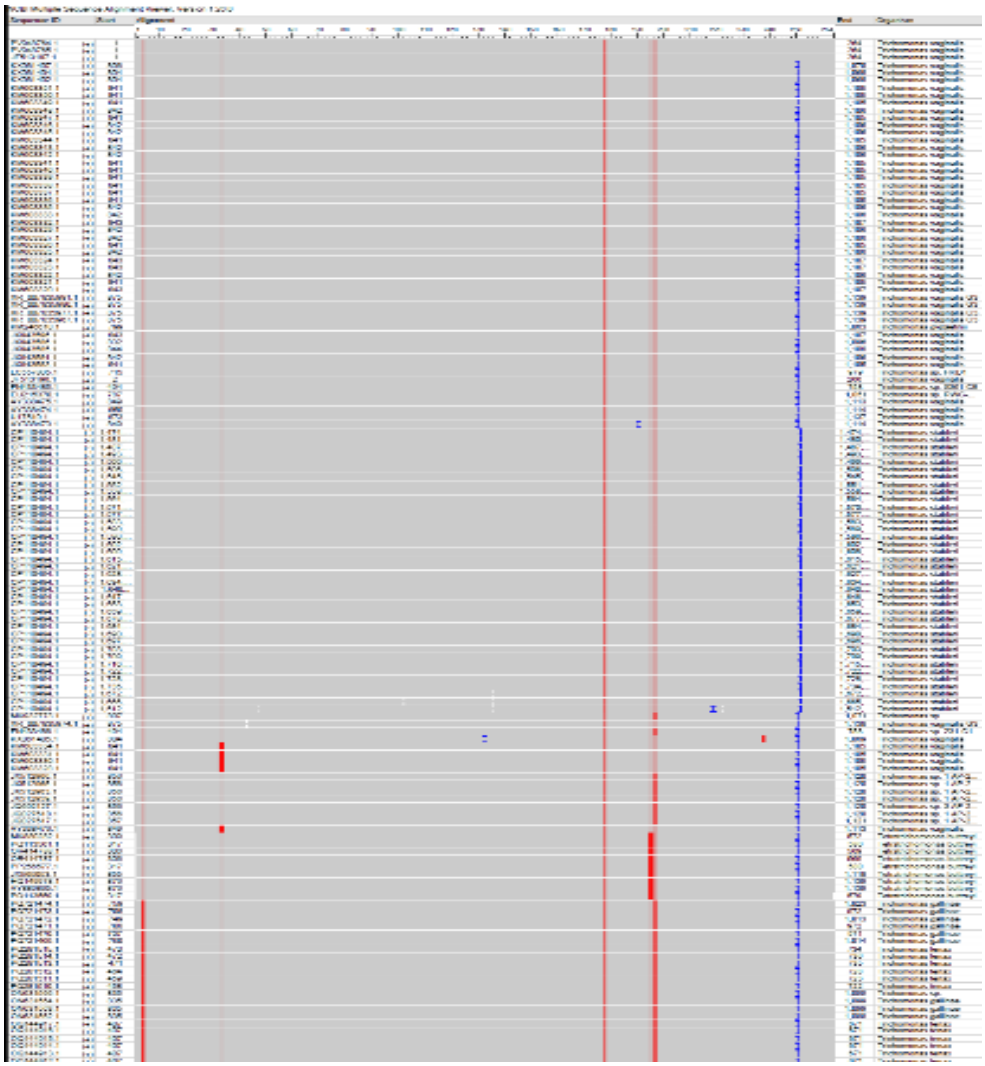

Figure 5. MSA of study and NCBI-GenBank *T. vaginalis* isolates / strains by the NCBI-Viewer software

