AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Detection of Prostate specific antigen and LDH in *Toxoplsama gondii* infected prostate Tissue using immunohistochemical and molecular techniques

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Article history
Received: 3/1/2026
Revised: 15/2/2026
Accepted: 28/2/2026

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Abstract: *Toxoplasma gondii* is a highly prevalent single-celled protozoan parasite that is estimated to infect billions of people worldwide, making it one of the most common human pathogens. Although humans are considered intermediate hosts,meaning the parasite cannot complete its full sexual life cycle, humans acquire the infection primarily through.
Mehods: A total of 100 paraffin embedded blocks containing fine needle aspiration tissues from patients suspected with prostatitis as per the histopathological report. A total of 50 paraffin embedded blocks containing healthy prostate tissues as confirmed by the histopathologist were used as control tissues. All samples underwent routine histological examination using hematoxylin and eosin (H&E) staining to assess overall tissue structure. Immunohistochemical (IHC) assays were performed to detect Prostate specific antigens and lactate dehydrogenase . Quantitative polymerase chain reaction (qRT-PCR) was used to assess the gene expression levels of PSA and LDH gene.
Results: the results of this study showed weak Immunohistochemical staining for Anti prostate specific antigens and Positive Immunohistochemical staining for anti LDH in *T. gondii* infected prostate tissue and elevated molecular gene expression of the PSA and LDH gene compared to control tissues.
Conclusion: the aim of this study is evaluate the pathogenic effect of *T. gondii* on prostate tissues in patients clinicaly diagnoses with Prostatitis.

Keywords: prostatitis, IHC, RT-qPCR, PSA, LDH, Najaf, Iraq.

1. Introduction

Toxoplasma gondii is the most widespread member of the Apicomplexa , causative agent of toxoplasmosis. It can infect humans as well as any warm-blooded animal and has a wide host range[1]. With significant variations between regions, the parasite is estimated to infect 25% of the world's population[2]. Ingestion of sporulated oocysts or raw or undercooked meat with tissue cysts are the main ways that transmission happens[1]. A complex equilibrium between the host immune response and parasite virulence factors determines the clinical outcome of toxoplasmosis[3].Invasion is a complex process that includes host cell contact, gliding movement, moving junctions (MJ), and the creation of the parasitophorous vacuolar membrane [4].The histological composition of the human prostate gland mostly comprises glandular epithelium and fibromuscular stroma. The epithelium has an only of three cell types: columnar luminal epithelial cells, basal cells, and neuroendocrine cells, whereas the stroma consists of many smooth muscle cells, fibroblasts, blood vessels, and nerves [5]. The human prostate is a walnut-

DOI: 10.36320/ajb/v18.i1.22842

sized gland located at the base of the urinary bladder [6].Inflammation is an immunological reaction to a number of things, including infections, toxic substances, and damaged cell components. The word inflammation was derived from the Latin word "inflammo [7]. Prostate inflammation, commonly referred to as prostatitis, is among the most prevalent conditions affecting men's prostates[8].Immunohistochemical analysis utilizes monoclonal or polyclonal antibodies to identify specific antigens in tissue samples. This technique is extensively utilized in various contexts, including cellular differentiation, characterization of a tumor's primary location, identifying metastases, assessment of prognostic factors, prediction of targeted therapy response, and identification of structures, organisms, and materials secreted by relevant cells [9]. Among the most important molecular technologies ever developed, the polymerase chain reaction (PCR), quantitative PCR (qPCR), and reverse transcription quantitative PCR (RT-qPCR) have had a significant influence on biological, medicinal, veterinary, and agricultural research [10]. kallikrein-related peptidase 3 (KLK3), also known as prostate-specific antigen "PSA" [11]. Prostate-specific antigen (PSA) is an enzyme encoded by the KLK3 gene. PSA levels may increase because of prostatic infection, inflammation, benign prostatic hyperplasia, advanced age, and increased prostate size[12]. The human PSA gene is on chromosome 19 [13]. Prostate-specific antigen (PSA) is one of the several substances secreted into the lumen by specialized cells called luminal cells [14]. (PSA) belongs to the kallikrein-related peptidase family and is released by the epithelial cells of the prostate gland. PSA is a trypsin-like serine protease that is part of a family of associated genes known as kallikrein-related peptidases. The family comprises 15 members, including 12 that are trypsin-like and 3 that are chymotrypsin-like, It helps sperm motility and liquefy ejaculate [15]. Lactate dehydrogenase (LDH) is a crucial enzyme in the anaerobic metabolic cycle. It is classified as an oxidoreductase. The enzyme catalyzes the reversible transformation of lactate to pyruvate, concurrently reducing (NAD+ to NADH) and vice versa. LDH is an enzyme found in nearly all bodily tissues. Two genes, (LDHA and LDHB), translate into the isozyme forms of lactate dehydrogenase enzymes, LDH-1 to LDH-5. The five main LDH isoenzymes (LDH-1 to LDH-5) each have unique catalytic characteristics [16].

