Assessment of risk factors and biomarkers of prostate disease

MARWA M. AL-ATTAR[1], OLA AMER JASIM[1,2] and ISRAA ALI ABOOD[3]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad 10052, Iraq; [2]College of Science, Al-Nahrain University, Baghdad 10070, Iraq; [3]Department of Microbiology, College of Science, Mustansiriyah University, Baghdad 10052, Iraq

Received December 3, 2025; Accepted July 23, 2026

DOI: 10.3892/wasj.2026.500

Abstract. Prostate diseases, including prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer (PCa), are among the most critical health issues among the older male population. Chronic inflammation, along with smoking and metabolic diseases such as diabetes, may contribute to the overall disease burden. The present study examined clinical risk factors and the levels of prostate-specific antigen (PSA), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), zinc, vitamin D3, ferritin, renal markers and select cytokines (IL-6, IL-8, IL-37, growth differentiation factor 15 and TGF-β) in the serum of male patients with different prostate diseases. The present study included 180 male patients: 135 patients with a prior diagnosis of PCa, BPH, or prostatitis (45 men per disease) and 45 healthy controls. Diabetes and a positive smoking status were prevalent among the patient groups compared to the healthy controls, with the highest prevalence of diabetes found in the PCa group (91.1%). Compared to the healthy controls, all patient groups had elevated levels of PSA, ESR, CRP, ferritin, urea and creatinine, and also had elevated levels of inflammatory cytokines, along with reduced levels of vitamin D3 and zinc. Among this group, patients with PCa had the highest levels of IL-6, IL-8, TGF-β and IL-37. While these preliminary findings suggest associations between prostate disease, and inflammatory and metabolic biomarker patterns, the limited sample size, single-center study and case-control nature of the present study suggest a degree of caution for interpretation. The proposed patterns of biomarkers are preliminary and require larger independent studies to confirm utility prior to probable, potential, or context of use for diagnosis or prognosis in clinical practice.

Introduction

The prostate is a male reproductive organ. It is approximately the dimension of a walnut and can be found near the base of the bladder. Prostatitis, benign prostate hyperplasia (BPH) and prostate cancer (PCa) are the three most frequent types of prostate diseases. Of note, ~25% of men aged ≥55 years have a prostate-related issues; the prevalence of such issues increases to >50% by the age of 70 years (1). The early stages of prostate disease may not exhibit any symptoms (1).

Prostatitis is an inflammation of the prostate gland caused by exposure to potentially harmful microorganisms. Chronic infection is considered to play a crucial role in the formation and development of BPH or PCa via oxidative stress and the generation of reactive oxygen species that cause mutations, or by inducing epigenetic modifications that promoting the transformation of neoplastic cells (2-4). PCa is the second most common type of cancer in terms of occurrence and fifth in terms of mortality globally, and it is notable for its geographical variability (5). Prostatitis and PCa are both major health concerns and a strong association has been demonstrated between the two conditions (6). Nicotine from cigarettes exacerbates prostate diseases and significantly increases the chance of developing urinary tract infections and enhances the dysfunction of the urinary system. Terminating smoking can reduce chronic prostatic inflammation and dysfunctional storage (7). There are several potential mechanisms of cigarette smoking for maximizing the prevalence and incidence rates of prostate diseases. Cigarette smoking alters the hormonal equilibrium, increases bioavailable testosterone and decreases bioavailable estradiol levels (8). Cigarettes have significant quantities of cadmium, which is considered an effective carcinogen related to cancer progression (9,10).

Furthermore, diabetes markedly increases the incidence of BPH and PCa, indicating that glucose metabolism is critical in the evolution of cancer of the prostate. Individuals with type 2 diabetes have been found to have larger prostate glands. Insulin and related factors stimulate prostate growth, changes in the expression of sexual hormones, the initiation of systemic inflammatory processes and oxidative stress (11,12).

A large family of low-molecular-weight proteins, known as cytokines, mediates cell-to-cell communications. They display complex roles in inflammation, tumor immunobiology, host defense, immunity and tumor pathogenesis (13). Interleukins (ILs), interferons, colony-stimulating factors, chemokines and tumor necrosis elements are among the primary subgroups of cytokines, and they are produced as membrane-bound or released proteins (14). Several recent discoveries support the essential role that IL-6 plays in the genesis of PCa. IL-6 has the ability to stimulate both pro- and anti-inflammatory

Correspondence to: Dr Marwa M. Al-Attar, Department of Biology, College of Science, Mustansiriyah University, North Street 13, Baghdad 10052, Iraq
E-mail: mar_alattar@uomustansiriyah.edu.iq

Key words: cytokines, prostate diseases, prostatitis, benign prostatic hyperplasia, prostate cancer, risk factors

responses, interact with a range of cell types, exert both autocrine and paracrine actions in the prostate, and trigger intracellular signals that mediate several biological responses in PCa (15). IL-6 mediates numerous crucial physiological processes, including controlling the acute-phase response at the onset of acute inflammation, regulating B-cell and T-cell differentiation and activation, and promoting cell proliferation and survival (16). Neutrophils, and endothelial and epithelial cells release the pro-inflammatory chemokine, IL-8, which is expressed in cancer cells and functions as both a chemoattractant and activator for granulocytes, thereby promoting cell proliferation, invasion, survival and chemoresistance. Its levels are elevated in the serum of men with PCa and has been related to adverse effects (17). IL-37 exerts both anti-inflammatory and immunomodulatory effects. According to previous research, it can reduce the inflammatory response in several disorders (18). IL-37 is a protein that is produced by a variety of tumor cells, as well as tissues and cells that are healthy in the body. It may reduce a host's immune response to malignancy and accelerate disease progression. However, several studies have uncovered its anticancer and host-protective properties; this cytokine may serve as both a crucial prognostic biomarker and a cutting-edge treatment option for a range of malignancies (19).

Members of the transforming growth factor (TGF)-β family play vital roles in regulating the differentiation, growth arrest and apoptosis of healthy epithelial cells. However, they can also promote cancer, depending on the stage and type of tumor. The signaling mechanism of TGF in cancer can serve as either a carcinogenic or tumor-suppressive mechanism. In the initial stages, it attenuates cell development as a tumor suppressor. However, in the later stages, it promotes invasion and metastasis. TGF-β signaling promotes bone metastasis in PCa (20,21).

The aim of the present study was to analyze critical clinical risk factors and blood levels of routine diagnostic markers [prostate-specific antigen (PSA), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), zinc, vitamin D3 and renal function] and inflammatory cytokines (IL-6, IL-8, IL-37 and TGF-β) in Iraqi male patients with various prostate disorders.

Subjects and methods

Study subjects. The present case-control study included 180 male subjects aged from 45 to 85 years. The patient group (n=135) included patients with prostate conditions. It was further classified into three groups with an equal number of patients, as follows: PCa (n=45), BPH (n=45) and prostatitis (n=45). For comparison, a healthy control group (n=45) was included which matched the patient groups in age range. A standardized demographic and clinical data collection sheet was utilized. The sheet captured data about patient identification code, age, medical history, smoking history, diabetes mellitus status, the use of antidiabetic medications, such as metformin, and other pertinent clinical details. The data collection process involved direct patient interviews and a comprehensive review of medical records at Al-Yarmouk Teaching Hospital, Baghdad, Iraq and from the period between January, 2024 to June, 2024.

