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Diagnostic Performance of Serum Transforming Growth Factor- β in *Toxoplasma gondii* Infection, Urinary Tract Infection, and Coinfection: A Comparative Cross-Sectional Study

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ABSTRACT: Background: TGF- β regulates diverse biological processes, including immune modulation, infection susceptibility, and tumor progression. However, serum TGF- β levels in patients co-infected with *Toxoplasma gondii* and UTI are unknown. This study assessed serum TGF- β as a diagnostic biomarker across four groups defined by cancer status and demographics.

Methodology: A cross-sectional study with 150 subjects across four groups: control (46), *T. gondii* (45), UTI (46), and coinfection (13). Serum TGF- β was measured via ELISA. Comparisons used the Kruskal-Wallis test with a Bonferroni correction; ROC analysis evaluated diagnostic performance. Correlations and logistic regression identified predictors of cancer.

Results: Serum TGF- β concentrations differed significantly ($H = 98.313$, $p < 0.001$), highest in coinfection (173.00 pg/mL) and lowest in controls (19.00 pg/mL). ROC showed perfect separation from controls (AUC = 1.00; 100% sensitivity and specificity). AUC for cancer detection was 0.921 (sensitivity 71.2%, specificity 91.8%) at a ≥ 152.0 pg/mL cutoff. TGF- β correlated positively with cancer ($r_s = 0.694$, $p < 0.01$). Independent predictors: female gender (AOR=3.190), positive IgG (AOR=4.217), older age (AOR=1.044). TGF- β was notably higher in *E. coli* UTI cases versus the no-growth group ($p < 0.001$).

Conclusions: Circulating TGF- β levels effectively indicate infection and detect cancer, especially in co-infections. It is recommended as part of diagnostic panels for infection-related malignancy, particularly in resource-limited settings.

INTRODUCTION

Transforming growth factor-beta (TGF- β) is a multifunctional cytokine that plays a key role in immunological homeostasis, inflammation, and tissue remodeling. It also regulates the activity of two opposing subsets of lymphocytes that are crucial for maintaining immunological tolerance and driving robust responses to infection, namely T helper 17 (Th17) cells and regulatory T cells (Tregs) [1]. The systemic action of TGF- β signalling is multifaceted across essentially all tissues and plays a key inhibitory role in early immune responses, yet paradoxically also promotes disease progression, such as fibrosis and malignancy, during chronic activation [2]. TGF- β dysfunction is connected to various pathological states, including autoimmune disorders and solid

tumors, leading to significant scientific and clinical interest in this cytokine. Oncology studies have linked elevated serum TGF- β levels to tumor growth, immunological suppression, and poor prognosis. In the tumor microenvironment, TGF- β activates cancer-associated fibroblasts, remodels the extracellular matrix, and inhibits cytotoxic T lymphocyte function [3, 4]. These findings suggest that alterations in TGF- β signalling may be a common immunological factor in various clinical states, including cancer and infectious illness. TGF- β 's immunomodulatory activity extends beyond its role in the tumor microenvironment to include infectious illnesses. One of the most prevalent human infections is caused by the obligate intracellular apicomplexan parasite *Toxoplasma gondii* (T. gondii). Seroprevalence estimates indicate that infection affects a large fraction of the global population, but they vary by geography and demographics [5]. Parasites control host cytokine responses. Enzyme-linked immunosorbent assay (ELISA) analysis has shown that IgG and IgM frequencies vary by region, demographics, and risk factors. Seropositive T. gondii individuals had different immunological profiles and higher mortality [6]. T. gondii infection may also partially restore tumor-associated immunosuppression by restoring effector immune cell activity, linking infectious and neoplastic pathways [7]. In innate immunity, urinary tract infection (UTI) is one of the most prevalent infections in clinical practice, especially in female patients, and involves extensive activation of the innate immune system at the uroepithelial surface. TGF- β is an important immunomodulatory factor in the host reaction to uropathogens and implicated in renal fibrosis after *Escherichia coli* pyelonephritis [8,9]. Infectious diseases (parasitic and bacterial) are a major public health concern, particularly in low- and middle-income countries such as Iraq [10]. Parasitic infections have been reported to pose a risk to blood safety via transfusion, emphasizing the importance of available diagnostic markers in these settings [11]. Despite the growing importance of TGF- β in the pathogenesis of infectious, inflammatory, and neoplastic diseases, studies of its circulating levels across clinical settings remain scarce. Little is known about differences in TGF- β expression in patients with T. gondii infection, UTI, or T. gondii-UTI coinfection. This study aimed to measure blood TGF- β levels in healthy controls, patients with T. gondii infection, UTI patients, and coinfection patients to assess its potential as a biomarker for differentiation.

Aim of study:

A study sought to evaluate serum TGF- β levels across four groups, assess its diagnostic efficacy using receiver operating characteristic analysis, and investigate correlations with demographic, immunological, and clinical variables, including cancer status, gender, age, and serological markers of T. gondii exposure.

MATERIALS AND METHODS

Study design and setting

It was designed as a cross-sectional analytical study between December 2025 and April 2026. This study included 150 participants classified into four groups: normal healthy controls, *Toxoplasma gondii* infection, urinary tract infection (UTI), and coinfection.

