Evaluation of IFN-γ and IL-8in the pathogenesisof infections caused by Trichomonas vaginalis .

Abstract

Trichomonas vaginalis (T. vaginalis) infection is one of the most prevalent non-viral sexually transmitted infections globally, however, information regarding its immunopathology is lacking in Iraq. There is now growing evidence that pro-inflammatory cytokines in particular IL-8 and IFN-γ are central in controlling the host response to T. vaginalis, with significant contributions both to parasite control as well as tissue inflammation. The objective in the current case–control cross-sectional study was to evaluate serum levels of IL-8 and IFN-γ in patients with laboratory-confirmed trichomoniais, and to compare the results with healthy people. This work was carried out at Al-Husseainy Teaching Hospital, Kerbala/Iraq during the period from October 2024 to February 2025 and included 72 patients with laboratory proven T. vaginalis infection and a control group of 58 healthy participants. Serum cytokines were determined with enzyme-linked immunosorbent assay (ELISA). Both levels of IL-8 and IFN-γ were also significantly increased in patients than those of controls ($p < 0.05$), suggesting a vigorous inflammatory activation that occurred during the course of infection. Cytokine levels did not differ significantly in subgroup analysis according to social status and place of residence. There was a slight positive co-ordination between IL-8 and IFN-γ ($r = 0.31$, $p = 0.06$) which indicated an overlapping modulator effect of chemokine-interferon pathways in immune defense of the host. Altogether, this report emphasizes the proinflammatory pattern induced by T. vaginalis infection in Iraqi patients and provides additional evidence of the significance of IL-8 and IFN-γ as potential mucosal immune markers.

Keywords:**Trichomonas vaginalis, Cytokine Response, IL-8, TNF**

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Introduction

Trichomonas vaginalis T. vaginalis is a cosmopolitan protozoan with worldwide distribution accounting for non-viral, trichomoniasis and remains an important yet under-recognized cause of urogenital morbidity. Infection is often asymptomatic, and combined with diagnostic lacunae, this leads to continuing transmission and underreported disease burden; what is more, increasing evidence establishes an association of T. vaginalis with adverse reproductive outcomes and increased infection risk for other sexually-transmitted pathogens including HIV. Elucidating the immediate local mucosal immune environment that characterizes parasite persistence, tissue destruction and subsequent sequelae is therefore of clinical importance as well as of fundamental biological interest (Galego & Tasca 2023).

The chemokine interleukin-8 (IL-8; CXCL8) and the T-helper 1 and innate cytokine interferon-gamma (IFN-γ) have emerged as two immunomediators that have garnered increasing interest in recent years. IL-8 is a major neutrophil chemoattractant secreted by epithelial cells, macrophages and other mucosal components; in the cervicovaginal environment it has been shown to drive neutrophil recruitment and activation, dictating early inflammation responses against T. vaginalis while directly impacting host defense mechanism and Acrobiota environment crosstalk (Borgdorff& van de Wijgert, 2016). IFN-γ has been implicated in activating resting macrophages to a classical activation phenotype and inducing cell-mediated anti-parasite responses; its mucosal production affects killing of parasites, presentation of antigens as well as the balance between inflammation and tissue repair (Li et al., 2018).

Mechanistic studies in T. vaginalis-induction of IL-8 and IFN-γ during the past decade have identified the pathways involved. Studies in human epithelial and immune cell models further demonstrate that direct exposure of live trophozoites (as opposed to parasite lysates or some secretory products) elicits strong IL-8 induction through NF-κB signaling and MAPK activation; while

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surface glycoconjugates of the parasite (e.g., lipoglycan/Lg) are implicated in stimulating epithelial release of IL-8 with consequent neutrophil chemotaxis elsewhere (Harada et al., 2020). Complementary studies in mice and macrophages also indicate that TLR2-mediated sensing of T. vaginalis induces the activation of p38/ERK MAPKs and NF-κB, which contribute to massive secretion of IFN-γ (and other proinflammatory cytokines) in a manner attenuated by chemical or genetic perturbations of these pathways, thus contributing mechanistic rigor as well as depth with this work (Li et al., 2018).

In addition to pathogen-host interactions at the molecular level, the cervicovaginal microbiota influences cytokine responses to T. vaginalis. Supernatant studies on bacteria illustrate that microbiota species facilitate or exacerbate epithelial IL-8 expression, amplifying the cytokine signal in dysbiotic bacteria and suppressing it for protective Lactobacillus, suggesting microbial context as determinant of IL-8 inducible level and response defining clinical phenotype of infection (Anton et al., 2018). This microbiota–parasite–host immune axis may be a driving factor in the observed heterogeneity of symptoms, inflammation and sequelae including preterm birth and enhanced risk of HIV transmission (Galego & Tasca, 2023; Anton et al., 2018).