Inclusion and exclusion criteria. The inclusion criteria defined participants aged between 45-85 years with a urologist-confirmed diagnosis of prostatitis and/or BPH/lower urinary tract symptoms (LUTS)/PCa. The exclusion criteria included those with any other types of cancer, autoimmune disease, any renal or thyroid disease, congenital heart disease, incomplete clinical and/or laboratory records, and the absence of confirmed prostate disease. The controls were men within the same age range, with no history or clinical evidence of any prostate disease, with low/normal PSA levels and no diabetes mellitus.

Diagnostic criteria for prostate diseases. Urology specialists at Al-Yarmouk Teaching Hospital made the patient diagnoses. Confirmed PCa diagnoses were made via the official guidelines on the diagnosis of PCa that detail the need for prostate biopsies to undergo histopathological assessments (22). Prostate enlargement due to BPH was assumed based on LUTS along the American Urological Association (AUA) recommendations for the assessment of LUTS/BPH, which involves taking a patient history, performing a digital rectal examination (DRE), obtaining a prostate ultrasound and a PSA assessment (23). Prostatitis was diagnosed based on the symptoms, a urine culture (if applicable), and the presence of inflammation along the European Association of Urology (EAU) guidelines on urological infections and male accessory gland infections (24). The healthy controls had no history of prostate diseases and/or had no symptoms of prostate disease, and had low and/or normal levels of PSA. Diabetes mellitus was treated as a clinical risk factor and diagnosed based on a fasting blood glucose level of at least 126 mg/dl (or) the current use of antidiabetic medications. Due to the effect of inflammation and metabolism, the use of metformin was documented as a treatment variable and analyses were carried out separately.

Sample size calculation. The sample size was determined using G*Power (version 3.1). For a one-way ANOVA with four groups, assuming a medium effect size (f=0.25), an alpha error probability of 0.05, and a statistical power of 80%, the estimated minimum total sample size was 180 participants, or ~45 per group.

Ethical approval. The study protocol was approved by the Research Ethics Committee of the College of Science, Mustansiriyah University, Baghdad, Iraq (Approval no. BC SMU/3024/00081M; January, 2024). In accordance with the approved protocol and within the framework of academic collaboration with Mustansiriyah University, blood samples and clinical data were collected from Al-Yarmouk Teaching Hospital in Baghdad. The treating medical team and hospital administration provided the records and clinical samples after obtaining the necessary ethical approvals (Approval no. 9274; December, 2023) and written informed consent from all participants.

Sample collection and preparation. The collection of blood samples from all individuals was divided into two parts: A total of 2 ml was added into EDTA tube for hematological tests [ESR, complete blood count (CBC) and CRP], and 3 ml

Table I. Demographic characteristics of the study subjects in the different groups.

Characteristic	Control (n=45)	Prostatitis (n=45)	BPH (n=45)	PCa (n=45)	P-value
Age range, years	65.30±0.98	68.04±1.08	66.17±0.55	64.93±1.06	0.086
Smokers, n (%)	1 (2.2)	41 (91.1)	43 (95.6)	29 (64.4)	<0.001
Non-smokers, n (%)	44 (97.8)	4 (8.9)	2 (4.4)	16 (35.6)	
Diabetic, n (%)	0 (0.0)	31 (68.9)	37 (82.2)	41 (91.1)	<0.001
Non-diabetic, n (%)	45 (100.0)	14 (31.1)	8 (17.8)	4 (8.9)	

Adjusted Tukey HSD pairwise P-values for the continuous variable are provided in Table SII. BPH, benign prostatic hyperplasia; PCa, prostate cancer.

were used to obtain serum, which was used for the estimation of serum level of diagnostic biochemical and immunological parameters (the serum was stored at -20˚C until use).

Laboratory measurements. As part of the routine clinical work-up of the patients at Al-Yarmouk Teaching Hospital, hematological and biochemical parameters [ESR, CBC, CRP, PSA, fasting blood sugar (FBS), hemoglobin A1c (HbA1c), ferritin, urea, creatinine, serum zinc and vitamin D3 levels] were collected in accordance with the data-collection permit of the hospital and are available retrospectively as clinical data. The research team then measured the serum cytokine levels of IL-6 (ID: SL1001Hu), IL-8 (ID: SL1004Hu), IL-37(ID: SL2231Hu), growth differentiation factor 15 (GDF-15) (ID: EL0270Hu) and TGF-β (ID: EL0003Mt) using enzyme-linked immunosorbent assay (ELISA) kits in the laboratories of the Department of Biology, College of Science, Mustansiriyah University. Kits were sold and shipped from Sunglong Biotech and, as per the manufacturer's instructions, measurements were taken using a microplate reader (BioRad Laboratories, Inc.) that detects absorbance.

Statistical analysis. IBM SPSS Statistics Version 27 was used for statistical analysis. Data are expressed as the mean ± standard error (SE). Data distribution was evaluated using the Shapiro-Wilk test for normality. One-way analysis of variance (ANOVA) was used to evaluate differences, and pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test. Categorical variables were evaluated using Pearson's Chi-squared test. Cytokine and clinical biomarker correlation/s within the disease groups of PCa, BPH and prostatitis were evaluated using Spearman's correlation analysis. The discriminatory performance of biomarkers within the present study cohort was evaluated using receiver operating characteristic (ROC) analysis. It was not intended to assess the clinical diagnostic validity of the biomarkers. A P-value ≤0.05 was considered to indicate a statistically significant difference. The Benjamini-Hochberg false discovery rate (FDR) correction was applied to the omnibus biomarker p-values. In this study, type I error was regarded as P≤0.05 (25). The effect size was calculated to provide better interpretation of the results. For one-way ANOVA, effect size was reported as eta squared (η^2), and for multinomial logistic regression, adjusted odds ratios were reported along with 95% confidence intervals (CIs). For ROC analysis, an area under the curve (AUC) along with 95% CI values are reported. When relevant, 95% CI values for group means were calculated.

Results

Patient demographic characteristics and risk factors. The demographic profile of the patients in the present study is presented in Table I. Each group included 45 participants and was matched within the same age range (45-85 years). Smoking and diabetes mellitus were significantly more frequent among patients with prostate diseases than among the healthy controls (P<0.001 for both). The highest frequency of smoking was detected in the BPH group (43/45, 95.6%) followed by prostatitis (41/45, 91.1%) and PCa (29/45, 64.4%) groups. Diabetes mellitus was most prevalent in the PCa group (41/45, 91.1%), followed by the BPH (37/45, 82.2%) and prostatitis (31/45, 68.9%) groups, whereas no cases of diabetes were recorded in the healthy control group. A multivariable multinomial logistic regression model was performed using the control group as the reference category. The overall model was statistically significant (likelihood ratio χ^2=70.38, P<0.001; R^2=0.140). Following adjustment for age, smoking and diabetes, smoking was independently associated with BPH [odds ratio (OR), 4.02; 95% CI, 1.10-14.66; P=0.035] and PCa (OR, 6.65; 95% CI, 1.75-25.26; P=0.005), while the association with prostatitis was borderline (OR, 3.40; 95% CI, 0.96-12.00; P=0.058). Diabetes was independently associated with prostatitis (OR, 5.71; 95% CI, 2.24-14.55; P<0.001), BPH (OR, 10.75; 95% CI, 3.76-30.70; P<0.001) and prostate cancer (OR, 76.48; 95% CI, 9.31-628.47; P<0.001). Age was not significantly associated with any disease group following adjustment (Fig. 1 and Table SI). These findings suggested that smoking and diabetes were associated with the prostate disease groups in the present study, particularly BPH and PCa; however, causality cannot be inferred from the case-control design.

