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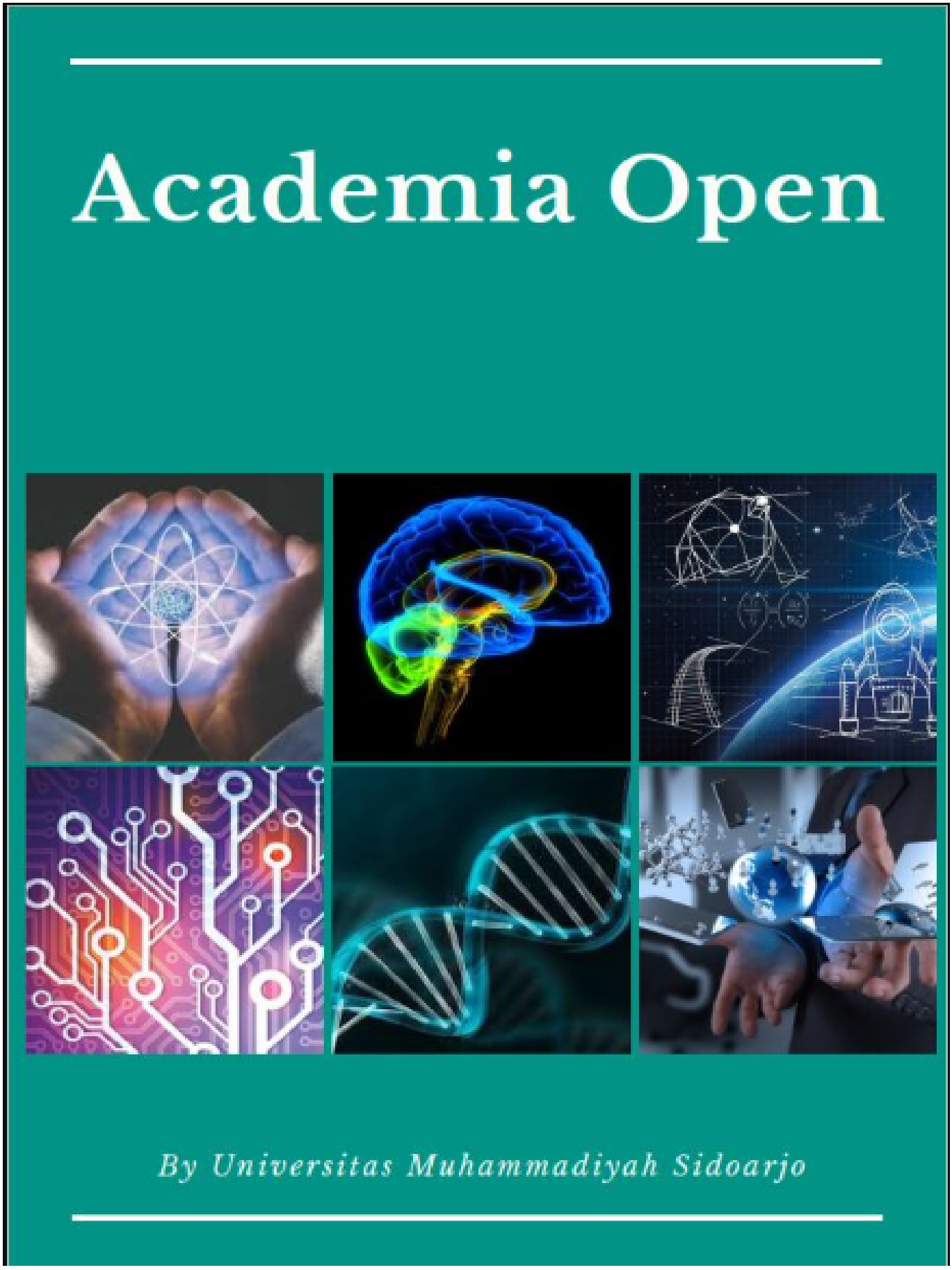

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Genetic Diversity and Host Immune Response of Trichomoniasis Among Women: Keanekaragaman Genetik dan Respons Imun Inang pada Trikomoniasis pada Wanita

Baraa Abdulsalam Hraija, babdulsalam@uowasit.edu.iq (*)

Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq

(*) Corresponding author

Abstract

General Background: *Trichomonas vaginalis* is one of the most prevalent protozoan sexually transmitted infections affecting women and is associated with reproductive and inflammatory complications. **Specific Background:** Accurate molecular detection and characterization of the parasite are essential for understanding its epidemiology and its interaction with host immune responses. **Knowledge Gap:** Despite the widespread occurrence of trichomoniasis, limited molecular and immunological data are available regarding the genetic diversity of local isolates and their relationship with inflammatory biomarkers in Iraqi populations. **Aims:** This study investigated the prevalence of *T. vaginalis* infection among women in Wasit Province, Iraq, using PCR targeting the internal transcribed spacer 1 (ITS1) region, examined the phylogenetic relationships of detected isolates, and assessed associated immune markers including cysteinyl leukotrienes, interleukin-8, and leukotriene B4. **Results:** Molecular analysis identified a 7.56% infection rate among 291 examined women. Sequencing and phylogenetic analysis of 22 isolates revealed strong similarity with previously reported Iraqi strains. Infected women demonstrated significantly elevated levels of CysLTs, IL-8, and LTB4 compared with non-infected individuals. **Novelty:** The study integrates molecular detection, phylogenetic characterization, and immunological biomarker evaluation of *T. vaginalis* isolates in a single population-based investigation. **Implications:** These findings contribute to improved epidemiological understanding of trichomoniasis and highlight the potential role of inflammatory mediators in the pathophysiology of infection.

Keywords: Trichomonas Vaginalis, ITS1 Region, Molecular Detection, Phylogenetic Analysis, Immune Biomarkers

Key Findings Highlights

1. PCR analysis identified trichomoniasis in 7.56% of examined women from Wasit Province.
2. Sequenced isolates clustered with previously reported Iraqi strains in phylogenetic analysis.
3. Infected participants showed marked elevation of inflammatory mediators including IL-8 and leukotrienes.

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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) directly by molecular PCR assay through targeting *ITS1*, and phylogenetic analysis of study *T. vaginalis* isolates. Association of positivity to immune response was aimed, also.

Materials and methods

Ethical approval

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

Samples

In total, 291 women with different socio-demographic characteristics and reproductive diseases were hospitalized with the private gynecological clinics in Wasit province (Iraq) in March-June (2025) and were picked to participate in the present study. Sampling of vaginal swabs was done on all of the study women that maintained frozen state of -20°C before being subjected to molecular examination. 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.

Molecular conventional PCR assaying

DNAs were purified out of the vaginal swabs after thawing in the water bath at 37° C with the g SYNC™ DNA extraction kit (Genaid, Taiwan), analyzed by the Nandrop spectrophotometer, and they were used to prepare MasterMix tubes at a final quantity of 25µl. One of the primers was specific to this study (*ITS1*): (IQF: 5′-CCT GCC GTT GGA TCA GTT CT-3′ and IQR: 5′-TTC CAG TTC AGC GGG TCT TC-3′) using NCBI-GenBank T. vaginalis isolates (https://www.ncbi.nlm.nih.gov/nuccore/PX147461.1). Subsequent post amplification was carried out in Thermal Cycler system after modified conditions (Table 1), PCR products electrophoresis was conducted in 1.5% agarose-gel, with 90 minutes of Amperometric input of 80 Am and Voltage of 100 V and the positive samples of *T. vaginalis* isolates were visualized at an approximate position within the UV illuminator at 364 bp.

Tool	Step	Temperature / Time	Cycle No.
Qualitative PCR	Initial denaturation	94°C / 5 min.	1
	Denaturation	94°C / 30 sec.	30
	Annealing	56°C / 30 sec.	
	Extension	72°C / 1 min.	
	Final extension	72°C / 5 min.	1

Table 1. **Table (1): Thermal cycler parameters of traditional PCR reaction.**

Sequencing and phylogeny

All the positive *T. vaginalis* isolates DNA were sequenced at the Macogen Company (Korea). The information was subm to the NCBI-GenBank database with accession numbers assigned, and it was analyzed by phylogenetic software with the NCBI-Viewer (*version 1.26*) and MEGA software (*version 11*). Homology sequence identity, multiple sequence alignment, and phylogenetic tree analysis were conducted to prove the strong correlation 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-Gharban, 2017).

Results

Molecular examination of 7.56% (22/291) of study women were positively infected by *T. vaginalis* using the conventional PCR and targeting the *ITS1* region (Figure 1). The sequence data of all the positive *T. vaginalis* isolates (total=22) were entered in the NCBI-GenBank database, named (IQ-BARHAS 1, IQ-BARHAS 2, IQ-BARHAS 3, IQ-BARHAS 4, IQ-BARHAS 5, IQ-BARHAS 6, IQ-BARHAS 7, IQ-BARHAS 8, IQ-BARHAS 9, IQ-BARHAS 10, IQ-BARHAS 11, IQ-BARHAS 12, IQ-BARHAS 13, IQ-BARHAS 14, IQ-BARHAS 15, IQ-BARHAS 16, IQ-BARHAS 17, IQ-BARHAS 18, IQ-BARHAS 19, IQ-BARHAS 20, IQ-BARHAS 21, and IQ-BARHAS 22) and got respectively specific access numbers (PX920213.1, PX920214.1, PX920215.1, PX920216.1, PX920217.1, PX920218.1, PX920219.1, PX920220.1, PX920221.1, PX920222.1, PX920223.1, PX920224.1, PX920225.1, PX920226.1, PX920227.1, PX920228.1, PX920229.1, PX920230.1, PX920231.1, PX920232.1, PX920233.1, and PX920234.1). Phylogenetic analysis with the NCBI-BLAST *T. vaginalis* isolates / strains revealed that all study *T. vaginalis* isolates having a similarity (*) ranged from 98.10% to 99.46% and mutation / changes ranged from 0.003-0.02% with the NCBI-BLAST *T. vaginalis* Iraqi strain (ID: PQ403668.1), (Table 2, Figures 2-4).