Study population and samples

Participants were recruited from Azadi Teaching Hospital, Kirkuk General Hospital, and the Kirkuk Oncology and Hematology Specialized Center, Kirkuk, Iraq. The biological materials used here were collected from an established, previously characterized clinical cohort. Previously, these identical samples were used for a different study investigating the kidney injury biomarker KIM-1 (manuscript submitted). This study is an independent analytical extension of the same cohort, addressing a different scientific question focused exclusively on serum TGF- β .

Sample collection and processing

All midstream urine samples were collected aseptically and cultured on blood and MacConkey agar plates, incubated aerobically at 37°C for 24–48 h. Significant bacteriuria was defined as ≥ 8 colonies of a single uropathogen; mixed or lower counts were considered contamination or negative and excluded. Gram staining was performed on positive cultures, with species identified using the VITEK 2 Compact system (bioMérieux, France). Venous blood was collected in gel-containing tubes, allowed to clot at room temperature for 30 minutes, and then centrifuged at 4,000 rpm for 15 minutes. After centrifugation, serum was separated, aliquoted into sterile Eppendorf tubes, and stored at -60°C until analysis.

Serological detection of *Toxoplasma gondii*

Antibodies to *Toxoplasma gondii* were measured by a commercial ELISA kit (Biovantion Inc., USA) for IgG and IgM, according to the manufacturer's instructions. IgM detection was conducted with a capture ELISA format, and IgG detection was conducted using an indirect ELISA method. The optical density was measured at a wavelength of 450 nm.

Measurement of serum TGF- β

Serum TGF- β was measured using a sandwich ELISA kit (Cat. 47410–74650-8 and SL1736Hu) according to the manufacturer's protocol. The assay's sensitivity was 0.8 pg/mL, with a range of 4–300 pg/mL. Coefficients of variation were < 10% (intra-assay) and < 12% (inter-assay). This ELISA uses a sandwich technique with a pre-coated strip plate containing an anti-TGF- β antibody. Standards or samples are added, followed by an HRP-conjugated anti-TGF- β antibody. After washing, TMB substrate is added, producing a blue color that turns yellow upon addition of stop solution; the OD at 450 nm indicates the TGF- β concentration, which is calculated by comparing the sample OD to the standard curve.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26. The Shapiro–Wilk test was used to assess normality. The data were not normally distributed; therefore, nonparametric tests were used. We performed group comparisons with the Kruskal–Wallis test and a Dunn–Bonferroni post hoc analysis. Categorical variables were evaluated using the chi-square test or Fisher's exact test. Associations between variables were assessed using Spearman's correlation. Receiver operating characteristic (ROC) curve analysis and logistic regression were used to assess TGF- β 's predictive ability. Statistical significance was defined as a p-value < 0.05.

Ethical approval

The study was approved by the Ethical Committee of the Center of Technical Research, Northern Technical University (Ref. No. 9, January 19, 2026), and administratively approved by the Kirkuk Health Directorate (Consent No. 134/2025). All participants provided written informed consent before enrollment. This study was conducted in accordance with the Declaration of Helsinki.

RESULT

The demographic and clinical profiles across the four study groups are summarized in **Table 1** and presented in **Figure 1 (A–E)**. A highly significant difference in gender distribution was observed ($\chi^2 = 45.271$, $p < 0.001$; **Figure 1A**), with females predominantly comprising the UTI (93.5%) and coinfection (84.6%) groups. Similarly, the prevalence of a history of cancer varied significantly among the cohorts ($\chi^2 = 59.761$, $p < 0.001$; **Figure 1B**); the highest oncological rate was observed in the isolated *Toxoplasma gondii* group (75.6%), followed by the coinfection group (15.4%). Serological analysis revealed distinct immunoprofiles across the cohorts. Anti-character-*Toxoplasma* IgG seropositivity showed an extremely significant variation ($\chi^2 = 137.795$, $p < 0.001$; **Figure 1C**), peaking in the isolated *Toxoplasma gondii* (95.6%) and coinfection (92.3%) groups, while being absent (0.0%) in both the Control and UTI groups. Conversely, active infection status, as indicated by anti-*Toxoplasma* IgM positivity, was lower overall but remained statistically significant ($\chi^2 = 9.078$, $p = 0.028$; **Figure 1D**) and was primarily observed in the Coinfection (15.4%) and *Toxoplasma gondii* (11.1%) cohorts. Furthermore, a significant discrepancy was noted in the clinical history of pregnancy loss ($\chi^2 = 32.641$, $p < 0.001$; **Figure 1E**). The highest rate of historical abortion was documented in the coinfection group (61.5%), followed by the UTI group (17.4%).

Table 1. Baseline Demographic and Clinical Characteristics Across Study Groups (N = 150)

Variable	Control (n=46)	T. gondii (n=45)	UTI (n=46)	Coinfection (n=13)	Total (N=150)	χ^2	p-value
Gender							
Male	32 (69.6%)	12 (26.7%)	3 (6.5%)	2 (15.4%)	49 (32.7%)	45.271	< 0.001
Female	14 (30.4%)	33 (73.3%)	43 (93.5%)	11 (84.6%)	101 (67.3%)		

IgG Status							
Negative	46 (100.0%)	2 (4.4%)	46 (100.0%)	1 (7.7%)	95 (63.3%)	137.795	< 0.001
Positive	0 (0.0%)	43 (95.6%)	0 (0.0%)	12 (92.3%)	55 (36.7%)		
IgM Status							
Negative	46 (100.0%)	40 (88.9%)	45 (97.8%)	11 (84.6%)	142 (94.7%)	9.078	0.028
Positive	0 (0.0%)	5 (11.1%)	1 (2.2%)	2 (15.4%)	8 (5.3%)		
Cancer Status							
No	46 (100.0%)	11 (24.4%)	30 (65.2%)	11 (84.6%)	98 (65.3%)	59.761	< 0.001
Yes	0 (0.0%)	34 (75.6%)	16 (34.8%)	2 (15.4%)	52 (34.7%)		
History of Abortion							
No	46 (100.0%)	40 (88.9%)	38 (82.6%)	5 (38.5%)	129 (86.0%)	32.641	< 0.001

Note. Data are presented as counts and percentages within each group. χ^2 = Pearson's Chi-square statistic; p-values are two-tailed. Significance was evaluated using Pearson's Chi-square test.

