RESEARCH ARTICLE

Prostatic Acid Phosphatase (PAP), Interleukin-8 (IL-8), and Cytotoxic T-Lymphocyte–Associated Protein 4 (CTLA-4) as Complementary Prognostic Biomarkers to Prostate-specific Antigen (PSA) in Prostate Cancer

Salahaldin Mohammed Fahad[1], Noor Hameed Hanoush[2,*], Ahmed Teen Ahmed[3]

[1]Department of Specialized Supervision Biology, Directorate of Education in Anbar, Iraq's Ministry of Education, Ramadi, Al-Anbar, Iraq
[2]Scientific Affairs Department, University of Anbar, Ramadi, Al-Anbar, Iraq
[3]Directorate of Health of Anbar, Anbar Cancer Center, Ramadi, Al-Anbar, Iraq

*Corresponding author. Email: noor.hameed@uoanbar.edu.iq

Received date: Jun 20, 2026; Revised date: Aug 2, 2026; Accepted date: Aug 6, 2026

Abstract

BACKGROUND: Prostate-specific antigen (PSA) is frequently used for prostate cancer screening and diagnosis, however its low specificity and incapacity to differentiate aggressive from indolent disease reduces its clinical utility. It is necessary to identify biomarkers that can complement PSA to increase the prognostic accuracy of prostate cancer. Emerging evidence suggests that androgen-related factors and immune mediators are involved in prostate cancer progression. Therefore, to identify more accurate markers of prostate cancer progression, several androgen-related and immunological parameters were evaluated alongside serum PSA to determine their potential as complementary prognostic biomarkers.

METHODS: A cross-sectional study was performed involving 60 prostate cancer subjects age ranged 50-74 years and 60 healthy men within the same age range as control. Blood samples from subjects and controls were collected, then testosterone, dihydrotestosterone (DHT), androgen receptor (AR), prostatic acid phosphatase (PAP), interleukin (IL)-8, cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), transforming growth factor-β (TGF-β), IL-35 and PSA were assessed using the enzyme-linked immunosorbent assay (ELISA) in serum.

RESULTS: All parameters showed significant differences between prostate cancer subjects and control, with higher PSA level in prostate cancer subjects (10.2±2.8 ng/mL). AR, PAP, IL-8, IL-35, and CTLA-4 were independently associated with PSA level in predicting prostate cancer progression (p<0.05). However, after multivariable analysis, only PAP, IL-8, and CTLA-4 had shown overall association with PSA serum, which all three show positive correlation.

CONCLUSION: After integrated model analysis, only PAP, IL-8, and CTLA-4 remained significantly associated with PSA levels, indicating that combination of PSA, PAP, IL-8, and CTLA-4 levels might account for a substantial proportion of progression and biochemical activity in prostate cancer patients, and may serve as potential combined biomarker.

KEYWORDS: interleukin-35, prostate cancer, interleukin-8, prostate-specific antigen, testosterone

Indones Biomed J. 2026; 18(4): 312-21

Introduction

Prostate cancer is the most frequently identified malignancy among males and a major cause of cancer-related death in industrialized countries.(1,2) Over 1.4 million new cases and ~375,000 deaths due to prostate cancer was reported in 2020.(3) The incidence rate of prostate cancer in Asia keep showing an upward trend in 2025.(4) Despite successful treatment at early stage in most patients, prostate cancer often reoccurs within 10 years after the treatment in 20-40% of cases.(5)

Copyright © 2026 The Prodia Education and Research Institute.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC-BY-NC) License.

Prostate-specific antigen (PSA) is a marker often used to screening for prostate cancer and early diagnosis.(6) However, serum PSA is known for its limited specificity and inability to distinguish aggressive from indolent disease, for instance PSA levels are also higher in histological inflammation and benign prostatic hyperplasia (BPH).(7) This issue reduces the clinical utility of serum PSA as a sole biomarker. To enhance cancer diagnosis, recognize prostate cancer staging, and predicting outcomes after the therapy (8), it is necessary to have other complementary biomarkers alongside PSA.

Emerging evidence suggests that androgen-related factors and immune mediators are involved in prostate cancer progression.(9) Testosterone is the main circulating androgen generated by the testes and act as the precursor for dihydrotestosterone (DHT), which most powerful androgen in prostatic tissue.(10,11) Modifications in serum testosterone and intraprostatic androgen signaling have long been embroiled in prostate cancer progress and spread. (10) DHT promotes cellular proliferation and inhibits apoptosis in prostate epithelial cells, and it has a greater affinity for androgen receptor (AR). AR is essential to normal prostate development and is a key driver of prostate cancer initiation and progression. Prostate cancer risk and tumor aggressiveness have been linked to elevated testosterone, DHT and AR, suggesting their potential use as predictive biomarkers.(11,12) Prostatic acid phosphatase (PAP), a prostate-derived secretory enzyme, was the first serum biomarker used clinically for prostate cancer. Although it has largely been replaced by prostate-specific antigen (PSA) for diagnosis, elevated PAP levels are strongly connected to a poor prognosis, bone metastases, and advanced disease, suggesting that PAP may provide complementary prognostic information when used alongside PSA.(13)