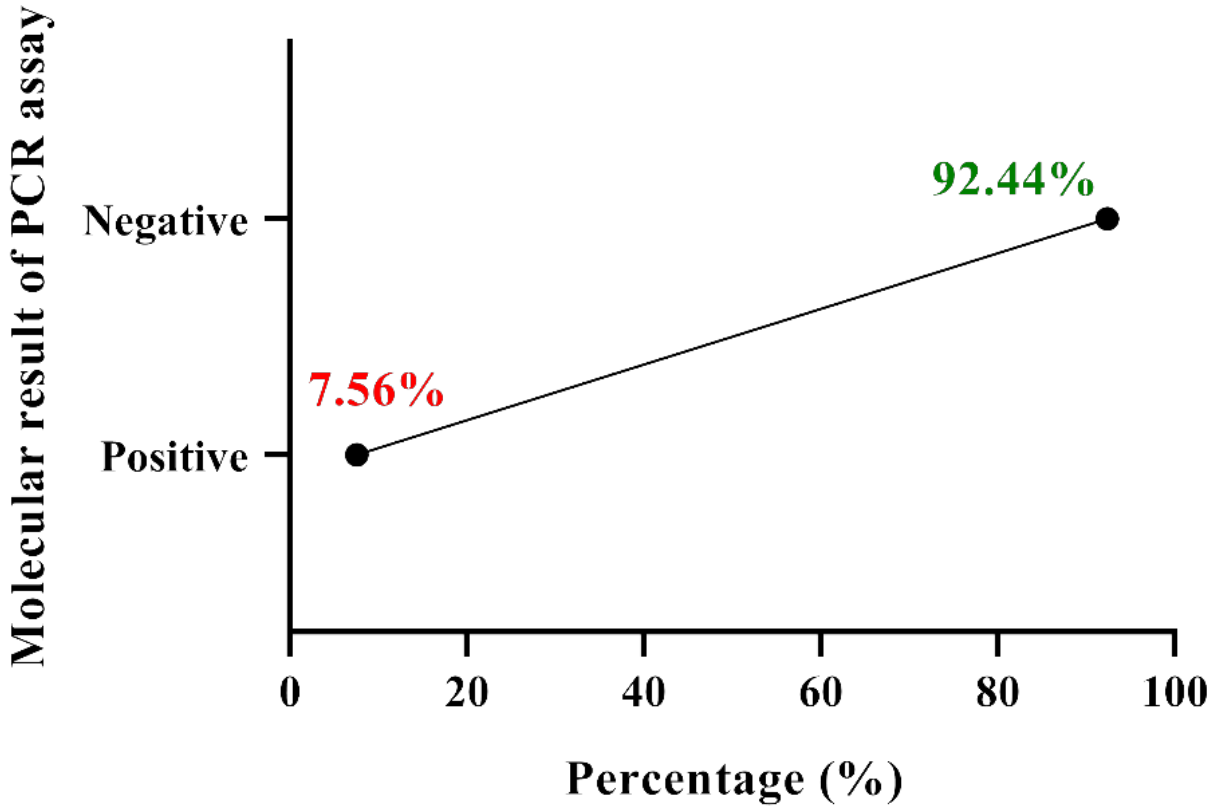

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Figure 1. **Figure (1): Total molecular results for testing 291 vaginal swabs by PCR assay targeting the *ITS1* region for *T. vaginalis***

Local isolate			NCBI-BLAST isolate			Identity (%)
Name	Access. No.	Strain	Source	Country	Access. No.	
IQ-BARHAS 1	PX920213.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.10
IQ-BARHAS 2	PX920214.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.83
IQ-BARHAS 3	PX920215.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.32
IQ-BARHAS 4	PX920216.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.31
IQ-BARHAS 5	PX920217.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.05
IQ-BARHAS 6	PX920218.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.79
IQ-BARHAS 7	PX920219.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.42
IQ-BARHAS 8	PX920220.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.51
IQ-BARHAS 9	PX920221.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.30
IQ-BARHAS 10	PX920222.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.87
IQ-BARHAS 11	PX920223.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.31
IQ-BARHAS 12	PX920224.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.33
IQ-BARHAS 13	PX920225.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.86
IQ-BARHAS 14	PX920226.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.47
IQ-BARHAS 15	PX920227.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.48
IQ-BARHAS 16	PX920228.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.61
IQ-BARHAS 17	PX920229.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.71
IQ-BARHAS 18	PX920230.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.19
IQ-BARHAS 19	PX920231.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.68
IQ-BARHAS 20	PX920232.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.49
IQ-BARHAS 21	PX920233.1	ZhSe2	Endocervix	Iraq	PQ403668.1	98.57
IQ-BARHAS 22	PX920234.1	ZhSe2	Endocervix	Iraq	PQ403668.1	99.16