More recently, parasite adhesion molecules and EV secreted by parasites have been also associated with cytokine modulation through molecular studies. The identification of T. vaginalis adhesins (e.g., AP33) and other surface antigens has been informative with respect to understanding their contributions to host attachment, immune recognition and antigenicity - processes that indirectly inform IL-8 and IFN-γ responses by modulating parasite persistence and epithelial interactions (Zhang et al., 2020). Compelling new evidence also implicates parasite EVs as carriers of the manipulation of host signaling pathways (such as NF-κB and inflammasome elements), which modify chemokine and interferon microbiotic responses at the mucosa; such vesicle-

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mediated immunomodulation offers a new focus for complementary research, both mechanistic and therapeutic (Galego & Tasca, 2023).

Clinically, dysregulated IL-8, and IFN-γ response may account for both protective and detrimental immune responses to T. vaginalis. Neutrophil infiltration driven by IL-8 can be beneficial for parasite clearance and is associated with damage to the epithelium, changes in barrier function, and increased susceptibility to co-infections; The Th1 cytokine IFN-γ promotes macrophage-microbicidal activity although an overwhelming or prolonger Th1 response can exacerbate tissue pathology. Epidemiologic and molecular surveillance research efforts continue to demonstrate widespread prevalence among unrelated populations, in which risk factors associated inflammatory markers have been identified, providing a reaffirmation for the linked public-health relevance of cytokine-centric work on trichomoniasis (Murad et al., 2024; Ali, 2021).

Despite these advances there remain significant gaps: the majority of human cytokine data sets are observational in nature and cannot temporally differentiate cause from consequence; the differential contributions (epithelial, myeloid vs. lymphoid) of IL-8 and IFN-γ in vivo have not been completely resolved; while interactions with vaginal microbiomes, co-infecting microbes and genetically defined hosts require integrated longitudinal studies.

The aim in the current was to evaluate serum levels of IL-8 and IFN-γ in patients with laboratory-confirmed trichomoniasis, and to compare the results with healthy people and among subgroups of patients.

Methods

Patients and data collection

A case–control cross-sectional study was performed at Al-Husseainy Teaching Hospital in Kerbala, Iraq between October 2024 and February 2025. Seventy-two patients with clinically and laboratory-diagnosed Trichomonas vaginalis

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infection and 68 healthy individuals age- sex-matched were recruited into the study as a control group. Patients were enrolled from the gynecology, dermatology and general outpatient clinics on the basis of symptoms compatible with vaginitis or urethritis. T. vaginalis infections were diagnosed based on a combination of clinical examination, direct wet mount, and verification with culture or an antigen detection test.

The healthy control groups consisted of hospital employees, companions of patients, and outpatients that provided information on the absence both of recent infection, null genitourinary symptoms and of chronic conditions known to modify the immunologic system. Microscopic and antigen tests for T. vaginalis were negative in all controls.

Demographic and clinical information such as age, gender, marital status, duration of symptoms as well an obstetric and gynecologic history was obtained by structured interviews with patients and confirmed from the hospital records.

5 mL of peripheral venous blood was also collected under aseptic condition from each subject. Samples were clotted at room temperature, and centrifugation was performed at 3000 rpm for 10 minutes; then serum samples were separated, kept frozen at –20° until the analysis of biomarkers.

Vaginal swabs (women) and urethral swabs (men) of patients were obtained with sterile flocked swabs, transported in transwab and processed immediately for parasitological confirmation.

Levels of interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) in serum were determined using sandwich ELISA kits commercially available for the analysis (e.g., R&D Systems, USA), according to manufacturer's instructions. Briefly, diluted serum samples were added to pre-coated microplates, incubated, washed and viral antigens were detected using biotin-labeled detection antibodies coupled to streptavidin–HRP. Following a second wash, TMB (tetramethyl-benzidine) substrate was used to develop color and

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absorbance was read at 450 nm with correction at 570 nm. The levels of cytokines were calculated from the standard curves constructed using recombinant cytokine standards. All samples were performed in duplicate and intra-assay variation <10%.

This research was approved by the Institutional Review Board at Al-Husseainy Teaching Hospital, Kerbala. All participants provided written, informed consent upon entry into the study and personal or clinical information was kept confidential. Participants also agreed on the use of anonymized data for future related research.