Clinical and biochemical parameters among the study groups. The differences detected in the examined groups from various diagnostic parameters yielded a considerable variation within the groups concerning the majority of the studied biomarkers (Table II). The commonly accepted reference ranges for selected biomarkers are as follows: PSA (<4 ng/ml), ESR (0-20 mm/h), CRP (<5 mg/l), vitamin D3 (20-50 ng/ml), zinc (70-120 mcg/dl) and ferritin (30-400 ng/ml) in adult males). In the present study, among the study groups, patients with PCa

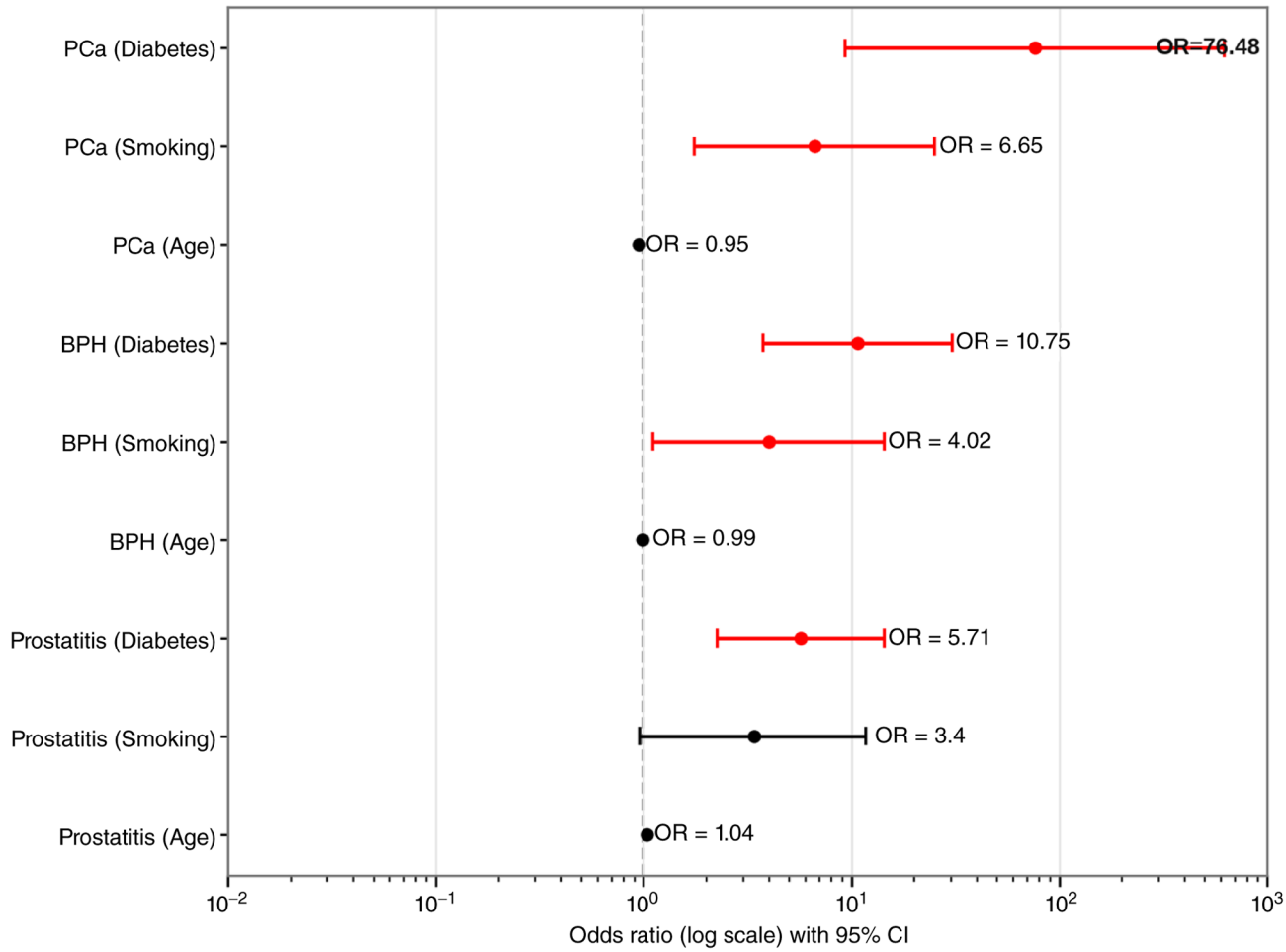

Figure 1. Forest plot of multivariable multinomial logistic regression analysis illustrating the association between clinical variables (age, smoking and diabetes) and disease groups (prostatitis, BPH, and PCa) compared to the control group. Data are presented as odds ratios and 95% confidence intervals. Red markers indicate statistically significant associations (P<0.05), while black markers indicate non-significant results (P>0.05). OR, odds ratio; CI, confidence interval; BPH, benign prostatic hyperplasia; PCa, prostate cancer.

had the greatest mean values of the aforementioned inflammatory and tumor-associated markers, such as ESR, white blood cell count (WBC) and CRP, in addition to PSA and ferritin; Tukey's HSD pairwise significance varied by marker, as detailed in Tables II and SII. The mean PSA level in patients with PCa was 51.75±4.33 ng/ml. This was significantly higher than that of patients with BPH (8.69±0.64 ng/ml), prostatitis (3.05±0.18 ng/ml) and the healthy controls (1.98±0.10 ng/ml). ESR and WBC were also markedly elevated in patients with PCa (52.95±4.02 mm/h and 15.80±0.67 x10^9/l, respectively). All the examined inflammatory biomarkers were lowest among the healthy participants. Both vitamin D3 and zinc levels were higher in the healthy controls (31.76±3.04 ng/ml and 97.86±2.16 mcg/dl, respectively) compared to the participants with prostate disease, who had significantly lower (P<0.001) levels. se findings suggest that the inflammatory and biochemical disturbance levels for PCa in the present cohort were higher compared to the other study groups. However, it is important to note that the markers in question are not specific to any one disease.

Glycemic data of the study groups. As displayed in Table III, there are notable differences in FBS and HbA1c levels across the study groups (P<0.001). Patients with PCa had the highest levels of glucose (FBS, 174.58±9.53 mg/dl; and HbA1c, 7.14±0.15%), followed by patients with prostatitis and BPH, and the healthy controls had the lowest levels (FBS, 121.55±2.92 mg/dl; and HbA1c, 5.61±0.05%). This pattern may be associated with prostate disease due to altered glucose metabolism.

Cytokine levels in the study groups. The differences in the levels of cytokines across the study groups are displayed in Table IV, while the 95% CI values are presented in Table SIII. Patients with PCa had the highest mean concentration levels of TGF-β, IL-6 and IL-8, with values of 107.33±4.98, 72.59±2.04 and 156.43±18.52 pg/ml, respectively. Tukey's HSD analysis revealed that there were no significant differences in IL-6 or IL-8 levels in those with PCa and BPH, while the remaining adjusted pairwise comparisons are detailed in Tables IV and SII. The IL-37 levels were also found to be higher than those in the healthy controls (11.43±0.50 pg/ml) in both PCa (15.38±0.33 pg/ml) and prostatitis (15.93±1.02 pg/ml) groups (P<0.001). GDF-15 levels also differed significantly among the study groups (P=0.040). The highest mean serum level of GDF-15 was detected in the PCa group (202.25±3.18 pg/ml), followed by the BPH

Table II. Serum levels of diagnostic parameters of prostate disease among the study groups.

