Research Articles Open Access

Expression ratio of PD-1 on the T cell surface and TGF-β levels in acute and chronic brucellosis: A marker of immune evasion

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Submission date: August 29th, 2025; **Acceptance Date:** September 22nd, 2025, **Publication Date:** October 15th, 2025

Please cite this article as: Al-Zamali S. K. S. , Jallod I. M. S., Mohammed S. S., Hassan S. A. Expression ratio of PD-1 on the T cell surface and TGF-β levels in acute and chronic brucellosis: A marker of immune evasion. *Functional Foods in Health and Disease*. 2025; 15(9): 754 – 768. DOI: https://doi.org/10.31989/ffhd.v15i10.1763

ABSTRACT

Background: Brucellosis is considered a zoonotic disease characterized by the ability of Brucella spp. to evade host immune responses and establish chronic infections. Key immune-regulatory molecules, such as transforming growth factor-beta (TGF-β) and programmed death-1 (PD-1), may play a central role in this immune evasion.

Objective: The present study had a gold target to compare the expression of PD-1 on $CD4^+$ and $CD8^+$ T cells, as well as serum levels of TGF-β level between patients with acute or chronic brucellosis relative to those in healthy controls. It also aimed to assess their prospective roles as immunological markers of advancing and persistent disease.

Methods: In the present study, we recruited a total of 60 confirmed brucellosis patients , about 30 of both acute and chronic, as well as 30 healthy controls. The enzyme-linked immunosorbent assay was reliant on the measurement of serum TGF-β levels, and flow cytometry assisted in the identification of PD-1 expression among $CD4^+$ and $CD8^+$ T cells. Correlation analyses were conducted to explore the relationship between TGF-β levels and PD-1 expression.

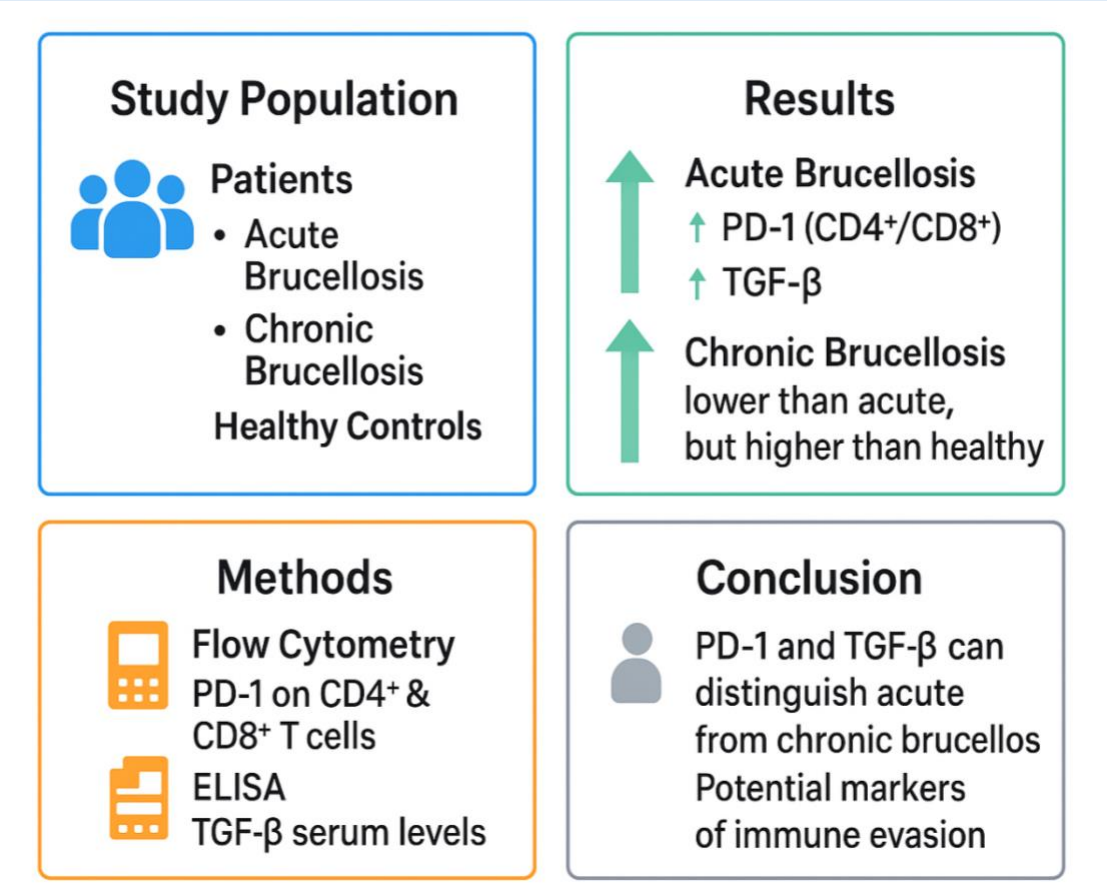
Results: Patients with acute brucellosis exhibited significantly higher levels of PD-1 on $CD4^+$ and $CD8^+$ T lymphocytes than that of chronic patients and healthy individuals ($P < 0.05$). Patients with chronic brucellosis also showed increased PD-1 levels compared with controls. No significant difference was found between both acute and chronic brucellosis stages

but serum level of TGF-β in both cases differed significantly from control ($P < 0.05$). Plasma TGF-β was highly positively correlated with PD-1 expression ($r = 0.99$, $P < 0.0001$) indicating that they could act co-operatively to suppress immunity.