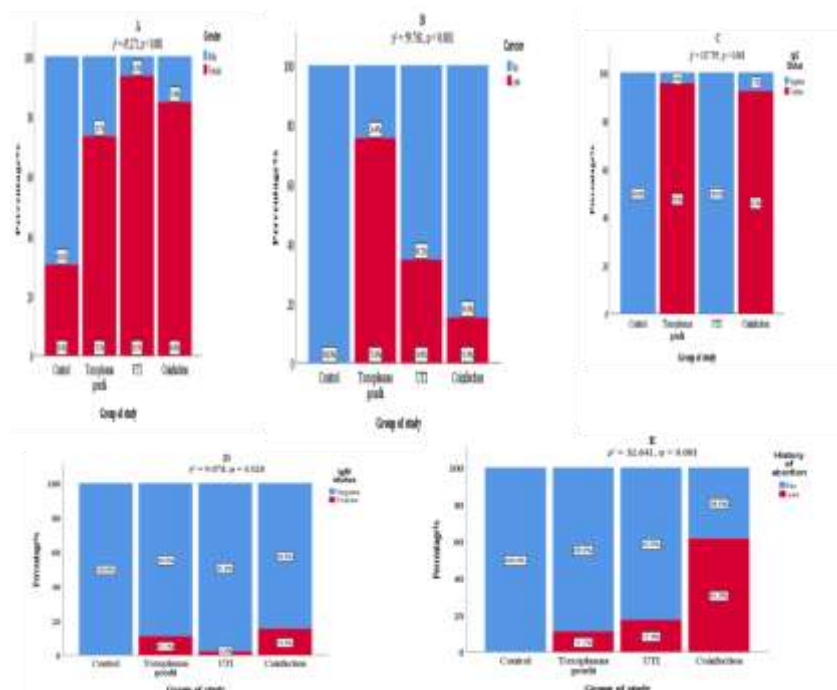


Figure 1: Demographic and clinical profile of the study groups. (A) Gender, (B) Cancer status, (C) IgG status, (D) IgM status, and (E) History of abortion. Percentages within bars show the distribution inside each group ($p < 0.05$ is significant).

Table 2 and Figure 2 illustrate that 150 participants were included in the analysis across the study groups. The control group exhibited markedly lower median TGF- β values (23.46 ± 9.33 pg/mL; Median = 19.00 pg/mL), with a narrow interquartile range, whereas all infected groups demonstrated substantially elevated and more variable TGF- β levels. The coinfection group presented the highest median concentration (184.38 ± 54.46 pg/mL, Median = 173.00 pg/mL), followed closely by the *Toxoplasma gondii* and UTI groups. The Kolmogorov-Smirnov and Shapiro-Wilk tests indicated significant non-normality ($p < 0.001$), justifying the use of non-parametric methods. The figure provides compelling visual confirmation of these statistically significant group differences and highlights the clear separation between control and infected participants.

Table 2. Sample Distribution and Descriptive Statistics of TGF- β

Variable / Group	n (%)	Mean $\pm$ SD (95% CI)	Median (IQR)	Range(Min-Max)
Control	46 (30.7)	23.46 ± 9.33 (20.69-26.23)	19.00(13.75)	15.00-44.00
<i>Toxoplasma gondii</i>	45 (30.0)	151.89 ± 61.85 (133.31-170.47)	154.00(106.00)	57.00-290.00
UTI	46 (30.7)	134.85 ± 68.03 (114.64-155.05)	100.00(104.75)	56.00-320.00
Coinfection	13 (8.7)	184.38 ± 54.46 (151.48-217.29)	173.00(105.00)	98.00-260.00
Total	150 (100.0)			
TGF- β (pg/mL)	150	110.09 ± 79.42 (97.28-122.91)	95.00(141.75)	15.00 – 320.00
Skewness / Kurtosis	0.555 / -0.688			
Normality (K-S / S-W)	$p < 0.001$ / $p < 0.001$			

Note: n = Frequency; SD = Standard Deviation; CI = Confidence Interval; IQR = Interquartile Range; K-S = Kolmogorov-Smirnov test; S-W = Shapiro-Wilk test.