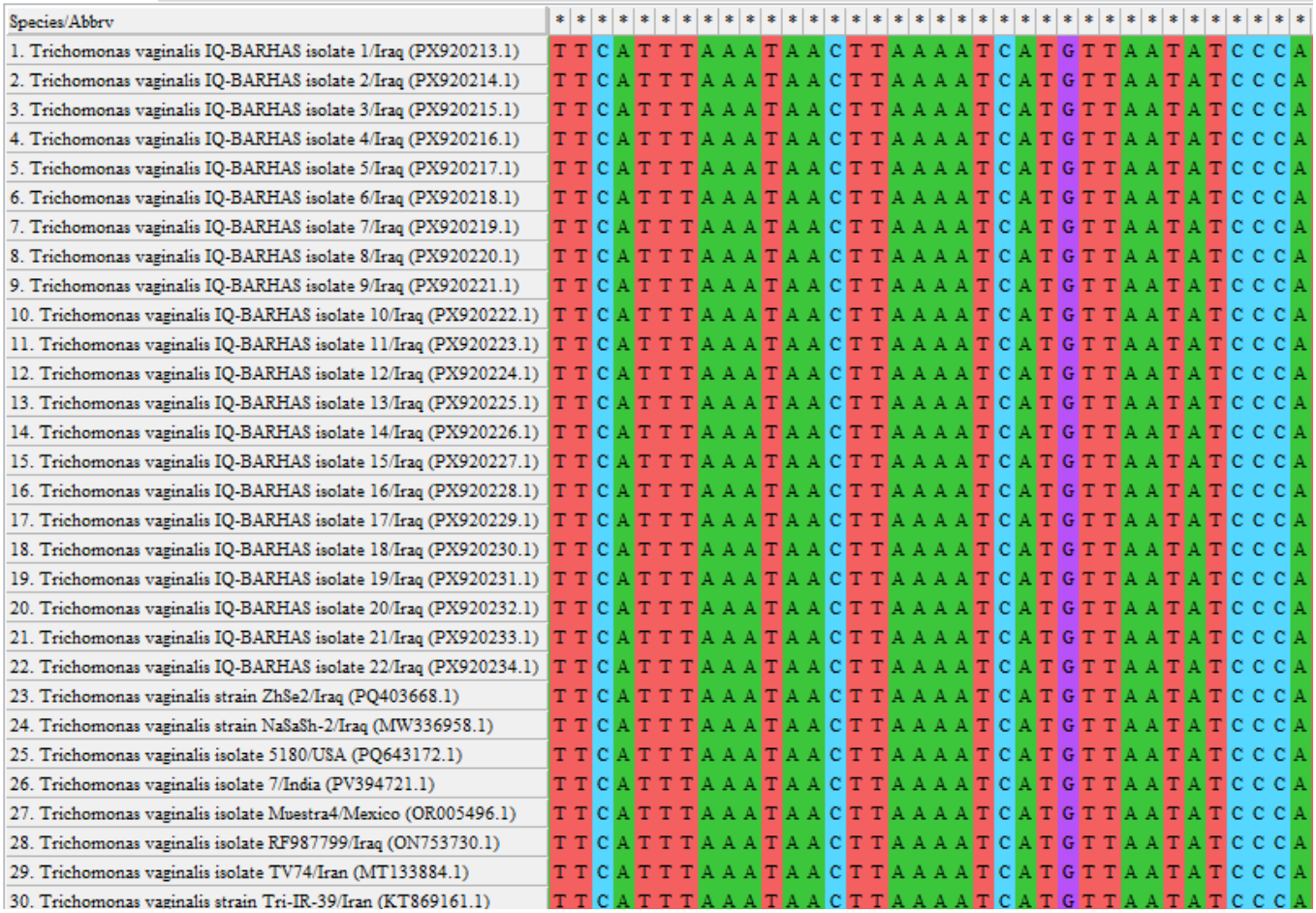

Figure 2.