Novelty: The present study sheds novel light on the immunological basis of brucellosis infection, especially about immune escape. It illustrates the correlation of PD-1 expression with TGF-β levels, as a biomarker for disease monitoring and also as a therapeutic target. These results provide a new insight into the host–pathogen interplay that may contribute to designing therapeutic interventions for reactivating antimicrobial immune responses in chronic brucellosis.

Conclusion: The ultimate conclusions of our study are that the immune suppression in patients infected with brucellosis infections is due to the product of higher levels serum TGF-β and increased expression levels of PD-1 on $CD4^+$ and $CD8^+$ T cells. The strong association of TGF-β and PD-1 suggests a controlling program that facilitates Brucella persistence. The examined markers look to be promising targets for therapy of immunological dysfunction in chronic brucellosis and could serve as the indicators for the monitoring of disease.

Keywords: Brucellosis, chronic infection (CI), immune evasion, PD-1, TGF-β, T cell exhaustion.



Graphical Abstract: Expression Ratio of PD-1 on the T Cell Surface and TGF-β Levels In Acute and Chronic Brucellosis: A Marker of Immune Evasion.

INTRODUCTION

Cases of brucellosis are characterized as a zoonotic disease brought on by bacteria belonging to the pathogenic genus Brucella, which are facultative intracellular bacteria that are Gram-negative and have the ability to enter and remain inside host cells. Humans, household animals, and wildlife are among the many species that these microbes can infect. Among the six classical *Brucella* species (*B. abortus, B. melitensis, B. suis, B. canis, B. ovis,* and *B. neotomae*), *B. melitensis, B. suis,* and *B. abortus* are considered the most pathogenic to humans [1–3].

Three clinical stages of brucellosis usually occur rapidly: an incubation period (the first two days after infection), an acute stage that is marked by the spread of bacteria in host tissues (2 days to 3 weeks), and a chronic phase that can last longer than six months and cause serious, occasionally fatal complications [4].Brucella can infiltrate the reticuloendothelial system and multiply among phagocytic immune cells after infection because the initial innate immune response is only partially successful. Due in significant part to the peculiar structural arrangement of its lipopolysaccharide (LPS) lipid A molecule, Brucella does not exhibit traditional endotoxin activity like many other Gram-negative bacteria [5].

Chronic brucellosis cases are defined by the persistence of Brucella within the host's cell, accompanied by an impaired or dysregulated immune response. The ability of the pathogen to evade immune detection is linked to alterations in key immunoregulatory pathways [6]. Among these, transforming growth factor-beta (TGF-β) and programmed death-1 (PD-1) play central roles.

TGF-β is a potent immunosuppressive cytokine involved in controlling inflammation and maintaining immune homeostasis. Regulatory T cells (Tregs), which promote immune tolerance, mediate suppression primarily through cytokines such as IL-10, TGF-β1, and IL-35 [7–12]. Elevated serum levels of TGF-β1 have been reported in patients with chronic brucellosis, suggesting a role in facilitating immune suppression during persistent infection [13]. Although Tregs are essential for preventing excessive immune activation, their suppressive functions may inadvertently support pathogen persistence by limiting effector T-cell responses [14, 15].

Similarly, PD-1 is a critical immune checkpoint receptor that negatively regulates T-cell activation and contributes to T-cell exhaustion in chronic infections. Sustained PD-1 expression on T cells diminishes overall T-cell function, impairing clearance of intracellular pathogens such as *Brucella* [16]. Single-cell sequencing studies have demonstrated that patients with chronic brucellosis exhibit features of T-cell exhaustion, including reduced expression of IFN-γ, decreased $CD8^+$/NK cell activity, and impaired effector functions, further supporting the role of persistent PD-1 expression in immune dysfunction [16].

Immune modulation can also be influenced by dietary bioactive compounds, which have been shown to regulate cytokine responses and immune checkpoint pathways. Recent studies highlight the interplay between nutrition and immune regulation, with functional foods and bioactive molecules capable of modulating pathways relevant to immune evasion in chronic infections such as brucellosis [17].

Although the importance of these immunosuppressive mechanisms is increasingly recognized, few studies have investigated PD-1 expression and TGF-β levels in parallel during the acute and chronic stages of brucellosis. The present study aimed to address this gap by evaluating PD-1 expression on $CD4^+$ and $CD8^+$ T cells, along with serum TGF-β levels, in patients with acute and chronic brucellosis. Understanding these immune parameters may help clarify how Brucella manipulates host immunity during infection and may provide insights into the development

of novel immunotherapeutic approaches to restore immune competence in chronic disease as a development of therapeutic manner.