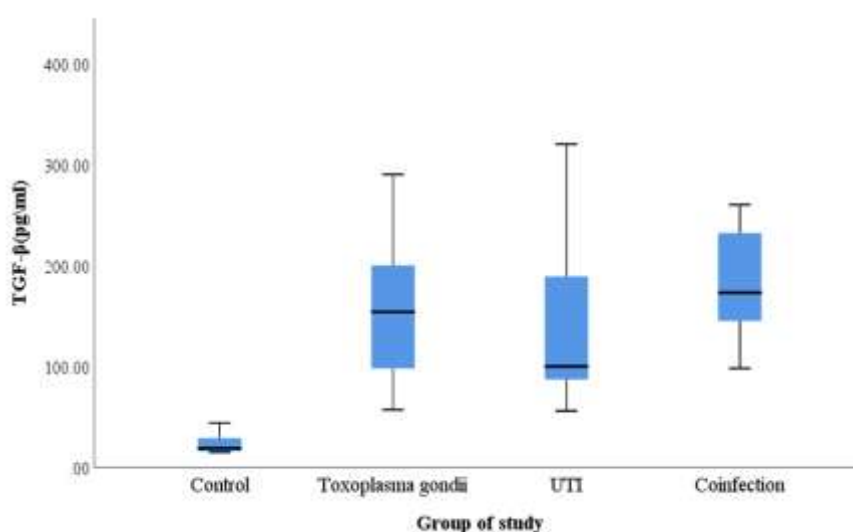


Figure 2: Box-and-whisker plots illustrating the distribution of serum TGF- β concentrations (pg/mL) across the four study groups (Control, *Toxoplasma gondii*, UTI, and Coinfection).

Table 3. A significant overall difference in TGF- β levels was detected across the four study groups ($H = 98.313$, $df = 3$, $p < 0.001$) using the Kruskal–Wallis test. Post hoc pairwise comparisons with Bonferroni correction showed that the control group differed significantly from all infection groups, whereas no significant differences were observed among the three infection groups.

Table 3. Post-hoc Pairwise Comparisons of Serum TGF- β Levels by Study Group (Kruskal-Wallis + Bonferroni)

Comparison	Test Statistic	Std. Error	Std. Test Statistic	p-value	Adjusted p *
Kruskal-Wallis: $H(3) = 98.313$, $df = 3$, $p < 0.001$					
Control vs. UTI	-67.34 4	9.045	-7.446	< 0.001	< 0.001
Control vs. <i>Toxoplasma gondii</i>	-76.62 2	9.045	-8.472	< 0.001	< 0.001
Control vs. Coinfection	-91.92 3	13.550	-6.784	< 0.001	< 0.001
UTI vs. <i>Toxoplasma gondii</i>	9.278	9.094	1.020	0.308	1.000
UTI vs. Coinfection	-24.57 9	13.583	-1.810	0.070	0.422
<i>Toxoplasma gondii</i> vs. Coinfection	-15.30 1	13.583	-1.126	0.260	1.000

Note. * p-values adjusted by Bonferroni correction for multiple comparisons. Asymptotic significances (two-tailed).

The performance and optimal threshold of serum TGF- β as a discriminatory biomarker were assessed using Receiver Operating Characteristic (ROC) curve analysis, presented in Table 3 and Figure 3. The AUC of serum TGF- β was excellent for distinguishing infectious cohorts (*Toxoplasma gondii* infection, urinary tract infection (UTI), and coinfection) from healthy controls, with an AUC of 1.000 (95% CI: 1.000–1.000; $p < 0.001$) across all infection-related comparisons. These ideal classification results should, however, be interpreted with caution, as they may reflect limitations in sample size and the likelihood of overestimating diagnostic performance in this sample.

The Youden Index was used to determine the optimal diagnostic cut-off, which ranges from 50.5 pg/mL for *T. gondii*, 50.0 pg/mL for UTI and 71.0 pg/mL for coinfection; all of these had 100% sensitivity and specificity in the study population. For oncological discrimination (cancer vs. non-cancer), the serum TGF- β had good but not perfect discriminatory power (AUC = 0.921; 95% CI: 0.878–0.964; $p < 0.001$), with an optimal cut-off value of 152.0 pg/mL, resulting in a diagnostic sensitivity of 71.2% and a specificity of 91.8%. Taken together, these findings indicate that serum TGF- β could be a valuable biomarker for distinguishing between infection-related immune responses and cytokine changes associated with malignancy, but should be validated in independent and larger cohorts.

Table 4. ROC Curve Analysis and Diagnostic Threshold Optimisation of Serum TGF- β Across the Investigated Cohorts

Comparison	n(+)	n(-)	Optimal Cutoff Values (pg/ml)	Sensitivity(%)	Specificity(%)	AUC	95%CI	P
Coinfection vs. Control	13	46	≥ 71.0	100%	100%	1.000	1.000 – 1.000	< 0.001

<i>Toxoplasma gondii</i> vs. Control	45	46	≥ 50.5	100%	100%	1.000	1.000 – 1.000	< 0.001
UTI vs. Control	46	46	≥ 50.0	100%	100%	1.000	1.000 – 1.000	< 0.001
Cancer (yes) vs. No cancer	52	98	≥ 152.0	71.2%	91.8%	0.921	0.878 – 0.964	< 0.001

Note: AUC = Area under the curve; CI = Confidence interval. (+): positive (diseased) group; (-): negative (control) group. Cutoff values were mathematically optimized by maximizing the Youden Index ($J = \{Sensitivity\} + \{Specificity\} - 1$). The diagnostic parameters (AUC = 1.000, 100% sensitivity, and 100% specificity) within infection-related groups indicate an operational separation between physiological baseline levels in healthy controls and the pathogen-induced cytokine response.