Groups	ESR (mm/h)	WBC (x10^9/l)	CRP (mg/l)	PSA (ng/ml)	Vitamin D3 (ng/ml)	Zinc (mcg/dl)	Ferritin (ng/ml)	Urea (mg/dl)	Creatinine (mg/dl)
PCa	52.95±4.02^a	15.80±0.67^a	30.09±14.13^a	51.75±4.33^a	12.10±1.06^c	15.59±0.58^d	597.93±58.11^a	47.03±2.30^a	1.21±0.06^a
BPH	28.13±1.77^b	11.61±0.57^b	6.36±0.34^{a,b}	8.69±0.64^b	23.39±1.13^b	33.48±1.07^c	421.59±36.97^b	44.14±1.67^a	1.16±0.05^{a,b}
Prostatitis	19.76±1.09^c	11.47±0.34^b	11.52±1.07^{a,b}	3.05±0.18^b	13.35±1.99^c	67.94±3.26^b	325.75±13.55^{b,c}	37.76±1.32^b	1.01±0.04^b
Control	10.07±0.53^d	6.40±0.24^c	2.67±0.09^b	1.98±0.10^b	31.76±3.04^a	97.86±2.16^a	206.61±9.57^c	34.60±0.64^b	0.84±0.02^c
P-value	<0.001	<0.001	0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are presented as the mean ± SE. Groups sharing at least one superscript letter (a, b, c) are not significantly different, whereas groups with no superscript letter in common differ significantly according to Tukey's honestly significant difference post hoc test (adjusted P<0.05). Adjusted P-values for all six pairwise comparisons are provided in Table SII. BPH, benign prostatic hyperplasia; PCa, prostate cancer; ESR, erythrocyte sedimentation rate; WBC, white blood cell count; CRP, C-reactive protein; PSA, prostate-specific antigen.

Table III. Serum levels of FBS and HbA1c of the investigated groups.

Groups	FBS (mg/dl)	HbA1c (%)
PCa	174.58±9.53^a	7.14±0.15^a
BPH	139.67±4.63^{b,c}	6.71±0.17^a
Prostatitis	144.56±4.92^b	6.05±0.14^b
Healthy control	121.55±2.92^c	5.61±0.05^b
P-value	<0.001	<0.001

Values are presented as the mean ± SE. Groups sharing at least one superscript letter (a, b, c) are not significantly different, whereas groups with no superscript letter in common differ significantly according to Tukey's honestly significant difference post hoc test (adjusted P<0.05). Adjusted P-values for all six pairwise comparisons are provided in Table SII. BPH, benign prostatic hyperplasia; PCa, prostate cancer; FBS, fasting blood sugar; HbA1c, hemoglobin A1c.

(179.59±21.93 pg/ml) and prostatitis (174.41±9.19 pg/ml) groups, whereas the control group exhibited the lowest level (150.65±2.80 pg/ml) (Table IV). Tukey's HSD test revealed a significant difference only between the PCa and the control group, as shown in Tables IV and SII. For instance, the FDR test was applied across the analyzed biomarkers, and all differences remained statistically significant, as shown in Table SIV. Effect size analysis revealed that large differences recorded between group, for TGF-β (η^2=0.633), IL-37 (η^2=0.159), IL-6 (η^2=0.848), IL-8 (η^2=0.478) and GDF-15 (η^2=0.045); these finding are supported by the data presented in Table SV. These findings suggested that IL-6, TGF-β and IL-8 strongly contributed to the differentiation among the study groups. These inflammatory cytokines activation may be related to PCa and may contribute to disease activity.

Impact of metformin treatment. All metformin-related evaluations were made comparing the metformin-treated participants with metformin-untreated participants within each group. The treated and untreated participants included the following: Prostatitis (4 treated; 41 untreated); BPH (9 treated; 36 untreated); PCa (21 treated; 24 untreated). The metformin-untreated participants of the prostatitis group had higher GDF-15 levels compared to the treated participants (147.00±0.71 vs.176.80±9.92 pg/ml; P=0.004). In the BPH group, IL-6 levels were lower among the participants treated with metformin (66.97±0.75) compared to those untreated (69.22±0.68; P=0.036). For PCa group, IL-37, TGF-β and IL-8 had no significant metformin-related differences. No significant differences in the levels of cytokines and GDF-15 were found in the PCa group. Particularly the metformin-treated prostatitis subgroup had a small sample size, and therefore were largely hypothesis-generating rather than confirmatory. The data supporting these findings are presented in Table SVI and Fig. 2.

Correlation analysis. Spearman's correlation analyses were performed within each disease group (PCa, BPH and prostatitis) to assess the correlations between cytokines and clinical biomarkers. Among the cytokines and clinical biomarkers, Spearman's correlation analysis revealed multiple statistically

Table IV. Levels of TGF-β , IL-37, IL-6 and IL-8 in the patients and healthy subjects.

Groups	TGF-β (pg/ml)	IL-37 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	GDF-15 (pg/ml)
PCa	107.33±4.98[a]	15.38±0.33[a]	72.59±2.04[a]	156.43±18.52[a]	202.25±3.18[a]
BPH	85.98±1.22[b]	12.51±0.34[b]	68.54±0.65[a]	147.87±6.38[a]	179.59±21.93[a,b]
Prostatitis	46.11±3.36[c]	15.93±1.02[a]	63.32±1.38[b]	108.16±1.17[b]	174.41±9.19[a,b]
Control	38.06±0.75[c]	11.43±0.50[b]	4.76±0.34[c]	35.95±0.70[c]	150.65±2.80[b]
P-value	<0.01	<0.001	<0.01	<0.01	0.040

Values are expressed as the mean ± SE. Groups sharing at least one superscript letter (a, b, c) are not significantly different, whereas groups with no superscript letter in common differ significantly according to Tukey's honestly significant difference post hoc test (adjusted P<0.05). Adjusted P-values for all six pairwise comparisons are provided in Table SII. BPH, benign prostatic hyperplasia; PCa, prostate cancer; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

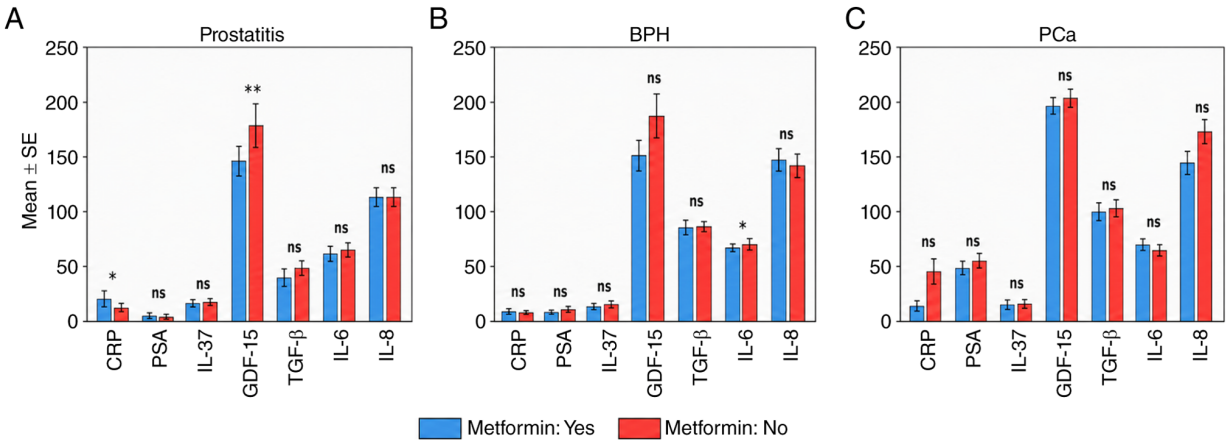

Figure 2. Comparing metformin-treated participants with metformin-untreated participants within the three patient groups [those with (A) prostatitis, (B) BPH and (C) PCa] according to diagnostic laboratory parameters of prostate disease. *P<0.05 and **P<0.01. ns, not significant; BPH, benign prostatic hyperplasia; PCa, prostate cancer; CRP, C-reactive protein; PSA, prostate-specific antigen; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