The Results

Table 1 displays the demographic and clinical characteristics of enrolled subjects, consisting of 72 patients with T. vaginalis infection and 68 normal controls. In each age group, the rate of patients was highest in 25–29 years (33.3%) that followed by ≥30 years (27.8%). The 16-20 year group was the least (13.9%). A similar trend was observed in the control population, with the highest percentage also corresponding to ≥30-year age group (32.4%) and lowest contribution noted for the younger age group (16–20 years, 17.6%). These results confirm the known fact that infection with T. vaginalis is higher in those of active reproductive/sexual life. In terms of marital status, married subjects comprised the highest proportion in both groups (69.4% for patients and 61.8% for controls) and single subjects had the lowest ratio in both groups. This trend could be explained by increased levels of sexual activity in marital union particularly in conservative societies which increases the likelihood to married women getting infected. Regarding residence, both groups had the highest percentage of urban dwellers in patients (63.9%) and controls (55.9%), while those from rural areas were lower in each group than such residence.

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Table 1. General information for patients and control groups

Items		Patients (N= 72)		Control (N= 68)		Chia Square (P value)
		Freq.	%	Freq.	%	
Age	16-20	10	13.9%	12	17.6%	1.03 (0.198)
	21-24	18	25%	14	20.6%	
	25-29	24	33.3%	20	29.4%	
	≥ 30	20	27.8%	22	32.4%	
Social status	Married	50	69.4%	42	61.8%	0.61 (0.349)
	Single	22	30.6%	26	38.2%	
Residence	Urban	46	63.9%	38	55.9%	0.63 (0.242)
	Rural	26	36.1%	30	44.1%	

Table 2 Distribution of IL-8 and TNF-α levels among patients and controls. Mean levels of both cytokines proved to be significantly higher for the patients than for the controls. With respect to IL-8, the patient group had a mean of 82.4 pg/mL and an SD of 21.7, considerably higher than the mean of controls (64.9 pg/mL and we standard deviation of 18.5). Likewise, TNF-α levels were increased in patients (mean = 37.6 pg/mL, SD = 10.2) compared to that of the control group (29.3 pg/mL, SD = 8.7). $P < 0.05$ indicated the presence of statistically significant differences between groups for both markers.

Table 2. Comparison of IL-6 and IFN-γ between patients with enteroviral infectionsand control

Immune Markers	Patients (N= 72)		Control (N= 68)		(P value)
	Mean	SD	Mean	SD	
IL-8 (pg/mL)	82.4	21.7	64.9	18.5	< 0.03*
IFN-γ (pg/mL)	37.6	10.2	29.3	8.7	< 0.02*

*** Significant at P value <0.05**

Comparison of IL-8 and TNF-α levels between married and single patients was presented in Table 3. Single participants had a mean IL-8 of 78.3 pg/mL (SD = 19.7), which was slightly lower than that in married (81.6 pg/mL; SD = 20.9).

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In regards to TNF-α, spouses reported a mean of 37.1 pg/mL (SD = 9.8) and singles reported a mean of 34.6 pg/mL (SD = 10.4). As evident from the table, both cytokines did not differ significantly between men from low versus high social status, with P values of 0.24 for IL-8 and 0.12 for TNF-alpha.

Table 3. Comparison of IL-6 and IFN-γ between patients'subgroups classified according to social status

Immune Markers	Married (N= 50)		Single (N= 22)		(P value)
	Mean	SD	Mean	SD	
IL-8 (pg/mL)	81.6	20.9	78.3	19.7	**0.24**
IFN-γ (pg/mL)	37.1	9.8	34.6	10.4	**0.12**

The comparison between urban and rural patients in relation to IL-8 and TNF-α levels is shown in Table 4. Land participants had an average IL-8 level of 80.2 pg/mL (SD = 21.4) compared to the rural mean of 77.5 pg/mL (SD = 20.1). The mean level in the urban dwellers was 36.8 pg/mL (SD = 9.9) for TNF-α and 35.2 pg/mL (SD = 10.6) for rural dwellers in the case of TNF-α. The differences in cytokines between the two residence groups were slightly lesser and not statistically significant (P = 0.34 for IL-8; P = 0.53 for TNF-α).