Some immunological parameters, such as transforming growth factor-β (TGF-β), interleukins (ILs), and cytotoxic t-lymphocyte–associated protein 4 (CTLA-4) also provide the useful information related to prostate cancer. Recent study has shown that prostate cancer cells produce more IL-8, which significantly inhibits apoptosis while promoting migration, invasion and proliferation.(14) Prior studies revealed that IL-8 concentration were greater in subject with prostate cancer than in health control, indicating that IL-8 could be a indicator for the genesis of prostate cancer as well as an indicator of tumor growth. (15) Several clinical studies have demonstrated elevated serum IL-35 concentration in prostate cancer subjects in comparison to controls and those with benign prostatic hyperplasia.(12,16) Plasma IL-35 expression may correlate with size of tumor, and the presence of nearby lymph node metastases in various cancer types, indicating a link to tumor development.(17) CTLA-4 is one of immune checkpoint inhibitors are found on tumor-infiltrating T cells, which are frequently malfunctioning and may be identified by low co-inhibitory surface receptor expression and activity.(18) Its clinical relevance is supported by the development of CTLA-4-targeted immunotherapies for advanced prostate cancer.(19) Meanwhile, TGF-β are known for its two roles in the development of prostate cancer, which is to suppresses tumors in the early stages of prostate cancer and to promotes tumors, invasion, and metastatic in the later, prostate cancer stages.(20) Several phases of the metastatic process of prostate cancer, including angiogenesis, basic tumor remodeling EMT, and tumor growth, have been associated with TGF-β.(21)

Although numerous biomarkers have been individually investigated for the prognostic and diagnostic of prostate cancer, evidence related to their combined clinical significance alongside PSA remains limited. Therefore, to identify more accurate markers of prostate cancer progression, several androgen-related and immunological parameters were evaluated alongside serum PSA to determine their potential as complementary prognostic biomarkers, especially in Iraqi prostate cancer patients.

Methods

Study Design

A clinical cross-sectional study was conducted between April 2024 and March 2025 at the Anbar Cancer Center/ Al-Ramadi Teaching Hospital. Initially, 82 men aged 50-74 years who had recently been diagnosed with benign prostatic enlargement based and had not previously undergone PSA testing were recruited. The prostate enlargement diagnosis was made based on clinical evaluation and imaging findings using transrectal ultrasonography (TRUS) and/or magnetic resonance imaging (MRI). Out of 82 recruited subjects, 66 of them had PSA levels ≥4 ng/mL, subsequently underwent prostate biopsy. After the biopsy, only 60 subjects were confirmed positive for prostate cancer (Figure 1). For the purpose of comparison, 60 men without prostate cancer in the same age range were recruited as the control group. Subjects having an acute sickness or had history of chemotherapy, radiation therapy, hormonal treatment (including androgen deprivation therapy), diabetes mellitus, kidney problems, smoking, other another malignancy, and benign prostatic hyperplasia, were excluded.

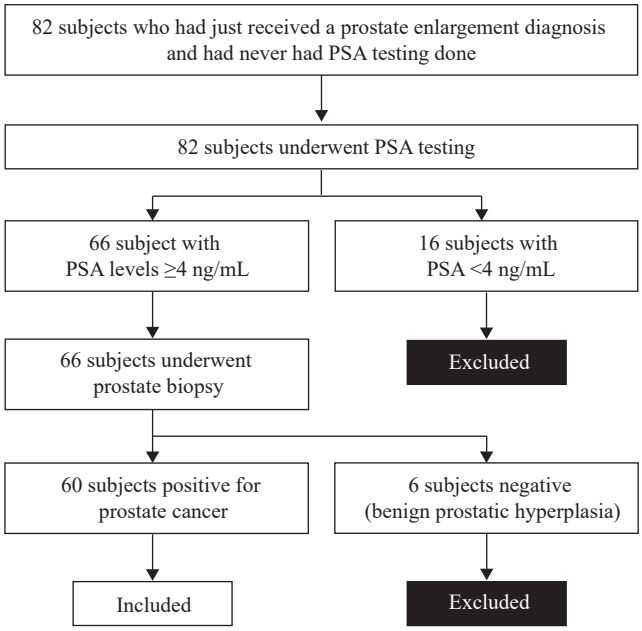

Figure 1. Flowchart of prostate cancer subjects recruitment.