2. Methodology

Study Design and Patients

A total of 100 paraffin embedded blocks containing fine needle aspiration tissues from prostatitis patients suspected with T. gondii as per the histopathological report between the periods of January and November 2025 from histopathology laboratories in Al-Najaf Al-Ashraf, Iraq. A total of 50 paraffin embedded blocks containing healthy prostate tissues as confirmed by the histopathologists were used as control tissues. Sectioning by rotary microtome (5µm thick sections). The tissues' sections were stained with hematoxylin and eosin.

Immunohistochemistry of anti-Toxoplasma gondii

Anti prostate specific antibodies were purchased from (BIO SB) was carried out using manufacturer protocol. Paraffin blocks tissues were sectioned and processed accordingly for H and E and immunohistochemistry. Charged Slides were marked with hydrophilic pen and Antibodies were applied at a dilution of 1:50 and incubated for 2 hours at room temperature. Slides were then washed with PBS 3 times for 2 minutes. Reagent 1 Bio SB (polymer helper) was applied to the slides and incubated for 20 minutes at room temperature and then washed with PBS 3 times for 2 minutes. Reagent 2 Bio SB (polyperioxidase-anti-mouse/ rabbit IgG) is then added and slides were incubated at room temperature for 20 minutes and slides were washed with PBS 3 times for 2 minutes. Slide colour development was carried out using DAB (3,3 diaminobenzidine) and slides were washed with deionized water. Slides were counterstained using haematoxylin stain. Mounting medium was applied before a cover slip was attached to each slide. Negative control slides received no primary antibody.

Immunohistochemical score

Positive stained cells were counted using image j, image processing software to identify and count the number of positively stained cells. Positive cells were counted and intensity of staining recorded. Each immunohistochemical slide was examined for 10 fields at a magnification of 100 X and mean data was recorded. Immuno reactive score (IRS) was worked out according to the following table and represented by plus system according to [20].

DOI: 10.36320/ajb/v18.i1.22842

Quantitative real-time polymerase chain reaction

The reaction was achieved using primers for PSA and LDHA genes (purchased from IDT). RNA was extracted from paraffin embedded tissues. The quantity and quality of RNA was evaluated using a Nanodrop 1000 Spectrophotometer (Thermo Fischer Scientific) and an Agilent 2100 Bioanalyzer Piimers for PSA F: 5-ACACAGGCCAGGTATTTCAG-3 R: 5-GTCCAGCGTCCAGCACACAG-3′ and LDHA F: 5-GGATCTCCAACATGGCAGCCTT-3 R: 5-AGACGGCTTTCTCCCTCTTGCT-3 were purchased and their sequence were optimized prior to analysis.

Statistical analysis

Each histological slide was photographed 10 times to assess the IHC intensity. Data were analyzed by comparing differences between values using a one -way analysis of variance (ANOVA) parametric test to determine the statistical significance. Value was considered significant at p ≤0.05. All those statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

The results of this study showed Weak positive Immunohistochemical staining for Anti prostate specific antigen as in Figure (1).Staining intensity of anti-prostate specific antigen antibody in control healthy tissue shows weak staining while negatively (no primary antibody) stained tissue shows moderate intensity higher than the healthy tissue. Significantly higher staining intensity is seen in positively stained prostate tissue as in Figure (2).