METHODS

Study Population: This study enrolled 60 patients with confirmed brucellosis who were admitted to Al-Hilla Teaching Hospital between May and November 2024. Patients were stratified into two groups: 30 in the acute stage (age range: 15–57 years; mean age: 39.31 ± 11.32 years; 22 males and 8 females) and 30 in the chronic stage (age range: 21–72 years; mean age: 43.21 ± 10.23 years; 20 males and 10 females). A control group of 30 healthy individuals (age range: 20–60 years; mean age: 41.81 ± 12.30 years; 21 males and 9 females) was also included.

Diagnosis and classification into acute or chronic stages were established according to the World Health Organization (WHO) 2022 diagnostic guidelines. Acute brucellosis was defined as disease duration of less than 6 months, whereas chronic brucellosis was defined as symptoms persisting for more than 6 months. Healthy controls had no clinical history or serological evidence of brucellosis.

Inclusion and Exclusion Criteria: Eligible participants met the WHO 2022 diagnostic criteria for brucellosis. Exclusion criteria included: (1) serious systemic illnesses affecting the cardiovascular, neurological, respiratory, hepatic, or renal systems; (2) history of malignancy or autoimmune disease; (3) use of immunosuppressive medications, corticosteroids, or immunomodulatory agents within the previous three months; and (4) any known immunodeficiency disorder.

Ethical Approval: Ethical approval was obtained from the Ethics Committee of the College of Medicine, University of Babylon (Approval Number: 39/2023). Written informed consent was secured from all participants before enrollment. For participants younger than 16 years, informed consent was obtained from their parents.

Enzyme-Linked Immunosorbent Assay (ELISA) for TGF-β Detection: Serum concentrations of TGF-β were measured using a commercially available human ELISA kit (Elabscience Biotechnology, USA; 96-well format), based on the sandwich ELISA principle. Briefly, microplate wells pre-coated with anti-human TGF-β antibodies were incubated with standards or serum samples. Biotinylated anti-TGF-β antibodies were added, followed by streptavidin-HRP conjugate. After a 60-minute incubation at 37 °C, the plate was washed five times with the supplied wash buffer to remove unbound components. Substrate solutions A and B were then added, and the plate was incubated in the dark at 37 °C for 10 minutes. The enzymatic reaction was terminated by adding stop solution, and optical density was measured at 450 nm using a microplate reader. A standard curve was generated from serial dilutions ranging from 15 to 240 ng/mL. The assay sensitivity was 0.49 ng/mL.

Flow Cytometric Analysis of PD-1 Expression: PD-1 expression on $CD4^+$ and $CD8^+$ T cells in whole blood samples was assessed by flow cytometry as follows:

1. **CD4 staining:** 5 µL PE-conjugated anti-human CD4 antibody (Catalog No. E-AB-F1109D; Elabscience) was added to 100 µL of whole blood. Each antibody lot was quality-controlled by immunofluorescent staining with flow cytometric analysis.
2. **CD8 staining:** 5 µL APC-conjugated anti-human CD8 antibody (Catalog No. E-AB-F1110C; Elabscience) was added under the same conditions as above.
3. **PD-1 staining:** 5 µL of surface-labeled anti-human PD-1 antibody was added to 100 µL of whole blood.
4. **Lysis and fixation:** Following 30 minutes of incubation, erythrocytes were lysed and leukocytes

fixed using the Unique-Lyse kit, which provides gentle red blood cell lysis and stabilization of leukocytes. The kit included:

- 50 mL Erythrocyte Lysing Reagent A (ready-to-use, sufficient for 50 tests).
- 50 mL Erythrocyte Lysing Reagent B (ready-to-use, sufficient for 50 tests). After treatment, samples were centrifuged, and the supernatant was discarded.

5. **Washing and acquisition:** Two milliliters of cell wash solution were added, followed by centrifugation at 500 × g for 5 minutes. The supernatant was discarded, and cells were resuspended in 500 µL of wash buffer. Samples were then acquired on a flow cytometer for analysis.

Statistical Analysis: Data analysis was performed using Microsoft Excel 2010 and SPSS software version 26 (IBM, USA). Continuous variables were summarized as means ± standard deviations. The Kolmogorov–Smirnov test was applied to assess data normality. The Mann-Whitney U test was used for data that was not normally distributed, while the independent-samples t-test was used for data that was normally distributed when comparing two groups. For comparisons across more than two groups, one-way ANOVA was used when normal distribution was verified. Categorical variables were analyzed using the Chi-square test. Correlations between continuous variables were assessed using Pearson's correlation coefficient (r). A p-value < 0.05 was considered statistically significant, and a p-value < 0.01 was considered highly significant.

RESULTS

Participant Characteristics

Exposure History: Among the 60 patients diagnosed with brucellosis, 42 (70.0%) reported a documented history of exposure to potential risk factors. Of these, 8 patients (13.3%) had occupational exposure through veterinary work, while 34 patients (56.7%) were engaged in livestock rearing (cattle and sheep), either through direct animal handling or consumption of unpasteurized dairy products. The remaining 18 patients (30.0%) reported no identifiable history of contact or known exposure to risk factors (Table 1).