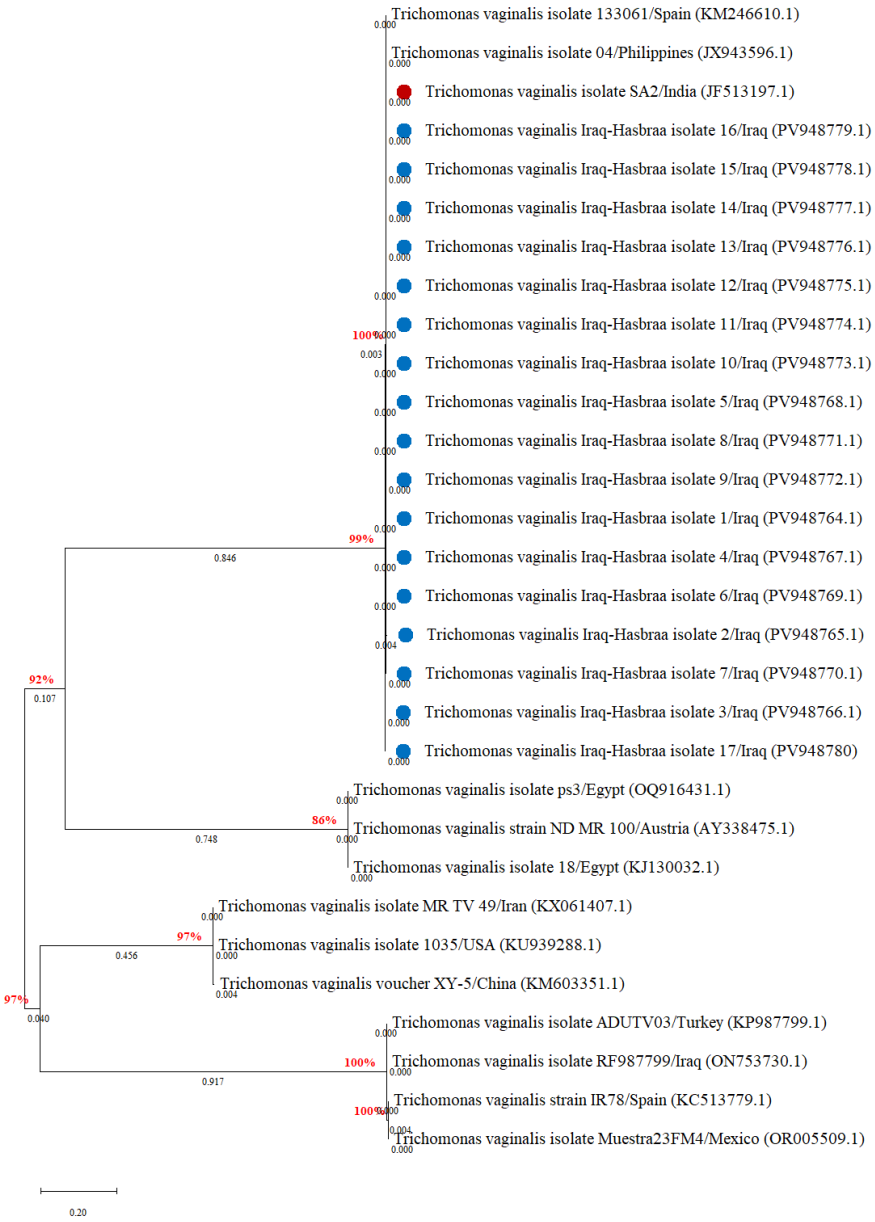

Figure 6. Phylogenetic tree analysis of study and NCBI-GenBank *T. vaginalis* isolates / strains

The findings of immunological markers have reported a significant variation ($p<0.05$) in their values among the positively (Figures 7-9). Significantly, the positively infected women were reported an elevation ($p<0.0001$; 95%CI: 1105 to 1454) in values of CysLTs (275.09 ± 17.09 pg/ml) compared to those of negatively infected individuals (74 ± 2.77 pg/ml). For IL-8, there was a significant increase ($p<0.0003$; 95%CI: 525.9 to 691.8) in values of positively *T. vaginalis* infected women (130.83 ± 6.04 pg/ml) in comparison with those of negatively result (35 ± 3.48 pg/ml). The positively infected *T. vaginalis* women (1512.059 ± 90.73 pg/ml) were shown the significant higher values ($p<0.0001$; 95%CI: 6206 to 8104) of LTB4 than those of negative values (385.93 ± 10.92 pg/ml).