significant correlations, as shown in Table V. In the PCa group, CRP negatively correlated with IL-6 (ρ=-0.316, P<0.05) and with GDF-15 (ρ=0.312, P<0.05). In the BPH group, there was a negative correlation between IL-37 and PSA (ρ=-0.337, P<0.05), a positive correlation between CRP and IL-37 (ρ=0.301, P<0.05), and a negative correlation between IL-37 and TGF-β (ρ=-0.305, P<0.05). In the prostatitis group, there were positive correlations between PSA and CRP (ρ=0.311, P<0.05), as well as between IL-37 and PSA (ρ=0.358 and P<0.05). The correlation heatmaps of all significant correlations analyzed in the PCa, BPH and prostatitis groups are presented in Fig. 3.

Analysis of ROC curves. ROC curves were calculated for measuring their discriminative performance with respect to inflammatory and cytokine markers. In the prostatitis cohort, the highest AUC value for IL-8 was 1.000, with IL-6 (AUC, 0.969) and CRP (AUC, 0.918) following. The AUC values for IL-37 and PSA were 0.763 and 0.764, respectively. In the BPH cohort, the AUC values for both IL-8 and PSA were 1.000, and the participants exhibited a large separation from the control group for TGF-β (AUC, 0.986), IL-6 (AUC, 0.978) and CRP (AUC, 0.967). In the PCa cohort, IL-6 and CRP, along with TGF-β (AUC, 0.983), IL-8 (AUC, 0.907), IL-37 (AUC, 0.811) and GDF-15 (AUC, 0.922), exhibited separation from the control group. Although the presented AUC values are data-driven exploratory measures, they require independent cohort replication to prove diagnostic feasibility. These findings are presented in Fig. 4 and Table VI.

Discussion

The present case-control study aimed to develop region-specific data on some of the clinical risks, as well as patterns of the inflammatory/metabolic biomarkers in Iraqi men diagnosed with PCa, BPH and prostatitis. The association of smoking and diabetes with the disease categories was maintained following the application of multivariable analysis; nonetheless, the authors of the present study recommend that the readers interpret the association they describe as an association and not as a causal association. There were no diabetic individuals in the control group, and the wide confidence interval for the comparison of diabetes in PCa indicates that this result may be unstable, and, therefore, the authors suggest interpreting the finding with caution.

The smoking variable was reported as yes/no only; thus, the authors were not able to report any smoking dose-response associations. These limitations should be considered as

Table V. Correlation between cytokines and studied biomarkers in the PCa, BPH and prostatitis groups.

A, PCa group

	Cytokine/biomarker						
Cytokine/biomarker	PSA	CRP	IL-6	IL-8	IL-37	TGF-β	GDF-15
PSA	1	0.030	-0.045	-0.113	-0.226	-0.036	-0.260
CRP	0.030	1	-0.316[a]	-0.151	-0.225	0.183	0.312[a]
IL-6	-0.045	-0.316[a]	1	0.063	-0.001	-0.009	-0.091
IL-8	-0.113	-0.151	0.063	1	0.026	0.088	-0.030
IL-37	-0.226	-0.225	-0.001	0.026	1	-0.115	0.123
TGF-β	-0.036	0.183	-0.009	0.088	-0.115	1	-0.066
GDF-15	-0.260	0.312[a]	-0.091	-0.030	0.123	-0.066	1

B, BPH group

	Cytokine/biomarker						
Cytokine/biomarker	PSA	CRP	IL-6	IL-8	IL-37	TGF-β	GDF-15
PSA	1	-0.213	-0.120	-0.034	-0.337[a]	0.213	0.257
CRP	-0.213	1	0.167	0.240	0.301[a]	-0.024	-0.160
IL-6	-0.120	0.167	1	-0.064	0.163	-0.184	0.103
IL-8	-0.034	0.240	-0.064	1	0.144	0.198	0.086
IL-37	-0.337[a]	0.301[a]	0.163	0.144	1	-0.305[a]	-0.066
TGF-β	0.213	-0.024	-0.184	0.198	-0.305[a]	1	-0.036
GDF-15	0.257	-0.160	0.103	0.086	-0.066	-0.036	1

C, Prostatitis group

	Cytokine/biomarker						
Cytokine/biomarker	PSA	CRP	IL-6	IL-8	IL-37	TGF-β	GDF-15
PSA	1	0.311[a]	-0.020	-0.151	0.358[a]	-0.085	-0.253
CRP	0.311[a]	1	-0.020	-0.151	0.166	0.132	-0.029
IL-6	-0.020	-0.020	1	0.002	0.019	0.207	-0.097
IL-8	-0.151	-0.151	0.002	1	-0.155	-0.061	-0.011
IL-37	0.358[a]	0.166	0.019	-0.155	1	0.109	-0.230
TGF-β	-0.085	0.132	0.207	-0.061	0.109	1	0.094
GDF-15	-0.253	-0.029	-0.097	-0.011	-0.230	0.094	1

Data are presented as Spearman's correlation coefficients (ρ). [a]P<0.05. Correlation plots are presented in Fig. 3. BPH, benign prostatic hyperplasia; PCa, prostate cancer; CRP, C-reactive protein; PSA, prostate-specific antigen; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

smoking, metabolic disease and chronic inflammation may be associated with aging, healthcare-seeking behavior and other diseases/conditions not included in the present study.

The higher PSA levels reported in PCa cases aligns with the role of PSA in the assessment of PCa; however, PSA is not specific and may be influenced by BPH, inflammation and other clinical factors. The assessment of PCa via PSA and DRE, along with imaging and a targeted tissue biopsy, is a stepwise process that incorporates a risk-based assessment, and a definitive diagnosis is rendered on histopathological assessment of the prostate biopsy (22). Thus, the elevated PSA in the present study supports the clinical categorization of the PCa group, but should not stand alone in evidenced-based prostate cancer diagnosis. Likewise, the ESR, CRP, WBC, ferritin, urea and creatinine levels should not be considered validated markers of prostate disease, as they may be markers of inflammation and a burden of disease, as well as a potential obstructive uropathy and other comorbidities.