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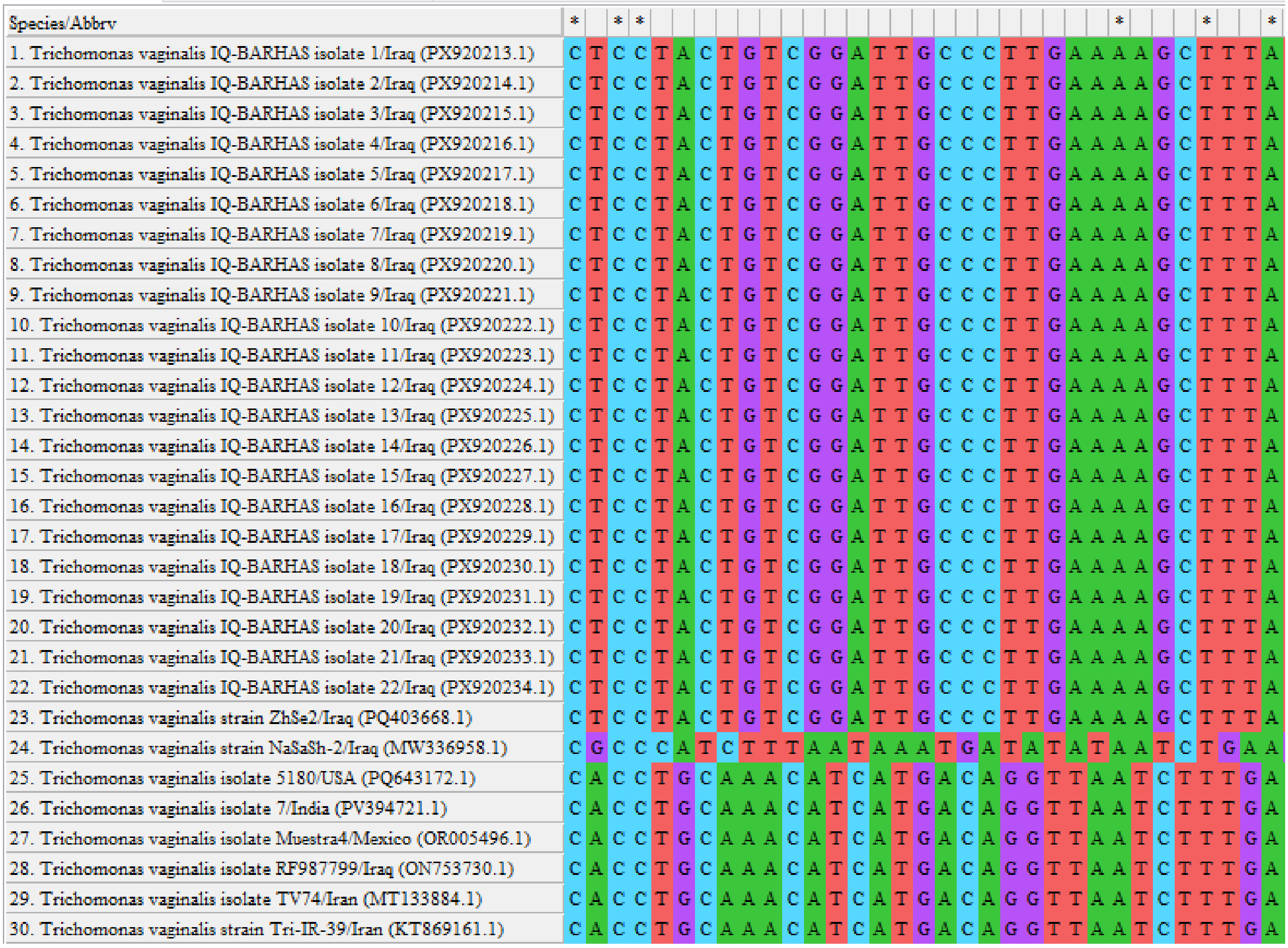

Figure 3. **Figure (2): MSA of study and NCBI-GenBank *T. vaginalis* isolates / strains targeting the *ITS1* region by the MEGA software showing nucleic acids similarity and differences**

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Figure 4.

Figure 5. **Figure (3): MSA of study and NCBI-GenBank *T. vaginalis* isolates / strains targeting the *ITS1* region by the NCBI-Viewer software showing nucleic acids similarity and differences**