Complete Blood Count (CBC) Measurement

Prior to the prostate biopsy, peripheral venous blood was drawn from subjects and control and put in ethylenediaminetetraacetic acid (EDTA) tubes to determine the hematological change, including the differential white cell count (basophils, neutrophils, eosinophils, lymphocytes and monocytes), lymphocyte to monocyte ratio (LMR), neutrophil lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR). These hematologic parameters were measured by using automated analyzers (Sysmex XN-1000, Kobe, Japan) that operates based on fluorescence flow cytometry, semiconductor laser technology, hydrodynamic focusing, and electrical impedance for the identification and counting of blood cells. Five mL of blood samples were injected into a gel tube (without anticoagulant), let it coagulate at room temperature for 20 min. After clotting, samples of blood were centrifuged at 402 × g for 10 minutes to obtain the serum. Immediately after collection, part of the serum was used for the evaluation of the study variables, while the remaining serum was stored at -80 °C until further analysis.

Enzyme-linked Immunosorbent Assay (ELISA) for Biomarkers Measurement

For the biomarker analysis, the obtained serum from subject' and control's blood samples were measured in serum using human Testosterone ELISA Kit (Cat. No. ab108666; Abcam, Cambridge, UK) with detection range of 0.2–16 ng/mL and sensitivity of 0.07 ng/mL; DHT(Dihydrotestosterone) ELISA Kit (Cat. No. EU2551; Fine Test, Wuhan, China) with detection range of 39.063–2500 pg/mL and sensitivity of 23.438 pg/mL; Human Androgen Receptor ELISA Kit (Cat. No. EEL028; Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA) with detection range of 0.31–20 ng/mL and sensitivity of 0.19 ng/mL; Human PAP ELISA Kit (ACPP) (Cat. No. ab267802; Abcam, Cambridge, UK) with detection range of 37.5–2400 pg/mL and sensitivity of 9.73 pg/mL; Human IL-8 ELISA Kit (Cat. No.E-EL-H6008; Elabscience) with detection range of 7.81–500 pg/mL and sensitivity of 4.69 pg/mL; Human IL-35 ELISA Kit (E-EL-H2443) (Cat. No. E-EL-H2443; Elabscience) with detection range of 15.63–1000 pg/mL and sensitivity of 9.38 pg/mL, Human CTLA4 ELISA Kit (Cat. No. E-EL-H2069; Elabscience) with detection range of 0.16–10 ng/mL and sensitivity of 0.1 ng/mL; and Human TGF beta-1 ELISA Kit (Catalog No. ab100647; Abcam, Cambridge, UK) with detection range of 18–4000 pg/mL and sensitivity of 18 pg/mL; Human PSA (Prostate Specific Antigen) ELISA Kit (Cat. No. E-EL-H0091; Elabscience) with detection range of 0.31–20 ng/mL and sensitivity of 0.19 ng/mL, according to the manufacturer's protocol.

Briefly, for all biomarkers, 100 µL of blank, standards and serum samples were added to the wells in duplicate and incubated for 90 min at 37°C. After adding 100 µL of the biotinylated detection antibody solution to each well without first washing, the mixture was incubated for 60 minutes at 37°C. Three washes were performed on the wells and soaked during each wash. Then, 100 µL of HRP conjugate solution was added to all well and incubate for 30 minutes at 37°C. The procedure was repeated 5 times before 90 µL of substrate reagent was added to each well and incubated for 15 min at 37°C. Finally, 50 µL of stop solution was added and the optical density was immediately estimated at 450±2 nm by a microplate reader. Each biomarker levels was calculated from standard calibration curves.

Statistical Analysis

For statistical analysis, SPSS software version 21 (IBM Corporation, Armonk, NY, USA) was utilized to determine the statistical significance. Sensitivities and specificities were measured using the receiver operating characteristic (ROC) curve, and the area under the curve (AUC), was also computed. The effectiveness of each variable in predicting prostate cancer was assessed using multiple linear regression analysis. Bootstrap resampling (1000 repeats) was used for internal validation of a predictive multivariable model.

Results

Characteristics of Study Subjects

Average prostate cancer duration of subjects was 1.65±0.58 years, with 10 out of 60 subjects also have a family history of prostate cancer. Prostate cancer subjects were classified into four stages (I, II III, and IV) according to Tumor, Node, and Metastasis (TNM) staging system, based on physical exams and biopsies results. There were significant differences of subject's distribution among prostate cancer stages (p=0.015), with most subjects classified as stage IV (33.33%) and least subjects as stage I (18.33%). Subjects were also classified based on the Gleason score classification and histopathological findings, with the majority of subjects (40.00%) were categorized as having intermediate-risk disease, while 33.33% and 26.67% were classified as high-risk and low-risk, respectively (p=0.026), (Table 1).