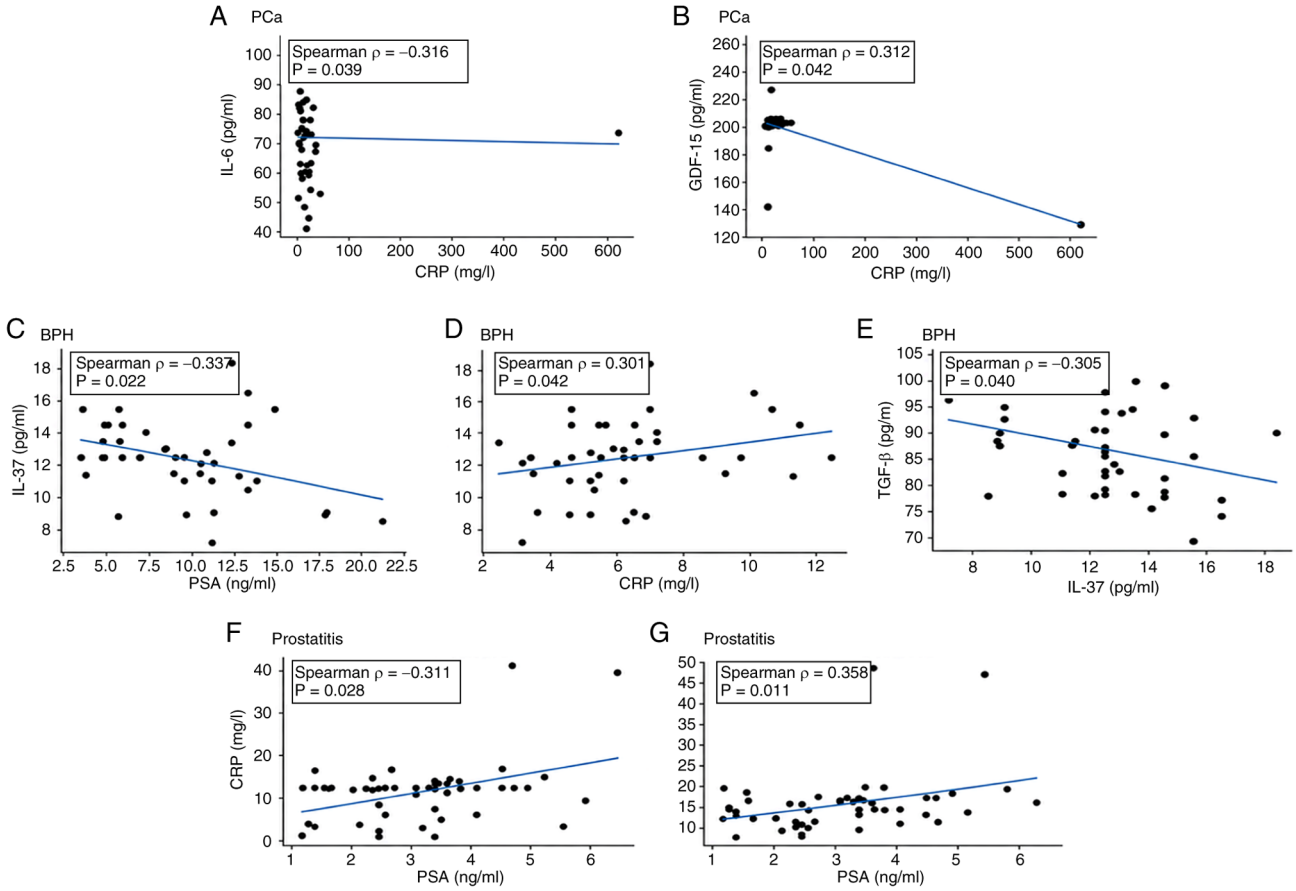

Figure 3. Correlation between cytokines and studied biomarkers in the (A and B) PCa, (C-E) BPH and (F and G) prostatitis groups. BPH, benign prostatic hyperplasia; PCa, prostate cancer; CRP, C-reactive protein; PSA, prostate-specific antigen; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

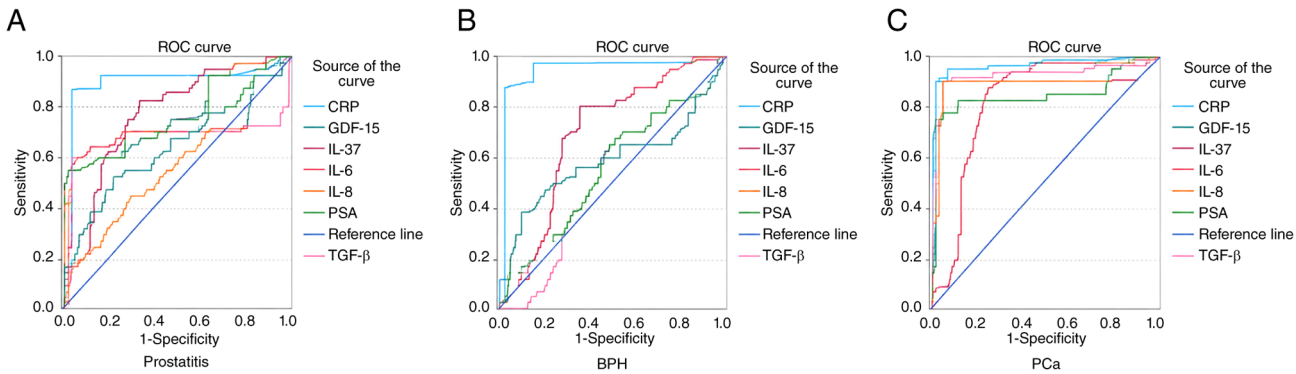

Figure 4. ROC analysis for inflammatory and cytokine marker levels in the patient groups [those with (A) prostatitis, (B) BPH and (C) PCa]. ROC, receiver operating characteristic; BPH, benign prostatic hyperplasia; PCa, prostate cancer; CRP, C-reactive protein; PSA, prostate-specific antigen; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

The decreased vitamin D3 and zinc levels in the patient groups may indicate the effects of nutrition and metabolism or the effects of inflammation and other diseases. Further studies are required to understand the possible interrelationships and determine whether the observed vitamin D3 and zinc levels are in a cause or consequence association with other factors.

The observed cytokine levels are also plausible; however, they remain exploratory. Several studies have linked the inflammatory pathways and the cytokines of the prostate microenvironment with tumor growth, the innate and adaptive immune responses, and the inflammation of prostate disorders (25-31). The context-dependent role of TGF-β levels in cancer explains the increased levels of TGF-β in PCa. In the early stages of other types of cancer, TGF-β may function as a suppressor; in the later stages, TGF-β may function as a facilitator of cancer (32). Concern should be warranted with the increased levels of IL-37. These may indicate a compensatory response of inflammation and leaves the interpretation of these levels open due to the anti-inflammatory

Table VI. ROC statistics for each disease group against the control group.

A, Prostatitis group

Marker	AUC	95% CI	P-value	Cut-off	Sensitivity, %	Specificity, %
PSA	0.763	0.667-0.860	<0.001	2.99	54.0	97.7
CRP	0.918	0.849-0.987	<0.001	4.20	84.0	100.0
IL-37	0.764	0.666-0.863	<0.001	11.80	82.0	65.9
GDF-15	0.626	0.512-0.741	0.031	159.40	52.0	79.5
TGF-β	0.656	0.532-0.781	0.014	46.84	60.0	93.2
IL-6	0.969	0.924-1.000	<0.001	45.22	100.0	95.5
IL-8	1.000	1.000-1.000	<0.001	91.53	100.0	100.0

B, BPH group

Marker	AUC	95% CI	P-value	Cut-off	Sensitivity, %	Specificity, %
PSA	1.000	1.000-1.000	<0.001	3.36	100.0	100.0
CRP	0.967	0.930-1.000	<0.001	4.20	87.0	100.0
IL-37	0.642	0.521-0.763	0.021	11.06	82.6	56.8
GDF-15	0.578	0.456-0.699	0.212	168.90	37.0	88.6
TGF-β	0.986	0.959-1.000	<0.001	68.46	100.0	97.7
IL-6	0.978	0.935-1.000	<0.001	62.21	97.8	97.7
IL-8	1.000	1.000-1.000	<0.001	66.89	100.0	100.0