Table 1. History of exposure to risk factors among patients with brucellosis (n = 60).

Exposure History	Number of Patients (n)	Percentage (%)
Documented history of exposure (total)	42	70.0
Veterinary occupational contact	8	13.3
Livestock rearing (with/without direct contact or consumption of unpasteurized dairy)	34	56.7
No identifiable exposure	18	30.0

Clinical Presentation: The study population comprised two groups: 30 patients diagnosed with acute brucellosis (disease duration ranging from 1 week to 6 months) and 30 patients with chronic brucellosis (duration ranging from 7 months to 1 year). Comparative analysis of clinical manifestations between the acute and chronic groups revealed distinct patterns. Patients with acute brucellosis predominantly presented with fever, generalized fatigue, musculoskeletal pain, and profuse sweating. In contrast, chronic brucellosis was more commonly associated with persistent fatigue, recurrent episodes of fever, and joint discomfort. The detailed distribution of clinical symptoms across both stages is presented in Table 2.

Table 2. Comparison of Clinical Features between Brucellosis stage Patients

Clinical manifestations	Acute stage (n=30)	Chronic stage (n=30)
Fever	29 (96.7%)	21 (90.0%)
Fatigue	26 (86.7%)	29 (96.7%)
Chills	16 (53.3%)	2 (6.7%)
Sweats	20 (66.7%)	7 (23.3%)
Joint pain	25 (83.3%)	24 (80.0%)
Headache	8 (26.7%)	4 (13.3%)
Muscle pain	26 (86.7%)	14 (46.7%)
Weight loss	4 (13.3%)	0
Cough	5 (16.7%)	0

Laboratory Investigations: All patients underwent laboratory evaluation, including the erythrocyte plate agglutination test and the standard tube agglutination test, both of which yielded positive results. Antibody titers for patients with acute and chronic brucellosis are summarized in Table 3. Notably, the prevalence of elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels was lower in chronic cases compared to acute cases, as detailed in Table 4.

Table 3. Antibody Titers ranges in Acute and Chronic Brucellosis Patients.

Clinical Group	Minimum Titer	Maximum Titer	Titer Range
Acute Brucellosis	1:50	1:600	1:50 – 1:600
Chronic Brucellosis	1:100	1:800	1:100 – 1:800

Table 4. Comparative Laboratory Profiles of Patients with Acute and Chronic Brucellosis

Clinical manifestations	Acute stage (n=30)	Chronic stage (n=30)
Eerythrocyte plate test (Positive)	30 (100.0%)	30 (100.0%)
Leukocytopenia	2 (6.7%)	1 (3.3%)
Anemia	4 (13.3%)	6 (20.0%)
Thrombocytopenia	2 (6.7%)	4 (13.3%)
Increased ESR	26 (86.7%)	20 (66.7%)
Increased C-reactive protein	22 (73.3%)	18 (60.0%)

Flow Cytometry Findings

Proportion of PD-1 Expression on $CD4^+$ and $CD8^+$ T Cells: Flow cytometric analysis demonstrated that PD-1 expression on $CD4^+$ and $CD8^+$ T lymphocytes was significantly elevated in patients with acute brucellosis, with mean values of 12.27 ± 3.37 and 4.26 ± 0.65, respectively. These levels were markedly higher than those observed in patients with chronic brucellosis, who exhibited mean PD-1 expressions of 8.73 ± 2.87 for $CD4^+$ cells and 2.10 ± 0.44 for $CD8^+$ cells ($P < 0.05$). Additionally, individuals in the chronic group showed significantly higher PD-1 expression on both $CD4^+$ and $CD8^+$ T cells compared to healthy controls, whose corresponding values were 5.70 ± 1.48 and 0.89 ± 0.29 ($P < 0.05$).

The comparative expression patterns of PD-1 on $CD4^+$ and $CD8^+$ T cells among the study groups are illustrated in Figures 1 and 2, respectively, while Figure 3 presents representative flow cytometry plots for $CD4^+$ and $CD8^+$ markers.

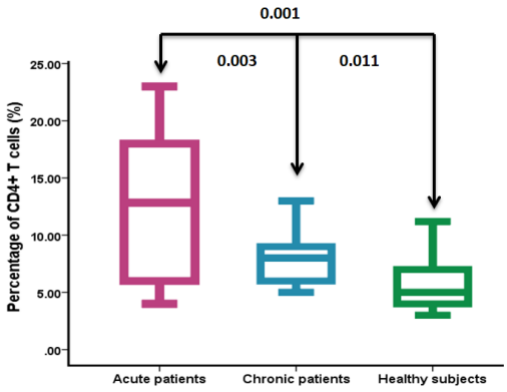


Figure 1. Proportion of PD-1 expression on $CD4^+$ T cells in peripheral blood across the different study groups.