Significant Differences of Androgen-Related Parameters between Subjects and Controls

As the standard biomarker, PSA level was significantly increased in prostate cancer subjects compared with control (10.2±2.8 *vs.* 1.45±0.16) ng/mL. Meanwhile, for the androgen-related factors, mean difference between the prostate cancer subjects and controls were also reported. Serum testosterone levels was demonstrated a significantly reduced in prostate cancer subjects compared with control (p=0.047). While, levels of DHT, AR, and PAP were significantly higher in prostate cancer subjects compared with control (all p<0.01) (Table 2).

Significant Differences of Inflammatory and Immunosuppressive Markers between Subjects and Controls

Significant differences in systemic inflammatory and immunosuppressive markers were observed in prostate cancer subjects compared with controls. The NLR and PLR were significantly increased in prostate cancer subjects compared to control (all p<0.01). In contrast, the LMR was significantly decrease in subjects with prostate cancer compared with control (p=0.000).

Concentrations of IL-8 and IL-35 were significantly raised in subjects with prostate cancer compared to control (all p<0.001). Likewise, level of TGF-β were significant higher in prostate cancer subjects than in controls, consistent with an immunosuppressive tumor microenvironment. Assessment of tumor dissemination markers revealed a significant increase in CTLA-4 in subjects with prostate cancer as opposed to controls (Table 2).

Univariate Linear Regression Analysis

Univariate linear regression analysis was estimated to evaluate the relationship between physiological and immunological biomarkers with serum PSA levels. Several parameters showed significant negative correlations with PSA level, including PAP (β=0.347, p=0.019), IL-8 (β=2.107, p=0.010), IL-35 (β=1.85, p=0.041), and CTLA-4 (β=1.34,

Table 1. The study subjects' demographic details.

Characteristics	Prostate Cancer (n=60)	Control (n=60)	p-value
Age range (years), mean±SD	66.63±7.87	66.4±8.21	0.150[a]
Prostate cancer duration (years), mean±SD	1.65±0.58	-	
Family history of cancer, n (%)	10 (16.67)	-	
BMI (kg/m^2), mean±SD	32.42±1.72	33.76±1.27	0.060[a]
TNM stage, n (%)			0.015*[,b]
I	11 (18.33)	-	
II	13 (21.67)	-	
III	16 (26.67)	-	
IV	20 (33.33)	-	
Gleason score, n (%)			
Low risk (≤6)	16 (26.67)	-	0.026*[,b]
Intermediate risk (7)	24 (40.00)	-	
High risk (8-10)	20 (33.33)	-	

Data are presented as mean±SD. [a]p-values were calculated using Student's t-test. [b]Categorical variables (%) were compared using the chi-square (χ^2) test. *p<0.05 is considered statistically significant. BMI: body mass index, TNM: Tumor Node Metastasis.

Table 2. Physiological and immunological predictors concentration of subjects.

Physiological Predictors	Mean±SD		*p*-value
	Prostate Cancer	Control	
Testosterone (ng/mL)	3.6±1.3	5.8±1.6	0.047*
DHT (pg/mL)	87±16	65±12	0.004*
AR (ng/mL)	10.3±1.3	3.5±0.88	0.000*
PAP (pg/mL)	94±3.4	39±2.6	0.000*
NLR	3.2±0.8	1.75±0.6	0.000*
PLR	177±36	112±25	0.005*
LMR	3.1±0.8	4.8±0.7	0.000*
IL-8 (pg/mL)	48.32±1.4	17.5±1.23	0.000*
IL-35 (pg/mL)	134.62±2.8	54.64±1.67	0.000*
CTLA-4 (ng/mL)	6.3±2.2	0.82±0.41	0.000*
TGF-β (pg/mL)	41.8±4.6	24±1.87	0.000*
PSA (ng/mL)	10.2±2.8	1.45±0.16	0.000*

Data are presented as mean±SD. *p*-values were calculated using Student's t-test. *$p<0.05$ is considered statistically significant.

p=0.022), Meanwhile AR was significantly negatively correlated with PSA level (β=-143, *p*=0.040) (Table 3). The biomarkers demonstrating significance relation ($p<0.05$) were then used to find independent predictors of PSA level in a multivariable linear regression model.

Integrated Multivariate Regression Analysis of Physiological and Immunological Predictors in Prostate Cancer Subjects

To identify independent predictors of biochemical disease activity, a multivariate linear regression analysis was performed with serum PSA as the dependent variable. Among the androgen-related factors, PAP was shown as the strongest independent predictor of PSA levels (β=+0.42, *p*=0.001), reflecting its association with disease progression. Meanwhile, among immunological markers, IL-8 showed as the strongest overall predictors of PSA levels (β=+0.51, *p*=0.001), highlighting its central role in inflammation driven tumor progression. While as the immune suppression marker, CTLA-4 (β=+0.32, *p*=0.001), remained independently associated with increased PSA levels (Table 4). The integrated model demonstrated strong

Table 3. Univariate analyses of variables associated with serum PSA levels in predicting the risk of prostate cancer.