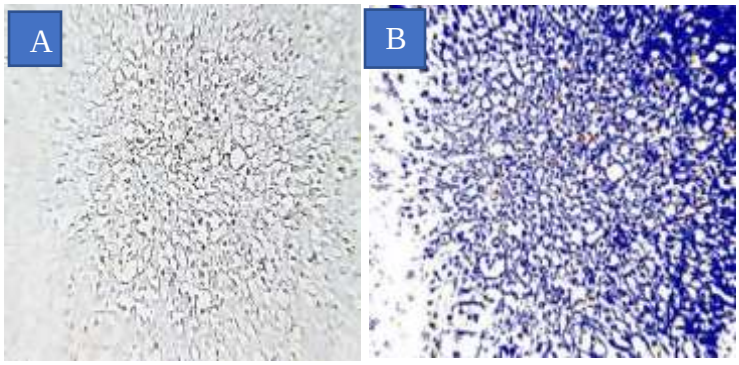

Fig1: Weak positive Immunohistochemical staining for Anti prostate specific antigen (A) brown prostate tissue. Immunoreactive analysis of Anti prostate specific antigen (B) showing low positively stained cells for PSA (yellow) surrounded by a large number of negatively stained cells (blue) 400X.

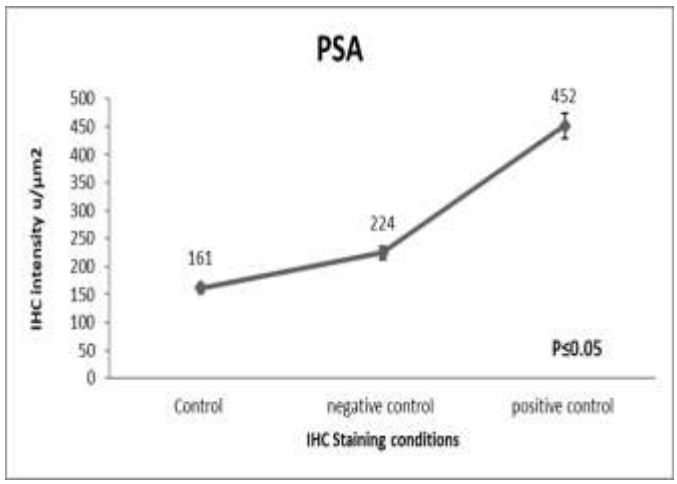

Fig 2: Staining intensity of anti-prostate specific antigen antibody in control healthy tissue shows weak staining while negatively (no primary antibody) stained tissue infected with *Toxoplasma gondii* shows moderate intensity higher than the healthy tissue. Significantly higher staining intensity is seen in positively stained prostate tissue. * P≤ 0.05.

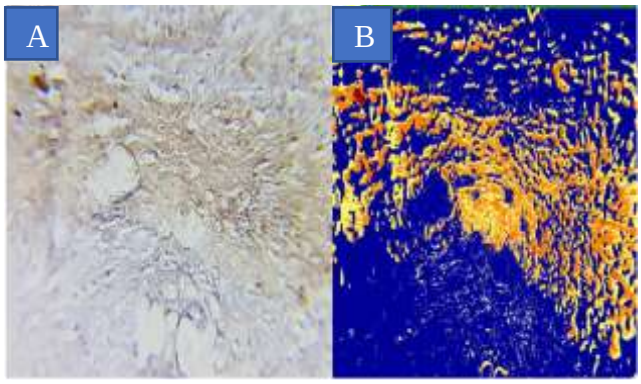

Fig 3: Positive Immunohistochemical staining for anti LDH (A) in Toxoplasma gondii infected prostate tissue indicated by brown stain. Immunoreactive analysis of Anti LDH receptor showing high positive staining for LDH receptor (yellow) surrounded by blue negatively stained cells 400X.

DOI: 10.36320/ajb/v18.i1.22842

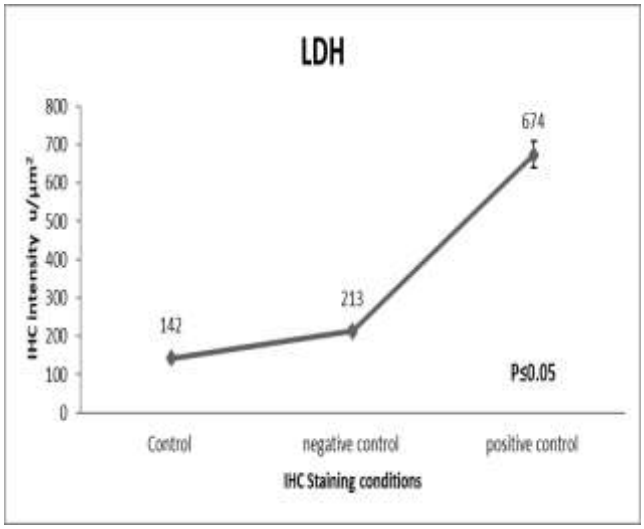

Fig 4: Staining intensity of anti-lactate dehydrogenase antibody in control healthy tissue shows weak staining while negatively (no primary antibody) stained tissue infected with *Toxoplasma gondii* shows moderate intensity higher than the healthy tissue. Significantly higher staining intensity is seen in positively stained prostate tissue. * P≤ 0.05.