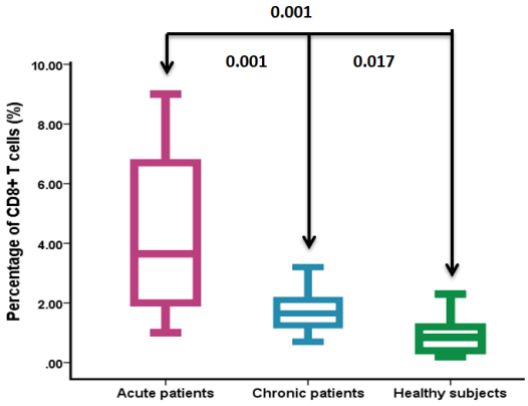


Figure 2. Proportion of PD-1 expression on $CD8^+$ T cells in peripheral blood among the various study groups.

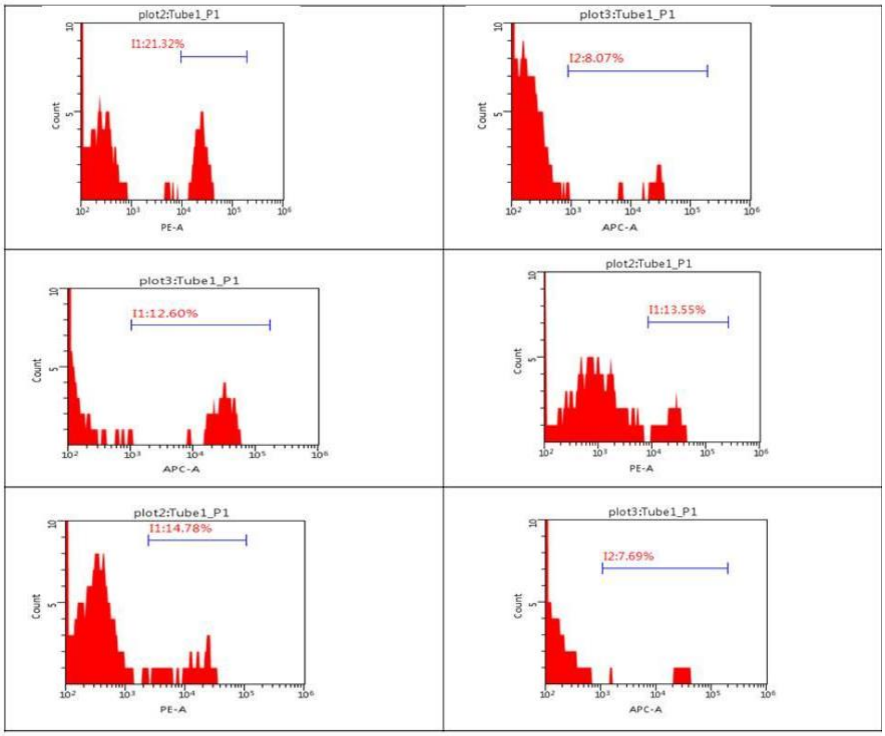


Figure 3. PD-1 expression levels on $CD4^+$ and $CD8^+$ T cells plots by flow cytometry in study groups.

Serum Levels of Transforming Growth Factor Beta (TGF-β): Serum concentrations of TGF-β were measured in patients with brucellosis and in healthy controls using ELISA. The results demonstrated that TGF-β levels were significantly elevated in both acute (2.32 ± 0.38 ng/mL) and chronic (2.15 ± 0.31 ng/mL) brucellosis patients compared with healthy controls (1.03 ± 0.28 ng/mL) ($P < 0.005$). No statistically significant difference was observed between the acute and chronic groups ($P > 0.05$) (Figure 4).

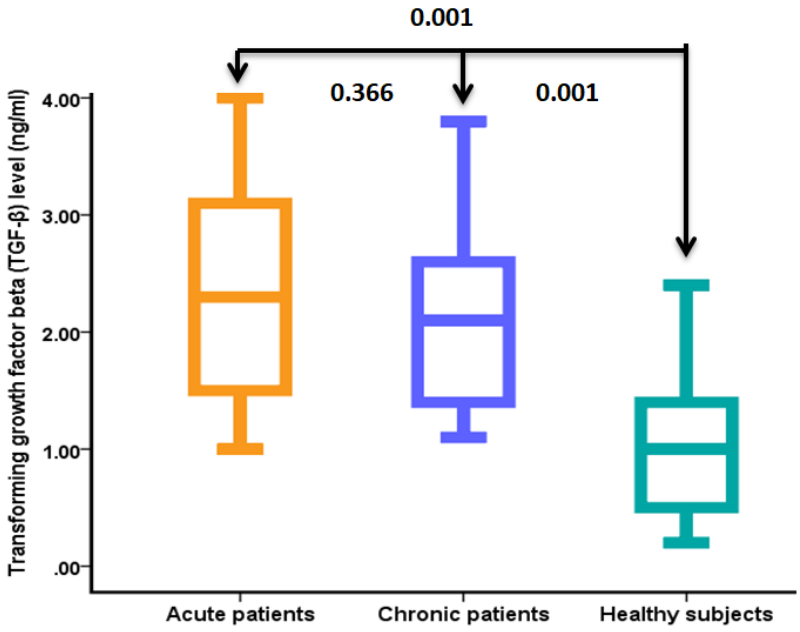


Figure 4. TGF-β cytokine level in the peripheral blood of different groups.