Variable	β coefficient	SE	95% CI	*p*-value
Testosterone	-0.107	0.052	-0.218–0.017	0.055
DHT	0.360	0.012	0.080–0.690	0.074
AR	-0.143	0.062	-0.217–(-0.057)	0.040*
PAP	0.347	0.013	0.245–0.417	0.019*
LMR	-0.637	0.165	-0.941–(-0.213)	0.051
PLR	0.618	0.135	0.452–0.821	0.120
NLR	0.141	0.067	0.034–0.267	0.066
IL-8	2.107	0.520	1.620–3.020	0.010*
IL-35	1.850	0.420	1.310–2.430	0.041*
TGF-β	0.820	0.240	0.451–0.980	0.060
CTLA-4	1.340	0.410	0.820–1.670	0.022*

p-values were calculated using the t-test for the regression coefficients in the univariate linear regression analysis.

Table 4. Multivariate regression analysis of physiological and immunological predictors associated with serum PSA levels in prostate cancer subjects.

Predictor	β Coefficient (Standardized)	Standard Error (SE)	95% CI for β	*p*-value
AR Expression	-0.62	0.06	-0.86–0.47	0.066
PAP	0.42	0.074	0.24–0.52	0.001*
IL-8	0.51	0.092	0.34–0.64	0.001*
IL-35	0.33	0.083	0.24–0.47	0.054
CTLA-4	0.32	0.081	0.17–0.42	0.001*

p-values were calculated using Student's t-test for the regression coefficients in the multivariate linear regression model.

explanatory power (R^2=0.71, p<0.001), indicating that combined physiological and immunological predictors account for a substantial proportion of biochemical disease activity in prostate cancer patients. After adjustment, it was found that AR and IL-35 were not statistically different (p=0.066 and p=0.054).

Multicollinearity Assessment and Internal Validation of the Final Regression Model

Multicollinearity among the independent biomarkers (PAP, IL-8, and CTLA-4) was estimated using tolerance and variance inflation factors (VIF) values. All three biomarkers showed acceptable VIF values (<10) and tolerance values (>0.10), indicating the absence of significant multicollinearity among the predictor markers to prostate cancer that included in the regression model (Table 5).

Internal validation using bootstrap resampling (1000 repeats) confirmed the stability of the multivariable model. After multivariate model and bootstrap validation, the strongest and stable independent predictors to prostate cancer was CTLA-4 (Bootstrap β: 0.039, p=0.017), followed by IL-8 (Bootstrap β: 0.074, p=0.032) and PAP (Bootstrap β: 0.088, p=0.050) (Table 6). The bias-corrected coefficients remained consistent, demonstrating the predictive mode's robustness.

Diagnostic Performance of PAP, IL-8, and CTLA-4

The diagnostic performance of physiological and immunological biomarkers was assessed using ROC curve analysis in discriminating prostate cancer subjects from healthy controls. Among androgen-related markers, PAP showed the pest cut-off value of ≥40 pg/mL and highest accuracy of diagnostic (AUC=0.84) with sensitivity 92.5% and specificity 88%. IL-8 and CTLA-4 also exhibited excellent stronger diagnostic performance between prostate cancer subjects and controls. The pest cut-off value of CTLA-4 was >3.9 ng/mL and IL-8 was >20.5 pg/mL, that demonstrated the highest accuracy of diagnostic (AUC=1.000 for CTLA-4 and AUC=0.991 for IL-8) with sensitivity of 99% and 95.6%, respectively, and specificity of 98.3% and 92%, respectively (Table 7, Figure 2).

Table 5. Study biomarkers that do not have multicollinearity issues.

Variable	VIF	Tolerance
PAP	4.621	0.216
IL-8	3.015	0.331
CTLA-4	1.143	0.714

Discussion

In the present study, a predictive model of prostate cancer progression in Iraqi subjects was constructed. The integrated model demonstrated strong explanatory power indicating that combined androgen-related and immunological parameters account for a substantial proportion of biochemical disease activity and predicting prostate cancer in patients. (20) These parameters were analyzed using multivariable linear regression to see the association with PSA levels as the current standard biomarker for prostate cancer. PSA is known to be independently influenced by DHT and PAP. While DHT is the most powerful intraprostatic androgen that activates AR, elevated PAP indicates higher tumor load and cellular turnover.(22,23) In this study, the elevated PSA level was followed by evelated DHT, AR, and PAP in prostate cancer subjects. Meanwhile, testosterone level was lower. This negative correlation between blood testosterone and PSA is consistent with earlier findings that aggressive prostate cancer can advance in spite of low systemic testosterone levels, most likely as a result of AR hypersensitivity and increased intratumoral androgen production. These results together highlight the significance

Table 6. Confidence interval for bias corrected accelerated bootstrap.