The study results indicate a clear difference in the intensity of immunohistochemical staining for prostate-specific antigen (PSA) among the different groups of prostate tissue (healthy tissue, tissue infected with T. gondii, and negatively stained tissue (without primary antibodies infected with *T. gondii*).

Weak staining in healthy tissue (Controls): The appearance of weak PSA staining in healthy tissue is expected, because normal prostate cells consistently express PSA but at a relatively low level in the absence of any inflammatory or pathological stimulation. Normal glandular cells in the prostate produce PSA physiologically [17]. In this case, the distribution of PSA is diffuse and homogeneous. The staining intensity observed in tissues infected with *T. gondii* that are negatively stained (without primary antibodies) is moderately higher than in healthy tissues this is possibly due to the increased inflammatory proteins in toxoplasmosis. These proteins may bind to a chromogen or secondary antibody and show higher staining even without a primary antibody. High staining in positively stained tissues (Positive PSA staining),This result is logical and expected. these may be When the primary PSA antibody is added, the remaining glandular cells in the prostate tissue, even if inflamed will show higher PSA expression and Inflammation (such as Toxoplasmosis) may increase membrane permeability, exposure to antigens, and stimulation of glandular cells, leading to increased staining intensity compared to healthy tissues [18].

The results of this study showed agree with [19] in which Prostatitis is a benign illness most commonly found in individuals with elevated (PSA) levels.(PSA) was postulated as a self-antigen that stimulates the inflammatory process in the prostate. The results of this study showed also agree with [20] in which increased (PSA) levels frequently occur in benign prostatic diseases. Examples include benign prostatic hyperplasia, acute prostatitis, chronic prostatitis and prostate abscess.

The results of this study showed Positive Immunohistochemical staining for anti LDH in *T. gondii* infected prostate tissue as in Figure (3). Staining intensity of anti-lactate dehydrogenase antibody in control healthy tissue shows weak staining while negatively (no primary antibody), stained tissue infected with *T. gondii* shows moderate intensity higher than the healthy tissue. Significantly higher staining intensity is seen in positively stained prostate tissue as in Figure(4). LDH in healthy tissues results in a weak dye intensity. This weak intensity means that the healthy cells in the prostate are producing basal and normal levels of the enzyme, sufficient for their normal metabolic functions. LDH in infected tissue (without primary antibodies) results in a moderate staining intensity, higher than in healthy tissue. This suggests a likely cause: the parasitic infection is causing inflammation and tissue damage. This intensity, higher than in healthy tissue, most likely reflects a nonspecific reaction resulting from the inflammation and tissue damage caused by the infection. LDH in infected tissues (positive staining) The result showed a significantly higher staining intensity compared to all other groups. This means that this large and specific increase proves that Toxoplasmosis infection leads to a real and significant increase in the amount of LDH enzyme within infected prostate tissues.

This increase in staining may be due to inflammation, hypoxia, and activation of metabolic shift (shift toward glycolysis) lead to increased LDH expression in host cells. This is observed in many

DOI: 10.36320/ajb/v18.i1.22842

infections and chronic inflammations and is explained by increased cellular energy requirements and lactate loading[21]. LDH staining is higher in prostate tissue infected with Toxoplasmosis compared to control tissue , this can be explained by a true increase in LDH expression (from infected cells or surrounding cells as an inflammatory response[22,23].

The reason for the higher staining intensity in infected tissue is that the parasite induces a metabolic pattern in the host cell or the parasite itself that requires more LDH (due to increased anaerobic metabolism/lactate conversion) leading to greater accumulation or expression of the enzyme or proteins detected by immunostaining or Immune cells (when activated or in the context of infection) alter metabolic pathways such as increasing glycolysis or changing energy utilization as needed [24,25]. Alternatively, the infection may induce histological changes (inflammation, hyperactivation of the cellular response, metabolic alterations) that cause host cells to express more (LDH) or decrease enzyme clearance or alter enzyme distribution within the cell, resulting in greater staining intensity [26].Elevated LDH levels are also associated with inflammatory diseases and severe infections, indicating tissue damage [27].