Correlation Between TGF-β and PD-1 Expression: In the statistical analysis, A strong positive correlation was observed between serum TGF-β levels and PD-1 expression (r = 0.99 under $P < 0.0001$). This association was consistently evident in both $CD4^+$ and $CD8^+$ T cell populations among brucellosis patients, suggesting a tightly coordinated upregulation of these two immunoregulatory markers.

DISCUSSION

The present study investigated the immunological profile of brucellosis patients by examining PD-1 expression on T cells and serum TGF-β concentrations in comparison to healthy controls. The findings discovered significant variations in studied immune markers according to disease stage and highlight the role of immune regulation in the pathogenesis of brucellosis.

Clinical and Epidemiological Characteristics: In this study, 70% of patients had a documented exposure history primarily to farming or veterinary practice. This finding is consistent with the known zoonotic transmission of brucellosis, which most commonly presents in persons in close proximity to animals or who consume unpasteurized milk products. Human infection is mostly derived from direct contact with infected animals or consumption of raw milk, especially among farmers, vets and workers handling animals in endemic areas. Most human brucellosis cases in Iran were also reported to occur in farmers and people living in rural area, with consuming of unpasteurized dairy products and close contacts with livestock considered as major risk factors [18]. In agreement with these results, 70% of patients in the present study reported a positive exposure history, mainly associated with working on livestock or veterinary work, supporting the zoonotic

route of transmission. It was also reported that eating of fresh milk or unpasteurized dairy products is one of the most important infection source in Human by Brucellosis, particularly in societies which tolerate such use [19].

The clinical presentation of the disease differed according to the stage. During the acute phase, patients predominantly presented with fever (96.7%), fatigue (86.7%), myalgia (86.7%), arthralgia (83.3%) and sweating (66.7%) indicating a systemic infection picture. In contrast, those with chronic brucellosis suffered primarily from fever (90%), fatigue (96.7%) and arthralgia 80% thus the spectrum of general inflammatory complaints shifted to localize musculo-skeletal symptoms. These results agree with the reports of [20, 21] on chronic brucellosis. Similarly, Bai et al. (2023) discussed that acute brucellosis is accompanied with systemic symptoms including fever, sweating while chronic brucellosis is marked by persistent musculoskeletal complains which include arthralgia and fatigue. [22].

Laboratory Findings: All patients were positive for ESR and agglutination tests. ESR and CRP levels tended to be higher in acute rather than chronic brucellosis cases, reflecting a stronger inflammatory response of the host at the acute stage. These are in line with the hypothesis that acute infection is characterized by an increased inflammation, whereas chronic infection might be associated with immune evasion or modulation.

In accordance with these results, Kılıç and colleagues (2024) found that there was a correlation between CRP and ESR levels with severity of acute and chronic brucellosis [23]. Also, Böncüoğlu et al. (2025) showed that patients with acute brucellosis had higher ESR and CRP levels compared to healthy subjects, indicative of an intense inflammatory response during the early phase [24]. Taken together, these results favor the notion that acute brucellosis is associated with a potent inflammatory microenvironment, while chronic forms may be characterized by phenomena of immune modulation or escape.

PD-1 Expression on T Cells: The expression level of programmed death-1 (PD-1) on $CD4^+$ and $CD8^+$ T cell in acute brucellosis, chronic brucellosis patients and healthy controls was markedly different as is evidenced by this study. In comparison with chronic patients, there were significantly higher levels of PD-1 expressions on $CD4^+$ and $CD8^+$ T cells in acute brucellosis ($P < 0.05$). This dramatic up-regulation of the acute stage may have indicated an early T cell exhaustion, possibly elicited by immunological hyperactivity caused by strong inflammatory responses that can injure host tissues during bacterial infection [25].

Elevated PD-1 expression during the early stages of infection refers to robust immune activation. As an inhibitory receptor, PD-1 is transiently expressed on T cells for 24–72 hours post-activation to limit effector T cell responses and prevent excessive inflammation and tissue damage. Therefore, PD-1 functions as a checkpoint to maintain immune homeostasis by regulating the magnitude of immune responses and preventing immunopathology [26]. However, while PD-1 upregulation serves a protective role, it may also facilitate bacterial persistence. Through acute Brucella infection, increased PD-1 expression can impair effector T cell-mediated bacterial clearance, enabling the pathogen to evade host immune surveillance [7].

The expression of PD-1 was lower in chronic brucellosis than acute cases, but significantly higher than that of the healthy controls ($P < 0.05$). This continued overexpression may, however, allow Brucella to persist in the host for prolonged periods and this form of low-level immunosupression. It serves as an inhibitory receptor, influencing active immunity and promoting immune evasion and chronic infection similar to the observed elevation in PD-1 upon chronic infection [7]. Chronicity is enhanced by the persistent expression that can be

associated with immune system dysregulation, lack of counter-regulation, and defect in T cell activation.