Variable	Bootstrap β	SE	Bias	95% Confidence Interval		*p*-value
				Lower	Upper	
PAP	0.088	0.025	1.555	0.021	0.098	0.050*
IL-8	0.074	0.018	0.378	-0.012	0.085	0.032*
CTLA-4	0.039	0.014	0.282	-0.039	0.107	0.017*

of local androgen metabolism.(24) After multivariate linear regression was performed, PAP was found to be the strongest marker among the androgen-related parameters associated with PSA level in prostate cancer.

The promotion of malignant cell characteristics, such as tumor carcinogenesis, metastasis, and angiogenesis in the tumor microenvironment, is significantly helped by immunological markers.(16) Systemic inflammatory indices, including NLR, PLR, and LMR, independently predicted PSA levels, supporting their utility as readily accessible markers serves as a basis for a more thorough evaluation as it not only indicates the existence of the tumor but also changes in the host immunological state.(25) In this study, higher NLR and PLR were found in prostate cancer subjects, while LMR was lower. The results of this study were supported by previous studies that mention that high blood PSA and high NLR are associated (26), as it may point to an issue with the host's adaptive capacity to manage inflammation (27). Meanwhile, elevated PLR suggesting that platelets participate to the growth and invasion of tumors and help cancer cells avoid immune monitoring.(28)

In this study, PSA, the main biomarker for prostate cancer screening, has a higher AUC of 0.981 than other inflammatory markers. Inflammatory indicators did, however, continue to show some promise in the prediction of metastatic prostate cancer. When inflammatory markers and PSA were used together in a prior research, the AUC for predicting prostate cancer increased to 0.791, indicating that inflammatory indicators offer extra information beyond PSA testing. Combining serum PSA with inflammatory markers might be able to address the restriction of using PSA alone or inflammatory markers, increase the diagnostic effectiveness for metastatic of prostate cancer, while offering important information for prostate cancer therapy and early detection.(29)

In this study, IL-8 concentration were raised in cases with prostate cancer compared with health control, this suggests that IL-8 activity directly amplifies tumor biological behaviour.(30,31) IL-8 emerged as the strongest overall predictor in the integrated model (β=2.107, p=0.010), emphasizing its central role in inflammation-mediated tumor progression.(31) IL-8 is promote epithelial mesenchymal transition, angiogenesis, and metastatic potential, while also enhancing AR signaling through ligand-independent pathways.(32,33) Previous studies examined the IL-8 expressions in patients with benign prostatic hyperplasia and prostate cancer and showed that urine retention was substantially more common in people whose condition was exacerbated by BPH alone than in those with prostate cancer.(6,34,35) Prostatic fluid contains key cytokines such as IL-6 and IL-8, which are implicated in the immunological inflammation of prostate hyperplasia.(36,37)

Immune suppression markers further reinforced the biological coherence of the model. Compared to healthy subjects, CTLA-4 level in prostate cancer subjects was significantly increased. Even after multivariate linear regression, CTLA-4 remained independently associated with PSA with high AUC values, sensitivity, and specificity. As an immune checkpoint molecule that suppresses T-cell activation and contributes to immune evasion in prostate cancer, elevated CTLA-4 level being associated with advanced disease and poorer clinical outcomes.(38) Although CTLA-4 and PSA reflect distinct biological processes, increased CTLA-4 expression may accompany

Table 7. ROC curve analysis of physiological and immunological predictors for discrimination of prostate cancer subjects.

Biomarker	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)	*p*-value
Prostatic Acid Phosphatase	0.84 (0.71–0.91)	≥40	92.5	88	<0.001*
IL-8	0.991 (0.980–1.00)	>20.5	95.6	92	<0.001*
CTLA-4	1.00 (0.998–1.00)	>3.9	99	98.3	<0.001*