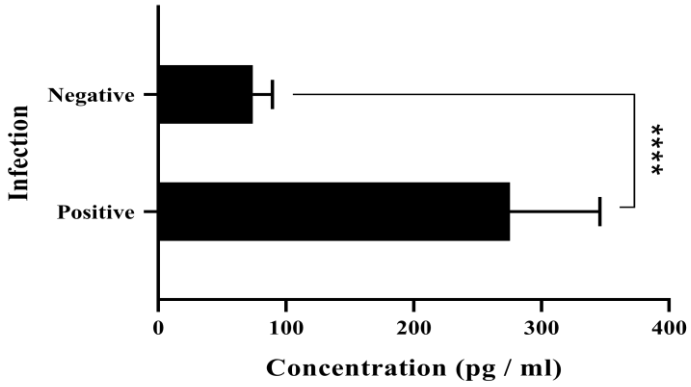

Figure 7. Quantitative result of serum CysLTs among study population using ELISA

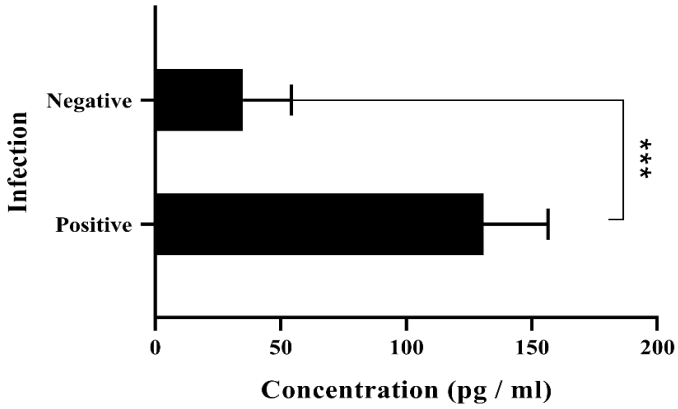

Figure 8. Quantitative result of serum IL-8 among study population using ELISA

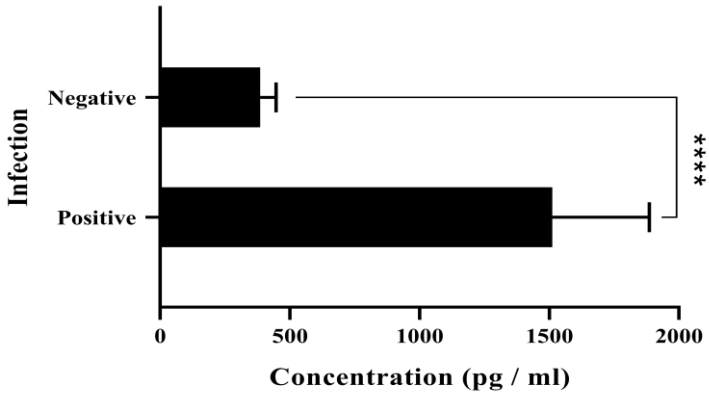

Figure 9. Quantitative result of serum LTB4 among study population using ELISA

Discussion

The findings of rapid test and molecular PCR recorded that the incidence rates of human *T. vaginalis* in women of Wasit province were 3.78% and 5.84%, respectively. In comparison to other local studies, the occurrence of human trichomoniasis was 38.1% in Al-Anbar using the microscopic examination of vaginal swab (Al-Ani et al., 2001), 1.66% in Sulaimania by direct microscopic examination and culture (Fattah and Kadir, 2010), 5.23% in Thi-Qar by wet method (Al-Abady and Al-Khazrajee, 2014), 8.33-21.67 % in Wasit by wet mount microscopy, Whif test, and RT-PCR (Rahi et al., 2014a,b), 12.41% in Diyala by wet mount examination (Hussein and Shaker, 2017), 62% in Tikrit by direct wet mount and liquid cultivate (Al-Ammash, 2017), 27.90% in Al-Najaf by wet mount examination (Al-Abbas and Radhi, 2019), 26-32% in Al-Muthana by wet smear and PCR (Al-Abodi et

al., 2019), 2.73-3.18% in Erbil by direct wet method and culture respectively (Nouraddin and Alsakee, 2019), 28.10% in Kirkuk by microscopic examination of endocervical and vaginal swabs as well as urine (Salman et al., 2019), 26.27% in Babylon by wet mount (Al-Kahfaji and Seher, 2020), 75.22% in Maysan by microscopic examination and cultivation (Al-Majidii and Alsaady, 2020), 25.5% and 29.5% in Al-Qadisiyah by wet smear and RT-PCR, respectively (AL-Khalidy and Al-abodi, 2020), 8.7-40.35% by microscopic examination of vaginal swabs and urine as well as indirect ELISA (Bedair and Ali, 2020), 0.5-1.6% in Basra by direct microscopic examination of vaginal swabs and urine (Kadhum et al., 2020), 5.5-8.62% in Karbala by wet mount, gram staining and PCR (Alhusseini and Alquraishi, 2021), 3.3% in Mosul by wet mount (AL-TAEI, 2022), 5.5% in Baghdad by PCR (Ghaima, 2022), and 9% in Duhok by microscopically wet mount examination (Murad et al., 2024). However, variations between the findings of the current study and the above mentioned studies could be attributed differences in diagnostic methods, study population (socio-demographic factors), geographical location, screening practices, sample size and type, and the presence of various *T. vaginalis* strains or other bacterial or viral infections that could interfere with the examination and interpretation of results.