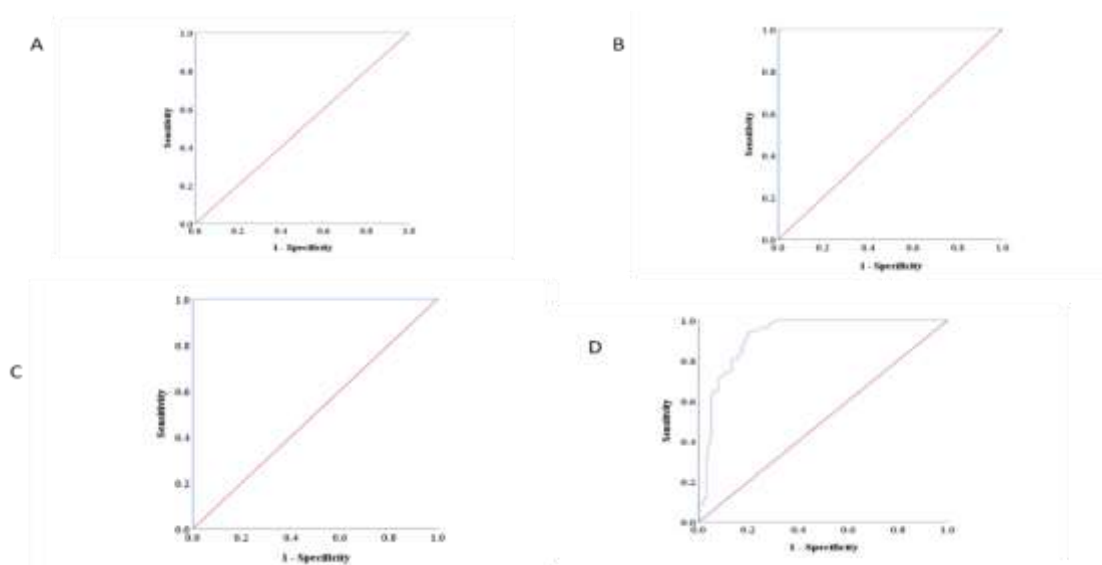


Figure 3. Receiver Operating Characteristic (ROC) curve analysis of serum TGF- β for diagnostic discrimination across study groups. (A) *Toxoplasma gondii* infection versus healthy controls. (B) Urinary tract infection (UTI) versus healthy controls. (C) Coinfection versus healthy controls. (D) Cancer versus non-cancer group. The diagonal reference line represents chance-level performance (AUC = 0.5). Serum TGF- β demonstrated excellent discriminative ability in infection-related groups (A–C) and strong diagnostic performance in cancer discrimination (D).

Table 5a. Binary logistic regression was performed to evaluate TGF- β as a predictor of cancer status. The overall model was statistically significant ($\chi^2 = 84.184$, $df = 1$, $p < 0.001$). TGF- β was a significant positive predictor ($B = 0.028$, $Wald = 41.528$, $p < 0.001$; $OR = 1.028$, 95% CI: 1.020–1.037). The model explained 42.9%–59.2% of variance in cancer status (Cox & Snell $R^2 = 0.429$; Nagelkerke $R^2 = 0.592$), with an overall classification accuracy of 83.3%.

Table 5a. Binary Logistic Regression Analysis: TGF- β Predicting Cancer Status

Variable	B	SE	Wald	P	OR (Exp B)	95% CI
TGF- β	0.028	0.004	41.528	$P < 0.001$	1.028	1.020 – 1.037
Constant	–4.106	0.619	44.065	$P < 0.001$	0.016	—

Model fit:	- 2LL = 109.424		Cox & Snell R ² = 0.429 Nagelkerke R ² = 0.592
Overall classification accuracy: 83.3%	Sensitivity: 75.0%	Specificity: 87.8%	

Note. B = regression coefficient; SE = standard error; OR = odds ratio; CI = confidence interval; -2LL = -2 log-likelihood.

Multinomial logistic regression was conducted to examine whether serum TGF- β levels could differentiate among the study groups, with the healthy control group serving as the reference category (**Table 5b**). The model demonstrated excellent fit (Nagelkerke R² = .778; McFadden R² = .490). TGF- β was a significant predictor for *Toxoplasma gondii* infection (B = 1.733, Wald = 133594, $p < .001$; OR = 5.655, 95% CI: 5.603–5.708) and UTI (B = 1.728, Wald = 123541, $p < .001$; OR = 5.630, 95% CI: 5.576–5.685) compared to controls. The co-infection group also showed markedly elevated odds (OR = 5.696), collectively indicating that elevated TGF- β strongly distinguishes all infected groups from healthy controls.

Table 5b. Multinomial Logistic Regression Analysis: TGF- β Differentiating Study Groups from Controls

Group vs. Control	B	SE	Wald	<i>p</i>	OR (Exp B)	95% CI
Toxoplasma gondii	1.733	.005	133,594	<.001	5.655	5.603–5.708
UTI	1.728	.005	123,541	<.001	5.630	5.576–5.685
Coinfection	1.740	.000	—	—	5.696	5.696–5.696
Model Fit Statistics						
Nagelkerke R²	.778					
McFadden R²	.490					

Note. B = regression coefficient; SE = standard error; OR = odds ratio; CI = confidence interval. Reference category = Control group. *p*-values are two-tailed. — indicates model convergence with near-perfect separation.

After adjustment for gender, IgG status, age, and IgM status, female gender (AOR = 3.190, $p = 0.021$), positive IgG status (AOR = 4.217, $p = 0.001$), and older age (AOR = 1.044, $p = 0.004$) remained significant independent predictors of cancer. IgM status was not significant after adjustment ($p = 0.287$). The model achieved an overall classification accuracy of 79.3% (Table 5c).