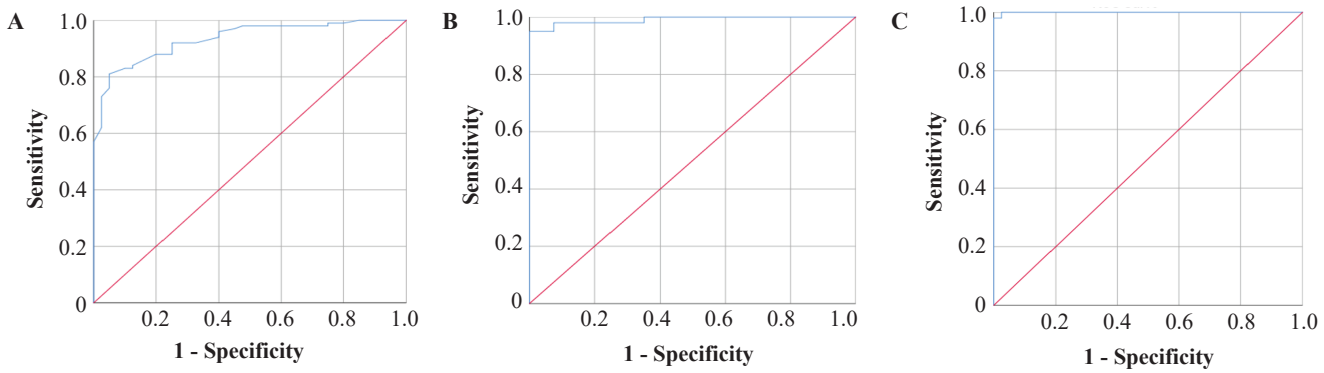

Figure 2. ROC curve demonstrating the diagnostic performance of physiological and Immunological biomarkers for distinguishing prostate cancer subejcts from controls. A: Prostatic acid phosphatase; B: IL-8; C: CTLA-4.

elevated PSA levels in advanced prostate cancer due to the formation of a tumor microenvironment that is immunosuppressive.(39,40)

Though this study provides a potential predictive model of prostate cancer progression, but this study has short follow-up period and lack of information on survival, metastasis, and recurrence. Further study that can overcome these issues are suggested to be perform with larger sample size and multiple study location. In spite of that, current study lestablishes the foundation for further investigation of the mechanisms underpinning immunological markers in prostate cancer and for the development of more comprehensive prospective investigations.

Conclusion

AR, PAP, IL-8, IL-35, and CTLA-4 are independently associated with PSA level in predicting prostate cancer progression. However, after integrated model analysis, only PAP, IL-8, and CTLA-4 remained significantly associated with PSA levels, indicating that combination of PSA, PAP, IL-8, and CTLA-4 levels might account for a substantial proportion of progression and biochemical activity in prostate cancer patients, and may serve as potential combined marker.

Acknowledgments

We would especially want to thank the whole Anbar Cancer Center staff in Al-Ramadi City and Oncology Hospital in Medical City for assisting ours in the collection and analysis of the samples. We owe profound thank to all patients and healthy who agreed to participate in the study. We extend our sincere thanks to Prof. Dr. Nasir N. for his important role in the statistical analysis of the results.

Authors Contribution

SM and AT were involved in the imagination and design of the study. SM, NH, and AT participated in data acquisition. SM and NH performed the data analysis. NH and AT contributed to the interpretation of the results and design of figures and tables. SM prepared the manuscript. The manuscript underwent critical revision by all authors, and the final version was authorized for publishing.

Ethical Statement

The ethical permission for the study was granted from Anbar University/College of Medicine Ethical Comittee (No:18 on February 20, 2024), and each subject gave their informed consent after being fully briefed about the methods. The study complied with the Declaration of Helsinki.

Conflict of Interest

The authors have no conflict of interests to disclose.

References

1. Ferlay J, Laversanne M, Ervik M, Lam F, Colombet M, Mery L, *et al*. Global Cancer Observatory: Cancer Tomorrow. Lyon (France): International Agency for Research on Cancer; 2020.