The same results were also found by Zheng et al. (2019) who demonstrated increased PD-1 expression on both $CD4^+$ and $CD8^+$ T cells in patients with brucellosis during the acute and chronic periods of illness. Alterations in T cell profiles and cytokines were observed with this increase that suggested a pivotal role for PD-1 in the immunopathogenesis of disease [27]. Analyses of PD1 in the context of chronic infections also lends support to a role for it in persistence [28, 29].

In the current study, PD-1 expression was highest in patients in the acute phase and significantly higher than expression levels seen in chronic cases. This finding implies that PD-1 up-regulation on peripheral T lymphocytes may be critically involved in immune regulation during Brucella infection. To date, exploring whether PD-1 blockade would act as a diagnostic biomarker or therapeutic strategy for intractable or chronic brucellosis would be the subject of further studies taking into consideration the widespread applications of PD-1 inhibitors to cancer immunotherapy [30].

In general, these results suggest that PD-1 may be a potential biomarker for brucellosis staging. Decreasing PD-1 expression from the acute to chronic phases, but still above that of controls suggests a dynamic relationship between persistent antigenic stimulation and immune controlling mechanisms during infection.

TGF-β Cytokine Levels: Serum levels of TGF-β were significantly increased in the patients of acute and chronic groups as compared to healthy controls ($P < 0.05$), while no significant difference was found between the acute and chronic group ($P > 0.05$). TGF- is an anti-inflammatory cytokine with two divergent faces, one that promotes immune tolerance and tissue repair [31] but also inhibits pathogen clearance. Its continued upregulation in both stages of disease may argue for an extended immunosuppressive environment among brucellosis patients [32].

Emerging evidence also indicates that TGF-β could be involved in immune suppression during infection. Mohammadnia-Afrouzi and Ebrahimpour (2018) have reported significantly higher serum TGF-β concentrations in patients with acute brucellosis compared to healthy controls [32]. Additionally, Sun et al. (2021) found that TGF-β1 level was elevated in patients with chronic brucellosis compared to healthy individuals. TGF-β1 might be involved in Treg-mediated immunosuppression in chronic brucellosis because these elevated levels were associated with higher frequencies of $CD4^+CD25^+$ regulatory T cells (Tregs) and CTLA-4 on activated Tregs [33]. In another investigation, TGF-β levels were also associated with a lower lymphoproliferative response to Brucella antigens in another study, and they were significantly increased in patients with chronic brucellosis compared to healthy participants. TGF-β has an inhibitory function as indicated by reversal of T cell proliferation in PBMCs following neutralization [34].

The dual significance of TGF-β in disease development is shown by the highest serum TGF-β levels found in both acute and chronic brucellosis. Although TGF-β helps control inflammation and promote tissue healing, its immunosuppressive properties may hinder the removal of pathogens, which could lead to a longer infection and related problems.

Correlation between PD-1 and TGF-β: In the statistical analysis, there was significant positive correlation between sera TGF-β and PD-1 expressions ($r = 0.99$ at $P < 0.0001$). These data indicate that Brucella may have a predetermined and orchestrated control on T cell exhaustion, actively promoting evasion from host immune surveillance. As both TGF-β and PD-1 play a central role in the induction of immune tolerance and T cell exhaustion, their simultaneously upregulation might fulfill different functions during brucellosis: **(**1) Immune

evasion strategy: The concurrent upregulation of TGF-β and PD-1 represents a strategic mechanism employed by pathogens during chronic infection. Continuous antigen exposure drives the induction of these molecules, promoting immune evasion. TGF-β signaling contributes to the generation of exhausted $CD8^+$ T cells with diminished effector functions and persistent expression of inhibitory receptors, including PD-1 [35]. Additionally, TGF-β supports the survival of PD-1^+ stem-like $CD8^+$ T cells, which maintain the T cell response while serving as a reservoir that may sustain pathogen persistence [36]. (2) Immunological signature of disease progression: Co-expression of TGF-β and PD-1 defines a distinctive immunosuppressive environment associated with disease chronicity. During prolonged inflammation, TGF-β1 can induce an immunoregulatory phenotype in macrophages by upregulating PD-1 via SMAD3/STAT3 signaling, thereby suppressing immune activation [36]. This immunological profile not only reflects chronic infection but also indicates immune dysfunction and exhaustion, similar to mechanisms exploited by tumors to evade immune surveillance. (3) Potential dual therapeutic target: Modulating both TGF-β and PD-1 pathways could restore immune competence and enhance host defense. Preclinical studies have shown that bispecific inhibitors targeting TGF-β and PD-1/PD-L1 can reinvigorate $CD8^+$ T and NK cell activity, reduce regulatory T cell expansion, and promote a favorable immune environment [37]. Clinically, bifunctional agents such as bintrafusp alfa, which combine TGF-β sequestration with PD-L1 blockade, have demonstrated promising antitumor immunity and are being explored in multiple cancer types [38].