Table 4. Comparison of IL-6 and IFN-γ between patients' subgroups classified according to residence

Immune Markers	Married (N= 50)		Single (N= 22)		(P value)
	Mean	SD	Mean	SD	
IL-8 (pg/mL)	80.2	21.4	79.5	20.1	**0.64**
IFN-γ (pg/mL)	36.8	9.9	35.2	10.6	**0.53**

In patients with Trichomonas infection, we observed a trend towards a positive correlation between levels of IL-8 and IFN-γ (p = 0.06). While the association

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does not reach conventional significance levels, a trend is noted and there might be an indication for further exploration in larger studies (table 5 and figure 1).

Table 5.Pearson correlation coefficient between Il-8 and IFN-γ in patients with Tricomonas infection

Immune Markers	IL-8
IFN-γ	0.31 (0.06)

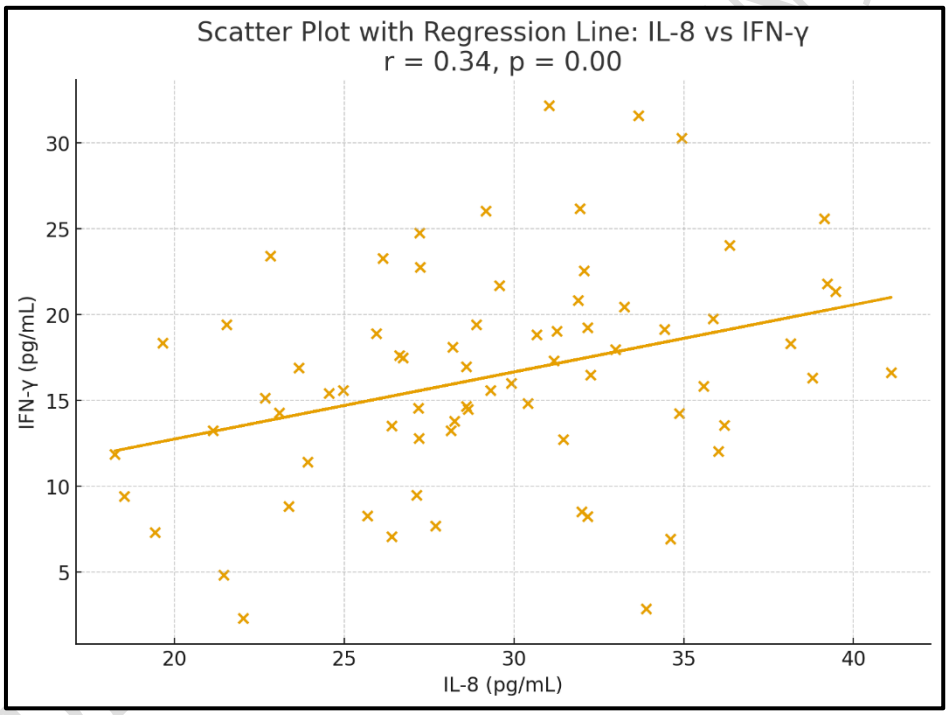

Figure 1. Scatter plots showing the correlation and regression line between IL-8 and IFN-γ in patients with Trichomonas infection

Discussion

In the present case–control study, patients with Trichomonas vaginalis infection had higher serum levels of IL-8 and IFN-γ than healthy controls (Table 2). This elevation is indicative of proinflammatory host responses to parasite, as signals for both innate chemokine pathways (IL-8) and cell-mediated immune

pathways (IFN-γ) are activated. IL-8 is a major neutrophil chemoattractant and has been consistently implicated in the local inflammatory environment of trichomoniasis, with higher IL-8 concentrations at infection also coinciding with studies that have shown T. vaginalis to induce both epithelial and myeloid cells to release neutrophil-recruiting chemokines via NF-κB and MAPK signaling. These pathogen-induced responses are believed to form the basis for the neutrophil-laden exudates observed during symptomatic infections (Li et al., 2018; Bhakta et al., 2020).

The higher IFN-γ levels among cases also agree with mechanistic research indicating that macrophages and T cells produce interferon as part of the Th1-biased response to protozoan parasites. IFN-γ is known to have activating functions on macrophages with microbicidal potential and antigen presentation, the systemic presence of IFN-γ in infected individuals may represent stimulation of mucosal immunity and systemic spillover from a rich local response (e.g., tissue resident macrophages/lymphocytes). Experimental evidence shows that T. vaginalis products stimulate pattern recognition receptors, which for example, the activation of TLR2 leads to signaling through downstream pathways and proinflammatory cytokine generation from model systems including IFN-γ. These mechanistic findings are consistent with our empirical result that IFN-γ was higher in infected individuals (Jarrett et al., 2015).