C, PCa group

Marker	AUC	95% CI	P-value	Cut-off	Sensitivity, %	Specificity, %
PSA	1.000	1.000-1.000	<0.001	4.20	100.0	100.0
CRP	0.978	0.942-1.000	<0.001	4.50	95.3	100.0
IL-37	0.811	0.710-0.912	<0.001	12.50	95.3	65.9
GDF-15	0.922	0.849-0.996	<0.001	186.80	93.0	95.5
TGF-β	0.983	0.962-1.000	<0.001	50.20	97.7	95.5
IL-6	0.980	0.949-1.000	<0.001	40.96	100.0	95.5
IL-8	0.907	0.819-0.995	<0.001	61.36	90.7	100.0

P<0.05, considered to indicate a statistically significant difference; P>0.05, no significant difference. BPH, benign prostatic hyperplasia; PCa, prostate cancer; CRP, C-reactive protein; PSA, prostate-specific antigen; IL, interleukin; GDF-15, growth differentiation factor 15; TGF, transforming growth factor.

and immunoregulatory role of IL-37, which varies in cancer of different types, stages and microenvironments (33,34). In the present study, the increased levels of IL-37 may be a compensatory response and should not be interpreted as a direct marker of disease severity.

The ROC results depicted the separation of defined cases and defined controls in the present dataset and should not be viewed as a validation of clinical diagnosis. The data presented in Table VI should not be interpreted as a substitutive threshold for screening or prognosis of clinical evaluation of PSA levels. Among other evaluated clinical pathways of PSA, DRE, imaging and histopathology, these unexplored biomarkers may serve as adjunctive candidates. These cut-offs would require to be validated in larger, prospective, independent cohorts with clinically relevant comparator groups, pre-specified outcome definitions, and adequate power before any use in screening, risk stratification, monitoring, or prognosis.

As a whole, the data of the present study are in agreement with additional literature (35-37) regarding the associations of inflammatory and metabolic pathways with the biology of prostate disease. Nevertheless, the results diverge from the clinically documented biomarker literature, as the present study was a single-center investigation with a case-control design and small and unproportionate subgroups. Thus, the results should be viewed primarily as hypothesis-generating regional data for an Iraqi population. Future studies are warranted to incorporate larger multicenter efforts, with the staging and grading of PCa and BPH, stratified classification of prostatitis, the classification of medications, the intensity of smoking, and the degree of follow-up, in order to seek and

identify any biomarker patterns which would be relevant to clinical practice and have potential for independent predictive power beyond current practice.

The present study had certain limitations which should be mentioned. Some limitations in design, data and methods constrain the interpretation and generalizability of the findings of the present study. First, due to the case-control design, all connections regarding risk factors, biomarkers and groups of prostate diseases were only associative observations. Second, cancer staging and Gleason/ISUP grading were lacking in the PCa cohort; thus, the extent of biomarkers and the grade of cancer were constrained. Third, BPH data lacked prostate volume and the International Prostate Symptom Score, constraining the interpretation of the severity of BPH. Fourth, smoking status was a yes/no variable only, and consequently, no analysis of the smoking dose-response was possible. Fifth, a lack of detail on other medications and comorbidities may have resulted in residual confounding and bias, particularly in the case of metformin. Sixth, the present single-center study, recruited only in Baghdad, limits generalizability to only that region of Iraq. Finally, with a rather small sample size of 45 participants per group, these findings need to be replicated in larger, multicenter, longitudinal studies with external validation of biomarker cut-offs.

In conclusion, in the present case-control study, smoking, diabetes, inflammatory markers, some cytokines, and lower levels of vitamin D3 and zinc among Iraqi men were found to be associated with some prostate diseases. The greatest elevations in markers of inflammation and a slow ESR, as well as in CRP, IL-6, IL-8 and TGF-β levels among the study population occurred in the patients with PCa. These findings suggest that the inflammation and metabolism of Iraqi patients with PCa warrants further attention. However, these findings fail to establish the value of these markers in diagnosis and prognosis. The markers assessed in the present study are candidate exploratory markers, and the ROC cut-off values derived from the present study are exploratory cut-off values. Further studies using larger populations are warranted in order to define these markers of inflammation, as well as make therapeutic and prognostic adjustments related to these markers, before they could be considered for assessment, monitoring, or risk stratification. These studies should include independent external validation, detailed clinical assessment and staging, medication adjustments, and analysis of the clinical course of the patients.

Acknowledgements

The authors are grateful to the College of Science at Mustansiriyah University, Baghdad, Iraq for providing the facilities for the present study.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MMAA conceptualized the study. In addition to drafting, revising and editing the manuscript, MMAA and OAJ contributed to the study methodology. All authors (MMAA, OAJ and IAA) were involved in data validation, investigation, and in the writing and preparation of the original draft of the manuscript. IAA contributed to the formal analysis, data curation and figure preparation. OAJ provided laboratory facilities, reagents, instruments and technical support. IAA and MMA supervised the study and involved in project administration. The published version of the manuscript has been read and approved by all authors. All authors (MMAA, OAJ and IAA) confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the College of Science, Mustansiriyah University, Baghdad, Iraq (Approval no. BCSMU/3024/00081M; January, 2024). In accordance with the approved protocol and within the framework of academic collaboration with Mustansiriyah University, blood samples and clinical data were collected from Al-Yarmouk Teaching Hospital in Baghdad. The treating medical team and hospital administration provided the records and clinical samples after obtaining the necessary ethical approvals (Approval no. 9274; December, 2023) and written informed consent from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Huang L, LaBonte MJ, Craig SG, Finn SP and Allott EH: Inflammation and prostate cancer: A multidisciplinary approach to identifying opportunities for treatment and prevention. Cancers(Basel) 14: 1367, 2022.
2. Sfanos KS, Yegnasubramanian S, Nelson WG and De Marzo AM: The inflammatory microenvironment and microbiome in prostate cancer development. Nat Rev Urol 15: 11-24, 2018.
3. Novysedlak R, Guney M, Al Khouri M, Bartolini R, Foley LK, Benesova I, Ozaniak A, Novak V, Vesely S, Pacas P, *et al*: The immune microenvironment in prostate cancer: A comprehensive review. Oncology 103: 521-545, 2024.
4. Tsang SH, Peisch SF, Rowan B, Markt SC, Gonzalez-Feliciano AG, Sutcliffe S, Platz EA, Mucci LA and Ebot EM: Association between Trichomonas vaginalis and prostate cancer mortality. Int J Cancer 144: 2377-2380, 2019.
5. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74: 229-263, 2024.
6. Dang T and Liou GY: Macrophage cytokines enhance cell proliferation of normal prostate epithelial cells through activation of ERK and Akt. Sci Rep 8: 1-9, 2018.
7. Larsson SC, Carter P, Kar S, Vithayathil M, Mason AM, Michaëlsson K and Burgess S: Smoking, alcohol consumption, and cancer: A mendelian randomisation study in UK Biobank and international genetic consortia participants. PLoS Med 17: e1003178, 2020.