Phylogenetically, high genetic identity between *T. vaginalis* from this study isolates and the Indian *T. vaginalis* isolate might be due to the shared phylogenetic lineage and the worldwide limited genetic diversity of the parasite suggesting the global spread and potential clonal evolution across these region, possibly facilitated by extensive human sexual contact and migration. Yet, the 18S rRNA gene targeted in this study demonstrates a high efficacy in identification of *T. vaginalis* due to its high conservation and repetitive nature, making it an excellent target for sensitive PCR-based detection methods (Liu et al., 2025; Shiluli et al., 2025). Recent studies reported that PCR targeting 18S rRNA gene achieves superior sensitivity and specificity compared to traditional methods like microscopy, accurately detecting infections even in asymptomatic individuals and facilitating timely diagnosis and treatment (Li et al., 2020; Shaukat et al., 2024; Nakhaei et al., 2025).

In comparison with the negatively infected *T. vaginalis* population, the positively infected women were reported a significant elevation in values of studied immune markers including CysLTs, IL-8, and LTB4. These results in agreement with observed by many researchers (Nam et al., 2012; Lee and Shin, 2024; Farhan, 2025). Nonetheless, the precise mechanisms by which *T. vaginalis* influences the synthesis and modulation of CysLTs remain an active area of investigation, but it is hypothesized that parasitic factors directly or indirectly trigger host inflammatory cascades that likely involving the activation of specific immune cells and releasing of various pro-inflammatory mediators, and subsequently affect the arachidonic acid pathway to produce leukotrienes (Jafarzadeh et al., 2023; Kou et al., 2024; Zhang and He, 2024). This intricate interplay between the parasite and host immune response could lead to an up-regulation of enzymes involved in leukotriene synthesis thereby contributing to the inflammatory pathogenesis observed in trichomoniasis (Rashidi et al., 2022). Also, *T. vaginalis* can induce the production of IL-8, a potent chemokine that attracts neutrophils and other immune cells to the site of infection, contributing to the inflammatory response and potentially exacerbating tissue damage (Bhakta et al., 2020; Zhang and He, 2024). This sustained pro-inflammatory cytokine secretion can contribute to chronic cervicitis and create a microenvironment conducive to persistence and progression of infections thereby increasing the risk of cervical intraepithelial neoplasia (Gargiulo Isacco et al., 2023). Moreover, this chronic inflammatory state and altered immune cell recruitment, partly mediated by IL-8, can promote genomic instability and activate oncogenic pathways, facilitating viral integration and subsequently leading to infertility due to damaging of reproductive tissues and impairment of normal physiological processes (Jarrett et al., 2015; Avitabile et al., 2024). LTB4, a potent lipid mediator derived from arachidonic acid metabolism, is implicated also in the inflammatory response orchestrated by *T. vaginalis* (Maddipati et al., 2016; Cheng et al., 2025). This chemotactic agent contributes to the recruitment of neutrophils and the perpetuation of localized inflammation within the cervicovaginal epithelium to further exacerbating tissue damage and modulating the immune response (Bhakta et al., 2020; Obeagu, 2024).

Conclusion

This study indicates that rapid test and/or molecular PCR can provide important data about the prevalence of T. vaginals in Iraqi population of various socio-demographic aspects who appeared with

or without clinical symptoms. Phylogenetically, this study showed that the local *T. vaginalis* isolates were markedly identical to the Indian *T. vaginalis* isolates; however, moreover sequencing and genetic information are greatly necessary as such data can be utilized in epidemiology of parasitic infection. Serological elevation of immune markers in positively infected women might further complicates the pathological landscape and enhance the inflammatory cascade with promoting the proliferation of pathogenic bacteria.

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