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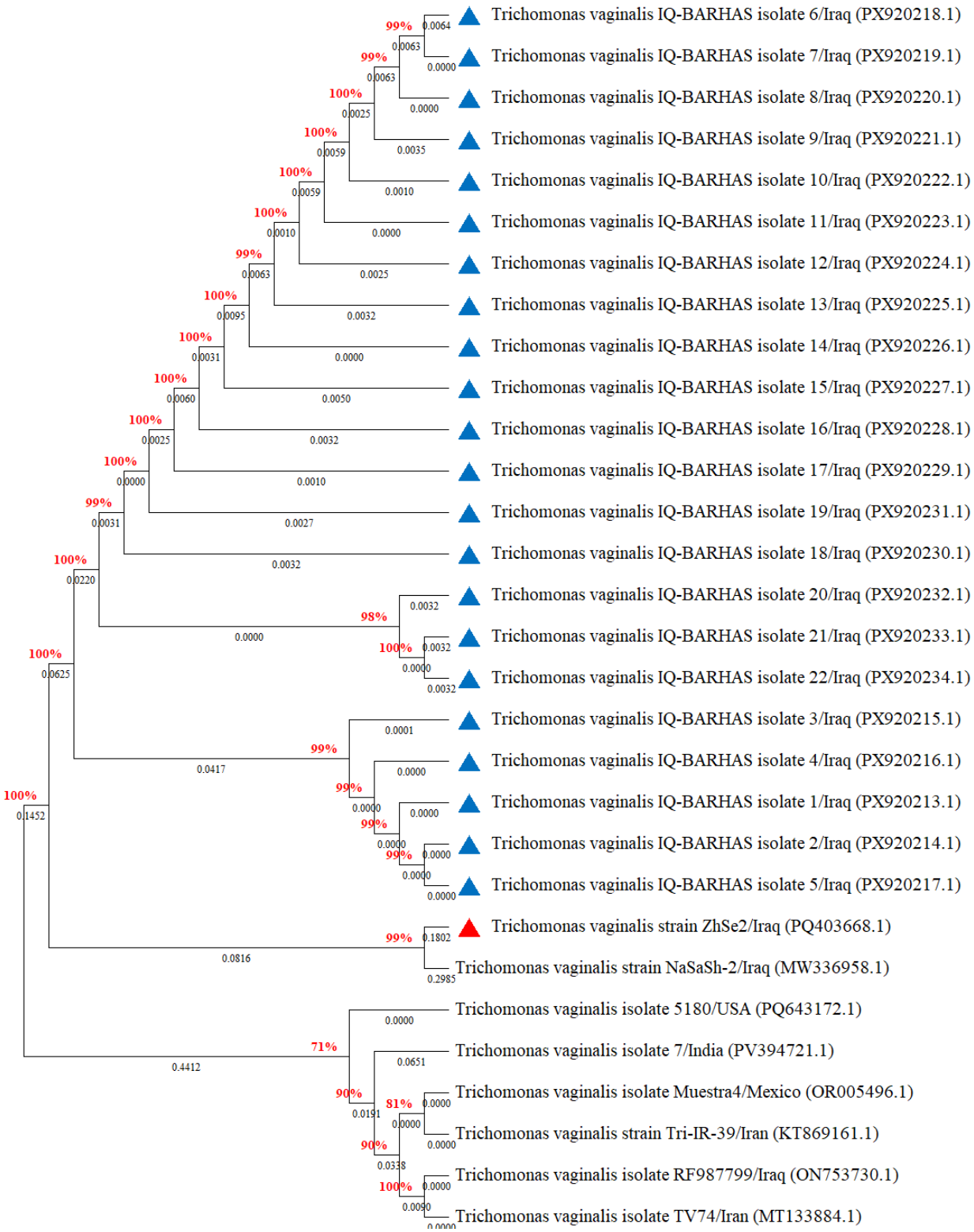

Figure 6. **Figure (4): Phylogenetic tree analysis of study and NCBI-GenBank *T. vaginalis* isolates / strains targeting the *ITS1* region**

The findings of immunological markers have reported a significant variation (p<0.0001) in their values among the positively (Figures 5-7). Significantly, the positively infected women were reported an elevation (95%CI: 1105 to 1456) in values of CysLTs (276.04±12.58pg/ml) compared to those of negatively infected individuals (74.48±2.77pg/ml). For IL-8, there was a

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significant increase (95%CI: 518.7 to 685.4) in values of positively *T. vaginalis* infected women (130.75±4.59pg/ml) in comparison with those of negatively result (35.98±3.48pg/ml). For LTB4, the positively infected *T. vaginalis* women (1496.5±70.06pg/ml) were shown the significant higher values (95%CI: 6115 to 7997) than negative values (385.93±10.92pg/ml).