These results agree with [28], in which (LDH) is confirmed to be higher in prostate tissue infected with T. gondii, this may indicate that the infection actually affects tissue metabolism or cellular response in the prostate, and may be associated with histological changes that lead to prostate diseases such as enlargement or even precancerous changes.

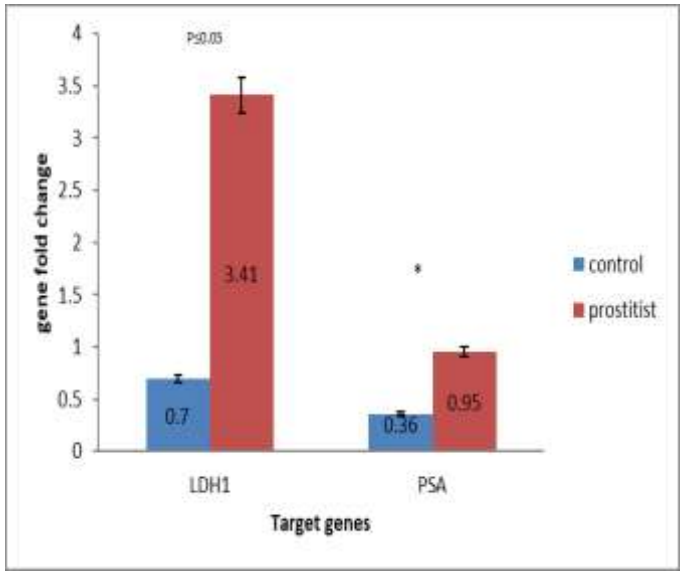

Fig 5: The gene expression data indicates a clear biological response in the prostititis group, characterized by a significant increase in the established marker LDHA and PSA. This pattern suggests a shift in cellular activity and metabolism within the affected tissue.* P ≤0.05.

The gene expression data indicates a clear biological response in the prostititis group, characterized by a significant increase in the established marker PSA. The presence of a high high PSA forms a consistent picture: elevated PSA gene levels in expression data as in Figure(5) indicate activation of prostatic luminal/epithelial cells or disruption of their barrier function. PSA is not an exclusive marker of cancer,it can be locally elevated in cases of benign prostatic hyperplasia, prostatitis, or when glandular irritation/damage occurs. Therefore, a modified PSA level suggests a glandular/inflammatory response in the prostate [29]. The results of current study agree with [30] in which From a clinical standpoint, (PSA) is frequently employed as a biomarker for prostate conditions, such as prostatitis and benign prostatic hyperplasia (BPH). The results of current study agree with [29] Those who found that the gene expression of the KLK3 gene is very high in those with benign prostatic hyperplasia, which may indicate that this gene is not specific to cancer.

Increased expression of the LDH 1 gene as in Figure (5). indicates a shift in cellular metabolism within inflamed prostate tissue. Cells (both prostate cells and invading immune cells) switch to anaerobic glycolysis to

rapidly produce energy. This shift is a natural response to inflammatory conditions that include oxygen deficiency and the need for rapid energy production. (Increased conversion of pyruvate to lactate) This is a well-known pattern in inflamed and transformed cells (as well as in tumor tissues), and indicates a rapid increase in energy demand or a change in the tissue environment (e.g., hypoxia or an inflammatory response [31]. The results of a current study agree [23]), who reported the explanation for the increased (LDH1) gene expression, metabolic shift, and inflammatory response lies in the fact that intracellular parasites, such as *T. gondii*, reprogram the host cell's metabolism to secure energy resources and vital structures.

Conclusion

Based on the results of IHC and RT-qPCR techniques for the PSA and LDH gene from patients with prostatitis showed significantly higher expression compared to healthy individuals. This increase suggests a link between inflammation and the stimulation of PSA expression and LDH at both the molecular and protein levels within prostate tissue.

Acknowledgement

All hospitals that provided efforts and facilities in the city of Najaf, Iraq.

Funding Information

This research was supported through the researchers' self-funding.

Author's Contributions

First Author: Professor Dr. Saleem Khteer Al-Hadraawy conceived the research idea and wrote the scientific plan.

Second Author: Assistant Professor Dr. Hydar Muhsin Khalfa designed the experiments and analyzed the data.

Third Author: Assistant Lecturer Wisam Aqeel Muslim conducted the experimental work.

Ethics

This study was conducted with the approval of the Medical Ethics Committee at the University of Kufa, 2025.

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