8. Engbang JPN, Sala TB and Abane ME: Risk factors of prostate cancer in douala (Cameroon): A case-control study. Cancer Sci Res 4: 1-7, 2021.
9. Rapisarda V, Miozzi E, Loreto C, Matera S, Fenga C, Avola R and Ledda C: Cadmium exposure and prostate cancer: Insights, mechanisms and perspectives. Front Biosci(Landmark Ed) 23: 1687-1700, 2018.
10. Bjurlin MA, Matulewicz RS, Roberts TR, Dearing BA, Schatz D, Sherman S, Gordon T and El Shahawy O: Carcinogen biomarkers in the urine of electronic cigarette users and implications for the development of bladder cancer: A systematic review. Eur Urol Oncol 4: 766-783, 2021.
11. Fleifel M and El Alam A: Diabetes mellitus and the prostate: The odd pairing. Cureus 17: e90712, 2025.
12. Murtola TJ, Syvälä H, Tolonen T, Helminen M, Riikonen J, Koskimäki, Pakarainen T, Kaipia A, Isotalo T, Kujala P and Tammela TLJ: Atorvastatin versus placebo for prostate cancer before radical prostatectomy-a randomized, double-blind, placebo-controlled clinical trial. Eur Urol 74: 697-701, 2018.
13. Coelho KBCA, Wosniaki DK, Pereira JL, Luz M, Albrecht L, Nardin JM, Aoki MN, Reis LO, dos Reis RB and Zanette DL: Comparative analysis of cytokine expression profiles in prostate cancer patients. Biology(Basel) 14: 505, 2025.
14. Adekoya TO and Richardson RM: Cytokines and chemokines as mediators of prostate cancer metastasis. Int J Mol Sci 21: 4449, 2020.
15. Ene CV, Nicolae I, Geavlete B, Geavlete P and Ene CD: IL-6 signaling link between inflammatory tumor microenvironment and prostatic tumorigenesis. Anal Cell Pathol 2022: 5980387, 2022.
16. Kaur S, Bansal Y, Kumar R and Bansal G: A panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. Bioorg Med Chem 28: 115327, 2020.
17. Gonzalez-Aparicio M and Alfaro C: Significance of the IL-8 pathway for immunotherapy. Hum Vaccin Immunother 16: 2312-2317, 2020.
18. Zhao J, Qiu L, Xu Y, Xu Y, Guo Y, Li P and Wang S: Role of the novel cytokine IL-37 in inflammations and tumors of human reproductive system. Obstet Gynecol Rep 2: 1-5, 2018.
19. Abulkhir A, Samarani S, Amre D, Duval M, Haddad E, Sinnett D, Leclerc JM, Diorio C and Ahmad A: A protective role of IL-37 in cancer: A new hope for cancer patients. J Leukoc Biol 101: 395-406, 2017.
20. Mirzaei S, Paskeh MDA, Saghari Y, Zarrabi A, Hamblin MR, Entezari M, Hashemi M, Aref AR, Hushmandi K, Kumar AP, *et al*: Transforming growth factor-beta (TGF-β) in prostate cancer: A dual function mediator? Int J Biol Macromol 206: 435-452, 2022.
21. Huang S, Wa Q, Pan J, Peng X, Ren D, Li Q, Dai Y, Yang Q, Huang Y, Zhang X, *et al*: Transcriptional downregulation of miR-133b by REST promotes prostate cancer metastasis to bone via activating TGF-β signaling. Cell Death Dis 9: 779, 2018.
22. Cornford P, van den Bergh RCN, Briers E, Van den Broeck T, Brunckhorst O, Darraugh J, Eberli D, De Meerleer G, De Santis M, Farolfi A, *et al*: EAU-EANM-ESTRO-ESUR-ISUP-SIOG guidelines on prostate cancer-2024 update. Part I: Screening, Diagnosis, and local treatment with curative intent. Eur Urol 86: 148-163, 2024.
23. Sandhu JS, Bixler BR, Dahm P, Goueli R, Kirkby E, Stoffel JT and Wilt TJ: Management of lower urinary tract symptoms attributed to benign prostatic hyperplasia (BPH): AUA guideline amendment 2023. J Urol 211: 11-19, 2024.
24. Kranz J, Bartoletti R, Bruyère F, Cai T, Geerlings S, Köves B, Schubert S, Pilatz A, Veeratterapillay R, Wagenlehner FME, *et al*: European association of urology guidelines on urological infections: Summary of the 2024 Guidelines. Eur Urol 86: 27-41, 2024.
25. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
26. Madan RA and Palena C: Behind the IL-8 ball in prostate cancer. Nat Cancer 2: 775-776, 2011.
27. Maynard JP, Ertunc O, Kulac I, Valle BD, Javier A, De Marzo AM and Sfanos KS: IL8 expression is associated with prostate cancer aggressiveness and androgen receptor loss in primary and metastatic prostate cancer. Mol Cancer Res 18: 153-165, 2020.
28. Cai T, Santi R, Tamanini I, Galli IC, Perletti G, Bjerklund Johansen TE and Nesi G: Current knowledge of the potential links between inflammation and prostate cancer. Int J Mol Sci 20: 3833, 2020.
29. Seok E, Lee DM, Yang H and Yoo TK: Interleukin-6 secreted by tumor-associated macrophages promotes proliferation and migration through JAK2/STAT3 signaling pathway in human prostate cancer cells. Prostate Int 74: 229-263, 2026.
30. Zhou T, Li Y, Liu Z, Zeng Z, Li T, Chen L, Zhao H, Wu X, Shen Y, Fan H, *et al*: IL-6/STAT3 signaling in prostate cancer: CAF-driven immune evasion and therapeutic opportunities. Front Immunol 16: 1736606, 2026.
31. Ghazi MH, Hassan EF, Ibrahim NH and Mohsein OA: Early detection of prostate cancer using novel ELISA-based biomarkers: Insights into inflammatory and tumor-specific pathways. Asian Pac J Cancer Prev 26: 3833-3839, 2025.
32. Xue VW, Chung JYF, Córdoba CAG, Cheung AHK, Kang W, Lam EWF, Leung KT, To KF, Lan HY and Tang PMK: Transforming growth factor-β: A multifunctional regulator of cancer immunity. Cancers(Basel) 12: 3099, 2020.
33. Gu M, Jin Y, Gao X, Xia W, Xu T and Pan S: Novel insights into IL-37: An anti-inflammatory cytokine with emerging roles in anti-cancer process. Front Immunol 14: 1278521, 2023.
34. Landolina N, Mariotti FR, Pelosi A, D'Oria V, Ingegnere T, Alicata C, Vacca P, Moretta L and Maggi E: The anti-inflammatory cytokine IL-37 improves the NK cell-mediated anti-tumor response. Oncoimmunology 13: 2297504, 2023.
35. Oseni SO, Naar C, Pavlović M, Asghar W, Hartmann JX, Fields GB, Esiobu N and Kumi-Diaka J: The molecular basis and clinical consequences of chronic inflammation in prostatic diseases: Prostatitis, benign prostatic hyperplasia, and prostate cancer. Cancers(Basel) 15: 3110, 2023.
36. Zhang L, Wang Y, Qin Z, Gao X, Xing Q, Li R, Wang W, Song N and Zhang W: Correlation between prostatitis, benign prostatic hyperplasia and prostate cancer: A systematic review and Meta-analysis. J Cancer 11: 177-189, 2020.
37. Zhu W, Xu H, Xiong X, Li Y, Feng D, Huang W, Qiang W and Yang L: Decoding GDF15: Impact on prostate cancer metabolism, chemoresistance, and clinical applications. Chin Med J (Engl) 139: 211-221, 2026.