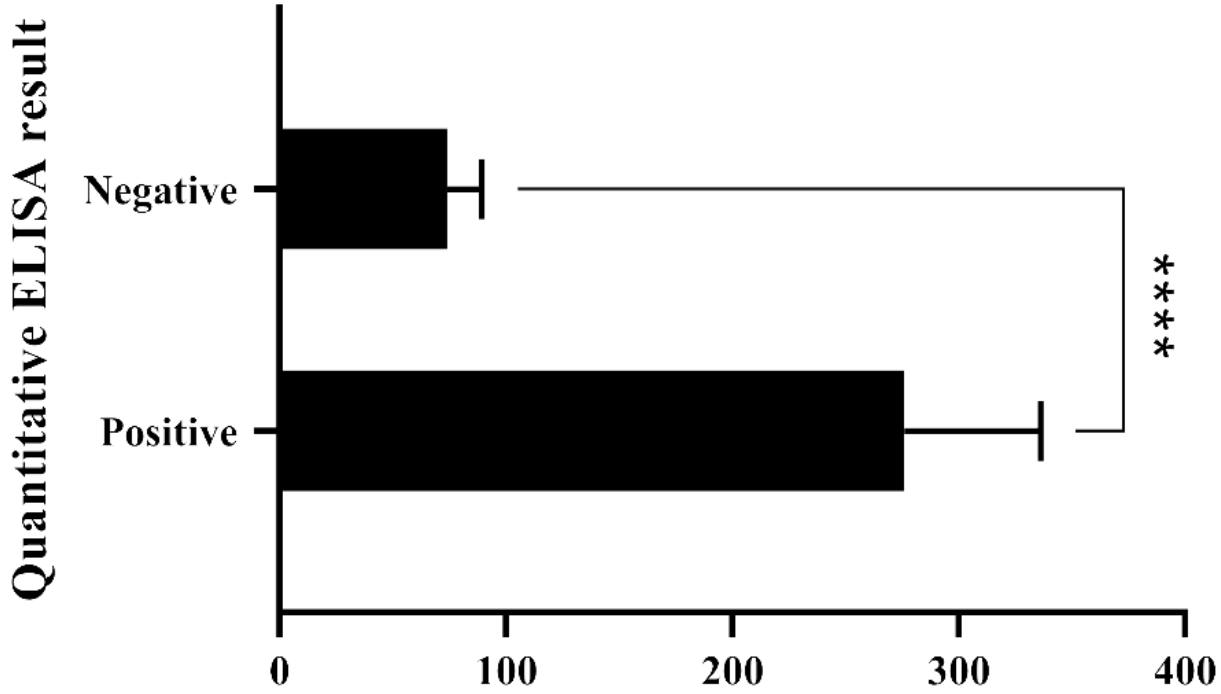

Figure 7. **Figure (5): Levels of serum CysLTs in positively and negatively study population to *T. vaginalis***

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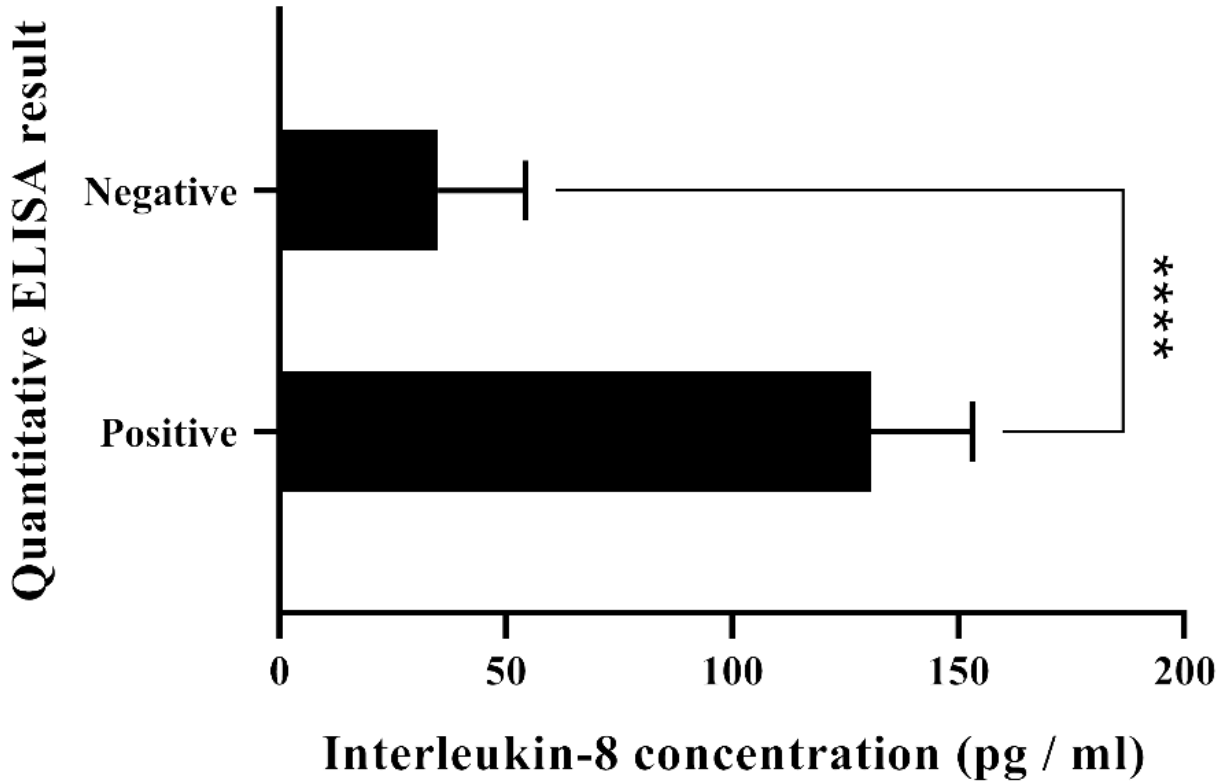