Table 5c. Adjusted Multivariate Logistic Regression: Predictors of Cancer Status

Predictor	B	SE	Wald	P	AOR	95% CI
Gender (Female vs. Male)	1.160	0.501	5.366	0.021	3.190	1.195–8.515
IgG Status (Positive)	1.439	0.428	11.313	0.001	4.217	1.823–9.754
Age (years)	0.043	0.015	8.415	0.004	1.044	1.014–1.075
IgM Status (Positive)	0.887	0.833	1.135	0.287	2.428	0.475–12.422
Constant	-5.322	1.091	23.818	< 0.001	0.005	—
Model fit: Cox & Snell R² = 0.276 Nagelkerke R² = 0.381 Overall Accuracy = 79.3%						

Note. AOR = Adjusted Odds Ratio; SE = standard error; CI = Confidence Interval.

Table 6 presents the Spearman's rank correlation matrix between TGF- β and demographic, immunoglobulin (IgG and IgM) ratio, and cancer state variables. In Spearman's rank correlation analysis, $r_s = 0.694$ ($p < 0.01$), indicating a strong, statistically significant positive correlation between cancer and TGF- β . Higher IgG ratio ($r_s = 0.298$, $p < 0.01$) and age status ($r_s = 0.454$, $p < 0.01$) were also significantly correlated with cancer status. Furthermore, gender showed a statistically significant positive correlation with cancer status ($r_s = 0.268$, $p < 0.01$). By contrast, there was no statistically significant correlation between cancer status and IgM ratio ($r_s = -0.161$, $p > 0.05$) nor between gender and either immunoglobulin ratio (IgG or IgM). Additionally, the TGF-beta and IgM ratios were negatively correlated ($r_s = -0.175$, $p < 0.05$), a weak but not insignificant correlation.

Table 6: Spearman's Rank Correlation Matrix of the Studied Variables and Cancer Status

Variable	TGF- β	Gender	Age (yr)	IgG ratio	IgM ratio	Cancer
TGF- β	—	0.473**	0.420**	0.311**	-0.175*	0.694**
Gender	0.473**	—	0.215**	0.138	-0.116	0.268**
Age (yr)	0.420**	0.215**	—	0.362**	-0.112	0.454**
IgG ratio	0.311**	0.138	0.362**	—	-0.063	0.298**
IgM ratio	-0.175*	-0.116	-0.112	-0.063	—	-0.161

Note. ** Correlation significant at $p < 0.01$ (two-tailed). * Correlation significant at $p < 0.05$ (two-tailed). N varies slightly due to missing data for the IgG ratio ($n = 146$) and the IgM ratio ($n = 131$).

Table 7 shows that statistical differences in the distributions of bacterial types across demographic and clinical characteristics were assessed using Mann–Whitney U tests. A marked difference was observed between males ($n = 49$) and females ($n = 101$) ($U = 1035.500$, $Z = -5.769$, $P < 0.001$), and a significantly different bacterial type was found between individuals with no history of abortion ($n = 129$) and those with a history of abortion ($n = 21$) ($U = 868.500$, $Z = -2.633$, $P = 0.008$). However, no significant difference was found between Gram-positive ($n = 13$) and Gram-negative ($n = 41$) groups ($U = 226.000$, $Z = -0.820$, $P = 0.412$). Additionally, a Kruskal-Wallis test comparing serum TGF- β levels across bacterial culture types ($N = 107$) revealed a highly significant difference among the groups ($H = 31.597$, $df = 7$, $P < 0.001$), as visually represented in the box-and-whisker plot (**Figure 4**). Post hoc pairwise comparisons with Bonferroni correction indicated that the "No growth" group exhibited the lowest median TGF- β levels, and Escherichia coli was the only bacterial type that differed significantly from this baseline group, showing a statistically significant elevation in TGF- β concentration (adjusted $P < 0.001$). All other pairwise comparisons failed to reach statistical significance (adjusted $P > 0.05$).

Table 7. Mann-Whitney U Tests and Kruskal-Wallis Test: Statistical Analysis of Bacterial Type Distribution across Demographic Variables and TGF- β Levels

Comparison	U / H Statistic	Z / df	P
Mann-Whitney U Tests			
Gender: Male vs. Female ($n=49$ vs. 101)	$U = 1035.500$	$Z = -5.769$	< 0.001
History of Abortion: No vs. Yes ($n=129$ vs. 21)	$U = 868.500$	$Z = -2.633$	0.008
Gram Stain: Positive vs. Negative ($n = 13$ vs. 41)	$U = 226.000$	$Z = -0.820$	0.412
Kruskal-Wallis: TGF- β by Bacterial Type ($N = 107$)	$H = 31.597$	$df = 7$	< 0.001
No growth vs. Escherichia coli (post-hoc, Bonferroni)	-34.909	—	< 0.001
All other pairwise comparisons	—	—	> 0.05 (NS)

Note. Mann-Whitney U: asymptotic significances (two-tailed). Kruskal-Wallis post-hoc: Bonferroni-corrected pairwise comparisons. NS = not statistically significant after correction. Only *Escherichia coli* differed significantly from the no-growth group.