Mechanistic studies indicate that TGF-β1 can directly enhance PD-1 expression on T cells via Smad3-dependent transcriptional activation. Although these studies were conducted in cancer models [39], similar pathways may be relevant in chronic infections such as brucellosis, where elevated TGF-β1 levels could contribute to PD-1-mediated T cell exhaustion, facilitating pathogen persistence.

Emerging evidence also suggests that nutritional interventions and functional foods may complement immune-based strategies to enhance host defense in chronic infections [40–43-44-45]. Bioactive dietary compounds can modulate cytokine production and immune checkpoints, including PD-1, offering potential adjunctive approaches to mitigate immune dysfunction during persistent infections such as brucellosis. Integrating immunological and dietary strategies could thus complement conventional therapies and improve outcomes in chronic infectious diseases.

Importantly, this study provides novel insights into immune evasion in brucellosis, emphasizing the strong correlation between PD-1 expression and TGF-β levels as potential biomarkers and therapeutic targets. These findings enhance our understanding of host–pathogen interactions and offer promising avenues for restoring immune function in chronic infections.

Limitations: Although the work provides valuable preliminary information, there are some limitations. The relatively small sample, even if adequate for an exploratory study, might limit generalizations, which would need to be replicated in larger and more heterogeneous sample. Furthermore, there were no functional assays (such as proliferation assays or cytokine gene expression) to clearly indicate the immunosuppressive effect of TGF-β and PD-1 on T cell activity.

Clinical Significance: The clinical implications of this study are noteworthy. The expression of PD-1 and elevated TGF-β levels may serve as candidate immune markers for evaluating disease stage and progression in patients with brucellosis. Therapeutic targeting of the PD-1/TGF-β pathway could represent a novel strategy to restore immune competence, particularly in chronic or treatment-resistant cases. Moreover, early detection and

monitoring of these markers may help identify patients at greater risk of developing chronic infection or immune-mediated complications, thereby supporting prognosis and guiding clinical management.

CONCLUSIONS

Our findings suggest that the immunomodulation of PD-1 expression, in combination with elevated TGF-β levels, plays a role in the immunopathogenesis of brucellosis. The observed upregulation of PD-1 on $CD4^+$ and $CD8^+$ T cells during the acute phase aligns with patterns of T cell exhaustion commonly associated with active infection. Although PD-1 expression was comparatively lower during the chronic phase, it remained elevated relative to healthy controls, indicating a persistent immunosuppressive state.

Additionally, the sustained elevation of serum TGF-β levels in both acute and chronic cases reflects the presence of an immunoregulatory environment that may facilitate Brucella persistence. The observed correlation between TGF-β and PD-1 expression further suggests a potential interactive regulatory axis contributing to immunosuppression.

Collectively, these observations highlight PD-1 and TGF-β as relevant biomarkers of immune dysregulation in brucellosis and suggest that modulation of this pathway could hold therapeutic promise. Nevertheless, future longitudinal and mechanistic studies are required to clarify their precise role in disease progression and immune regulation.

DECLARATIONS

Abbreviations: PD-1: Programmed death-1; TGF-β: Transforming Growth Factor Beta; $CD4^+$ T cells: Cluster of Differentiation 4 positive T lymphocytes; $CD8^+$ T cells: Cluster of Differentiation 8 positive T lymphocytes; Tregs: Regulatory T cells;IL-10: Interleukin 10; IL-35: Interleukin 35; ELISA: Enzyme-Linked Immunosorbent Assay; LPS: Lipopolysaccharide; PBMCs: Peripheral Blood Mononuclear Cells; SMAD3: Mothers Against Decapentaplegic Homolog 3 (signaling molecule); STAT3: Signal Transducer and Activator of Transcription 3; CRP: C-reactive protein; ESR: Erythrocyte Sedimentation Rate; SPSS: Statistical Package for the Social Sciences; ANOVA: Analysis of Variance; WHO: World Health Organization; HRP: Horseradish Peroxidase; APC: Allophycocyanin (fluorescent label); PE: Phycoerythrin (fluorescent label)

Availability of Data and Materials: Any competent researcher will have unrestricted access to the raw data used to support the results of this work from the authors.

Competing Interests: The authors declare that they have no conflicts of interest related to this article.

Authors' Contributions: All authors contributed to the study design and conceptualization, data interpretation, manuscript drafting and revision, and approved the final version of the article.

Acknowledgments: The authors would like to express their gratitude to all participants in this study, particularly those who contributed voluntarily.

Ethical Approval and Consent to Participate: Written informed consent was obtained from all patients prior to participation. For participants under the age of 16 years, informed consent was obtained from their parents. The Ethics Committee of Hammurabi College of Medicine, University of Babylon, Iraq, approved the study protocol (Approval number: 39/2023). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Guidelines on Human Research.

Consent to Publication: All authors have read and approved the final version of the manuscript and consent to its submission and publication in this journal.

Clinical Trial Number: Not applicable.

Funding: Not applicable.

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