Figure 8. **Figure (6): Levels of serum IL-8 in positively and negatively study population to *T. vaginalis***

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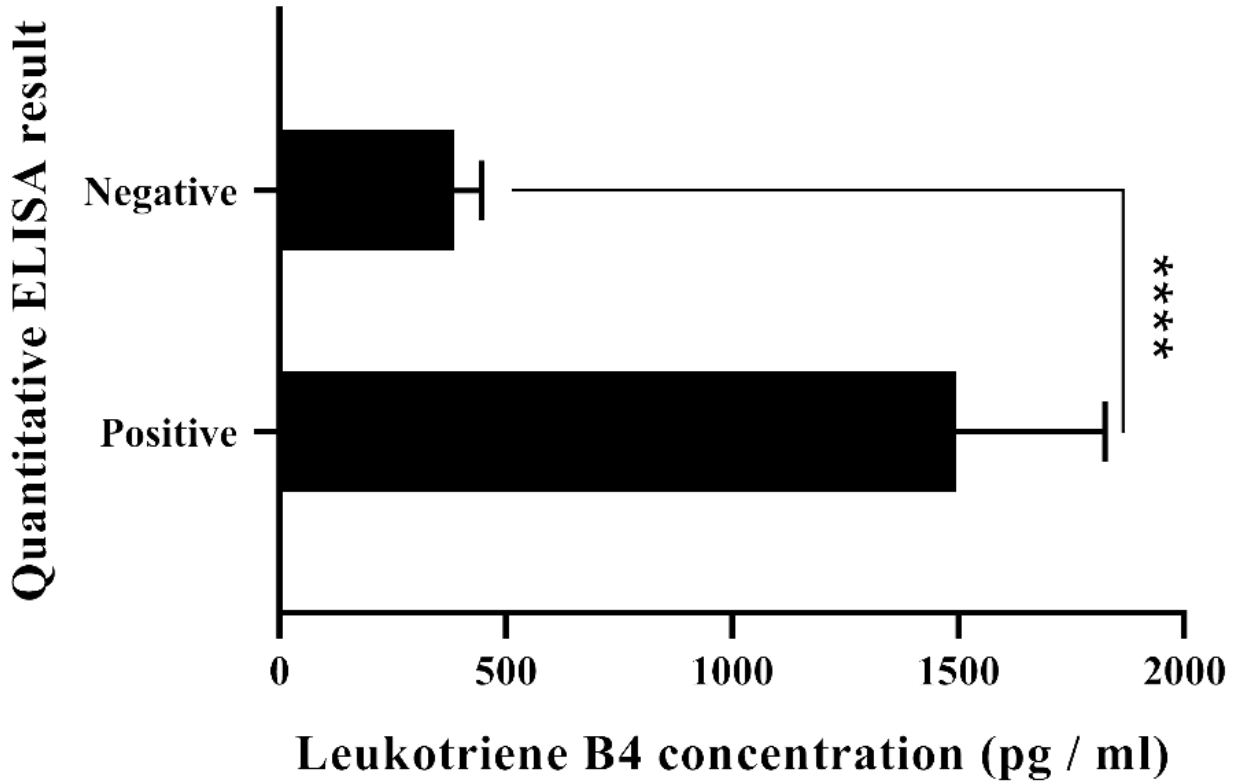

Figure 9. **Figure (7): Levels of serum LTB4 in positively and negatively study population to *T. vaginalis***

Discussion

The findings of molecular PCR recorded that the incidence rates of human *T. vaginalis* in women of Wasit province were 7.56%. 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 was shown between the current study *T. vaginalis* isolates and the NCBI-BLAST Iraqi *T. vaginalis* strain that isolated from endocervix of non-married and married women in Duhok province suggesting that this strain might be circulated in Iraqi population women, and its responsible for infections across different locations in Iraq. However, *ITS1* region that situated between the *18S rRNA* and *5.8S rRNA* genes is offered substantial variability that facilitates the taxonomic identification and subtyping of closely related protozoan parasites (Choudhary et al., 2015; Somasundaram and Yu, 2025). This genetic locus is present in multiple copies within the genome, which enhances the sensitivity of PCR amplification compared to single-copy targets (Ellingham et al., 2019). Consequently, this multi-copy nature not only increases diagnostic sensitivity but also provides sufficient sequence divergence to discriminate between

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distinct strains and subspecies during phylogenetic characterization (Mochizuki et al., 2017; Jarquín-Díaz, 2021).

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, the local *T. vaginalis* isolates were markedly identical to another local *T. vaginals* strain indicating the importance of this strain and the possible its role in women infections. Also, targeting of *ITS1* region offered a high sensitivity and specificity in molecular detection of infection and phylogenetic detection of local isolates. Therefore, additional molecular sequencing data based on *ITS1* regions appears 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.

Conflict of interests

Author declares no conflict of interest.

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