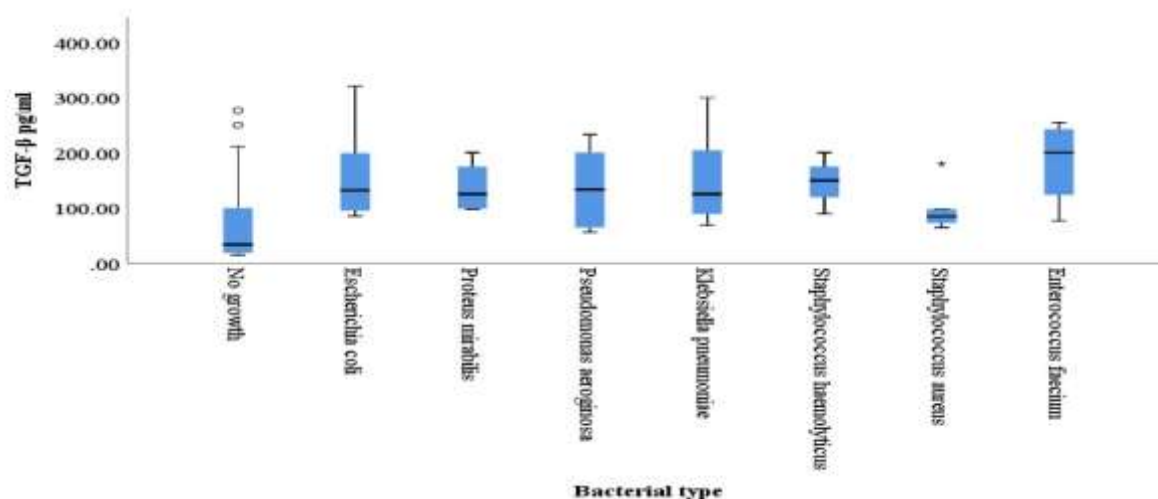


Figure 5. Box-and-whisker plots of serum TGF- β (pg/mL) by bacterial culture type (Kruskal-Wallis, $H(7) = 31.597$, $p < 0.001$, $N = 107$). The no-growth group exhibited the lowest median TGF- β ; *Escherichia coli* was the only group that differed significantly after Bonferroni correction ($p < 0.001$).

The Relationship of Age with Serum TGF- β . To analyze the correlation between patient age and serum TGF- β levels ($N = 150$), a Spearman's rank correlation coefficient analysis was performed. As shown in the scatter plot below (Figure 4), a statistically significant, moderate positive correlation was observed between the two parameters ($r_s = 0.420$, $P < 0.001$). The rather strong positive association indicates that increasing age in this patient material is significantly associated with higher serum TGF- β levels.

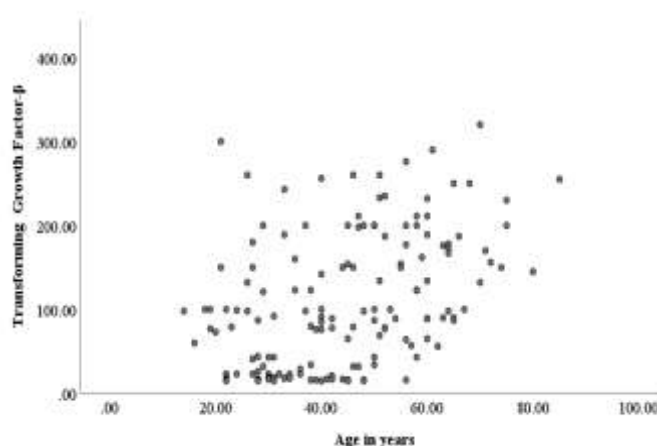


Figure 5. Scatter plot depicting the relationship between serum TGF- β (pg/mL) and patient age in years (Spearman's $\rho = 0.420$, $p < 0.001$, $N = 150$), indicating a moderate positive association between advancing age and elevated TGF- β levels.

A multinomial logistic regression analysis was conducted to determine how serum TGF- β levels, age, and gender differentiate the specific infection groups, utilizing the coinfection group as the reference category (**Table 8**). The overall model demonstrated an excellent fit ($\chi^2 = 212.985$, $p < .001$, Nagelkerke $R^2 = .819$). After adjusting for age and gender, the analysis revealed that TGF- β was a significant independent predictor for distinguishing the UTI group from the Coinfection group ($B = -0.011$, Wald = 4.635, $p = .031$, OR = 0.989, 95% CI: 0.979–0.999). Conversely, TGF- β did not significantly differentiate *Toxoplasma gondii* continuous membership from the Coinfection baseline ($p = 0.133$). Furthermore, demographic factors such

as age and gender did not emerge as statistically significant predictors for distinguishing between the infection groups relative to the coinfecting cohort ($p > .05$). Detailed estimates are presented in **Table 8**.

Table 8. Multinomial Logistic Regression: Group Differentiation (Reference: Coinfection)

Group vs. Coinfection	Predictor	B	SE	Wald	P	OR	95% CI
Toxoplasma gondii							
	TGF- β	-0.008	0.005	2.263	0.133	0.992	0.983–1.002
	Age	0.023	0.023	1.073	0.300	1.024	0.979–1.070
	Gender	0.514	0.885	0.337	0.562	1.671	0.295–9.471
UTI Group							
	TGF- β	-0.011	0.005	4.635	0.031	0.989	0.979–0.999
	Age	-0.033	0.022	2.200	0.138	0.967	0.926–1.011
	Gender	-1.431	1.022	1.960	0.161	0.239	0.032–1.772
Model fit: -2LL = 176.447 $\chi^2 = 212.985$, $p < 0.001$ Nagelkerke $R^2 = 0.819$							

Note. AOR = Adjusted Odds Ratio; CI = Confidence Interval. Reference category = Coinfection.