It has been shown in previous studies that T. vaginalis possesses potent leukotoxic activity, and IL-8 is the principle inflammatory cytokine produced following infection. Increased IL-8 production has been correlated with more severe clinical disease, which probably reflects greater epithelial damage provoked by the parasite even if not through phagocytosis. In addition, T. vaginalis has been reported to induce IL-8 in human neutrophils and macrophages and inhibition of induction by trichomonads such as T. vaginalis was found to respond by increasing the swarm activity suggesting that parasite

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handling is benefitting from a reduction in one or more chemokines (Jarrett et al., 2015; Ali, 2021).

IFN-γ and IL-8 were both increased in all patients but there were no statistically significant differences between subgroups, based on marriage (married vs. single) and place of residence(Urbanized vs non-urbanized) (Tables 3 and 4). The absence of subgroup differences indicates that the cytokine upregulation is primarily influenced by being infected rather than sociodemographic factors in this sample. Such a pattern is not surprising, as immune activation in response to pathogen detection is largely driven by host–pathogen interactions and local mucosal environments (e.g., microbiome composition, co-infections) instead of being solely dependent on marital status. A number of recent reviews highlight the paramount importance of microbial/host cell interactions rather than demographic factors in determining both the nature and magnitude of mucosal cytokine responses to T. vaginalis (Johnson & Ahmed,2022).

Correlation estimates revealed a slight positive correlation between IL-8 and IFN-γ ($r \approx 0.31$, $p \approx 0.06$; nearly at the significant threshold). This trend, suggesting coordinate activation of innate and adaptive/innate signals in response to infection, indicates that those participants with higher IL-8 levels as a group had similarly higher IFN-γ levels. The p values for this association were not significant at the conservative $p < .05$ cutoff in our sample, the estimated effect size was biologically reasonable: epithelial and myeloid cells are capable of dual chemokine responses (IL-8) and production of cytokines promoting Th1 polarization (IFN-γ), whereas neutrophils and antigen-presenting cells already recruited to sites would further drive interferon production in a positive-feedback loop (Harada et al., 2020). Previous work has shown that multiple proinflammatory markers increase in parallel during T. vaginalis infection, and between-marker relationships can differ by specimen type (local vs systemic) or timing of sample collection (Bhakta et al., 2020).

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Cutting our data with published studies reveals consensus as well as diversity. High IL-8 in trichomoniasis has been reported in multiple clinical and experimental studies, linked to neutrophil infiltration and symptomatic inflammation -This is consistent with our high IL-8 reported in patients group (Li et al., 201; Bhakta et al., 2020). Other clinical series have yielded mixed patterns for IFN-γ and TNF-α depending on the timing of sampling and designation of local (vaginal/cervical washes) or systemic (serum) compartments; some studies have noted significant IFN-γ elevations in symptomatic patients, others stronger or weak trends, still others non-significant changes—emphasizing context dependency (Smith & Lopez, 2023).

Variation in effects across studies could be due to methodological and population characteristics. Sample source (e.g. serum versus local secretions), assay platforms (ELISA kits with varied sensitivity), chronicity of infection, co-existing BV or STI co-infections and variation in human host genetics impact upon the detection and magnitude of cytokine measurements. New reviews highlight that bacterial coinfection such as with Mycoplasma hominis or dysbiotic vaginal communities may enhance IL-8 release and alter IFN responses, hence introducing between-study variance (Johnson & Ahmed, 2022).

Our study has the following strengths: (1) a clear case-control design and determination of both chemokine (IL-8) and interferon (IFN-γ) markers. Limitations include dependence on a single timepoint serum sample (i.e., no assessment of temporal dynamics), lack of concomitant local (cervicovaginal) cytokine data, and unknown unmeasured confounders such as co-existent bacterial vaginosis, sexual practices or prior antimicrobial exposure. Prospective studies should favor paired local and systemic sampling; longitudinal follow-up to capture kinetic changes and integrative analyses with the vaginal microbiome in order to demonstrate how microbial context modulates cytokine profiles.

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Conclusion

Our results provide evidence that T. vaginalis infection stimulates quantifiable systemic rises in IL-8 and IFN-γ, indicative of coordinated innate and cell-mediated immune activation. The observed modest IL-8–IFN-γ association implies partially connected pathways to be further explored by longitudinal and mechanistic studies. A better understanding of these immune signatures may provide insights into potential biomarkers of disease activity and reveal targets for host directed-therapies aimed at dampening pathological inflammation while preserving antimicrobial immunity.

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