DISCUSSION

In the present study, serum TGF- β levels were significantly elevated in all infected groups compared with healthy controls, with the highest levels observed in patients coinfecting with *T. gondii* and UTI. These findings align with the well-known immunoregulatory role of TGF- β in immune homeostasis and in balancing the regulatory T cell (Treg) and T helper 17 (Th17) populations [12]. This elevation may represent a compensatory mechanism to regulate inflammation while minimizing tissue damage during infection. Increased TGF- β levels were observed in patients with *Toxoplasma gondii* infection compared with those without infection. *T. gondii* is known to elicit a potent Th1-type immune response, in which the cytokines IL-12 and IFN- γ play key roles in controlling the parasite [13,14]. At the same time, upregulation of TGF- β could act as an immunosuppressive feedback loop, minimizing tissue damage during chronic infection [14,15]. Additionally, latent toxoplasmosis has been linked to markers of systemic inflammation over time [15]. Serum TGF- β was also significantly higher in patients with urinary tract infection (UTI) than in healthy controls. TGF- β is a key regulator of inflammation and tissue repair and remodeling in host defense against uropathogenic bacteria [16,17]. Experimental evidence indicates that TGF- β signaling plays a protective role during the inflammatory response and a pathogenic role in the progression of renal disease following *Escherichia coli* infection [17]. Interestingly, the coinfection group had the highest median TGF- β level of all the study groups. No significant difference was found between infected groups, but this trend suggests an additive or synergistic immunologic response in the presence of mixed parasitic and bacterial infections. This co-infection can exacerbate immune dysregulation and increase the secretion of regulatory cytokines compared with a single infection [14,16]. ROC analysis revealed a good discriminatory ability of serum TGF- β from infected and healthy controls. The near-optimal diagnostic accuracy, however, should be interpreted with caution because it may be affected by sample size limitations, and population-specific bias may lead to overestimation of accuracy [12]. Moreover, there was a strong correlation between cancer status and high expression of TGF- β . *Toxoplasma gondii* infection has also been suggested to regulate tumor-associated immunosuppression and the immune response against tumors by reprogramming the immune microenvironment [22]. Logistic regression and correlation analyses were used to confirm that TGF- β is an independent predictor of malignancy. The results are consistent with the growing body of evidence that dysregulated TGF- β signaling is associated with tumor growth, immune escape, angiogenesis, ECM remodeling, and resistance to anti-tumor immunity [18–21]. Elevated TGF- β levels have also been linked to poor prognosis and are now emerging as a therapeutic target and biomarker in oncology [19–21]. In addition, a positive relationship between age and serum TGF- β levels has been observed, suggesting that immunosenescence and age-related chronic low-grade inflammation may contribute to cytokine production. Immune system remodeling has been linked to aging and may be one reason for the increased

risk of infectious and malignant diseases. In summary, these data demonstrate that TGF- β is a complex molecule with multiple functions in infectious and neoplastic diseases. Serum TGF- β levels were significantly higher in *T. gondii*-infected, UTI-coinfected, and cancer patients, indicating its potential as a non-invasive biomarker for immune dysfunction and disease monitoring. However, further studies are required to validate its diagnostic and prognostic utility in large, multicenter, long-term follow-up studies.

LIMITATIONS

There are some limitations to consider when interpreting the results of this study. First, the relatively small sample size of the coinfection group ($n = 13$) may have reduced statistical power and led to unstable models in some regression analyses, especially in cases of near-perfect separation. Second, the cross-sectional study design does not allow causal inference or analysis of changes in serum TGF- β levels over the course of the disease. Third, significant differences in gender distribution and cancer rates between the study groups could have necessitated statistical adjustment for residual confounding. Fourth, the high diagnostic accuracy reported in the ROC analyses ($AUC = 1.000$ for comparisons between infection and non-infection) should be viewed with caution, as these were calculated from the same study group, and the actual diagnostic accuracy in the real world could be higher. Extensive testing in independent populations is therefore necessary. Lastly, the use of multiplex platforms to measure additional cytokines and immune mediators, such as IL-6, IL-10, IFN- γ , and TNF- α , in parallel with quantification of serum TGF- β would provide a more complete characterization of the immune response associated with *T. gondii* infection, UTI, and coinfection.

CONCLUSION

Serum TGF- β levels were significantly higher in patients with *Toxoplasma gondii* infection, urinary tract infection, and especially in those with coinfection, compared with healthy controls. These results demonstrate the relevance of TGF- β in immunoregulatory mechanisms during infectious and inflammatory processes. Serum TGF- β showed good discriminative power between infected and healthy subjects and was significantly associated with cancer status in the studied population. Taken together, these findings indicate that TGF- β could be a useful noninvasive marker of immune activation and/or disease-associated immune dysfunction. However, larger multicenter longitudinal studies are needed to confirm the suggested diagnostic cut-offs and the clinical value of TGF- β as a tool in clinical practice.

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Data availability statement

The corresponding author can provide data upon reasonable request.

Author Contributions

Tara Saeed Aziz's areas of expertise include conceptualization, methodology, research, formal analysis, project management, resources, data curation, original draft writing, review, and editing.

Hiro Mohammed Obaid: Writing, Review and Editing, Conceptualization, and Supervision. Co-supervision is by Berat Cinar Acar.

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