2. Meilana A, Wijaya A. Identiication of biomarkers for prostate cancer. Indones Biomed J. 2014; 6(3): 123-36.
3. Budaya TN, Daryanto B, Seputra KP, Fabrianta DM, Ekaputra AA, Dewi RARK, *et al.* SARM Rad140 increases osteoblasts, muscle fiber size, myonuclei, and reduces osteoclasts in orchidectomized Wistar rats. Mol Cell Biomed Sci. 2025; 9(1): 30-8.
4. Hansen SB, Unal B, Kuzu OF, Saatcioglu F. Immunological facets of prostate cancer and the potential of immune checkpoint inhibition in disease management. Theranostics. 2024; 14(18): 6913-30.
5. Suárez JF, Zamora V, Garin O, Gutiérrez C, Pont À, Pardo Y, *et al.* Mortality and biochemical recurrence after surgery, brachytherapy, or external radiotherapy for localized prostate cancer: A 10-year follow-up cohort study. Sci Rep. 2022; 12(1): 12589. doi: 10.1038/s41598-022-16395-w.
6. Foenna YG, Calapoğlu NS, Sancer O, Tepebaşı MY, Calapoğlu M. miR-29 family as epigenetic regulators of DNMTs in prostate cancer and benign prostatic hyperplasia. Indones Biomed J. 2025; 17(2): 134-42.
7. Mohammed Al-Fartosy AJ, Ati MH. A predictive clinical markers to make prostate cancer and benign prostate hyperplasia easy diagnosis. Biochem Cell Arch. 2021; 21(2): 2939-47.
8. Baston C, Preda A, Iordache A, Olaru V, Surcel C, Sinescu I, *et al.* How to integrate prostate cancer biomarkers in urology clinical practice: an update. Cancers. 2024; 16(2): 316. doi: 10.3390/cancers16020316.
9. Alemany M. The roles of androgens in humans: biology, metabolic regulation and health. Int J Mol Sci. 2022; 23(19): 11952. doi: 10.3390/ijms231911952.
10. Michaud JE, Billups KL, Partin AW. Testosterone and prostate cancer: An evidence-based review of pathogenesis and oncologic risk. Ther Adv Urol. 2015; 7(6): 378-87.
11. Arnold JT, Le H, McFann KK, Blackman MR. Comparative effects of DHEA vs. testosterone, dihydrotestosterone, and estradiol on proliferation and gene expression in human LNCaP prostate cancer cells. Am J Physiol Endocrinol Metab. 2005; 288(3): E573-84.
12. Sharma P, Zargar-Shoshtari K, Pow-Sang JM. Biomarkers for prostate cancer: Present challenges and future opportunities. Future Sci OA. 2016; 2(1): FSO72. doi: 10.4155/fso.15.72.
13. Koehler D, Maurer T. Prostatic acid phosphatase: A rediscovered target in prostate cancer. Nat Rev Urol. 2026 (Online ahead of print). doi: 10.1038/s41585-026-01156-5.
14. Meiliana A, Wijaya A. Combining epigenetic and immunotherapy in cancer: Molecular mechanisms. Indones Biomed J. 2023; 15(1): 1-24.
15. Ullah A, Jiao W, Shen B. The role of proinflammatory cytokines and CXC chemokines (CXCL1-CXCL16) in the progression of prostate cancer: insights on their therapeutic management. Cell Mol Biol Lett. 2024; 29(1): 73. doi: 10.1186/s11658-024-00591-9.
16. Zhu J, Yang X, Wang Y, Zhang H, Guo Z. Interleukin-35 is associated with the tumorigenesis and progression of prostate cancer. Oncol Lett. 2019; 17(6): 5094-102.
17. Zhao Z, Chen X, Hao S, Jia R, Wang N, Chen S, *et al.* Increased interleukin-35 expression in tumor-infiltrating lymphocytes correlates with poor prognosis in patients with breast cancer. Cytokine. 2017; 89: 76-81.
18. Fallah J, Agrawal S, Gittleman H, Fiero MH, Subramaniam S, John C, *et al.* FDA approval summary: Lutetium Lu 177 vipivotide tetraxetan for patients with metastatic castration-resistant prostate cancer. Clin Cancer Res. 2023; 29(9): 1651-7.
19. Agrawal S, Arora S, Amiri-Kordestani L, de Claro RA, Fashoyin-Aje L, Gormley N, *et al.* Use of single-arm trials for US Food and Drug Administration drug approval in oncology, 2002-2021. JAMA Oncol. 2023; 9(2): 266-72.
20. Chen D, Chen W, Ye R, Li L, Miao F, Kong X, *et al.* Identification of prognostic biomarkers and development of a prediction model for prostate cancer. Front Immunol. 2025; 16: 1709264. doi: 10.3389/fimmu.2025.1709264.
21. Srinivasan S, Stephens C, Wilson E, Panchadsaram J, DeVoss K, Koistinen H, *et al.* Prostate cancer risk-associated single-nucleotide polymorphism affects prostate-specific antigen glycosylation and its function. Clin Chem. 2019; 65(1): e1-e9.
22. Aurilio G, Cimadamore A, Mazzucchelli R, Lopez-Beltran A, Verri E, Scarpelli M, *et al.* Androgen receptor signaling pathway in prostate cancer: from genetics to clinical applications. Cells. 2020; 9(12): 2653. doi: 10.3390/cells9122653.
23. Kumar R, Sena LA, Denmeade SR, Kachhap S. The testosterone paradox of advanced prostate cancer: mechanistic insights and clinical implications. Nat Rev Urol. 2023; 20(5): 265-78.
24. Flores JM, Bernie HL, Miranda E, Nascimento B, Schofield E, Benfante N, *et al.* The relationship between PSA and total testosterone levels in men with prostate cancer. J Sex Med. 2022; 19(3): 471-8.
25. Arianmanesh F, Bagheri S, Karimi MA, Izadi S, Ahmadi MH. The evaluation of diagnostic, prognostic, and predictive role of hematologic inflammatory indices NLR, PLR, and LMR in common solid tumors. Cancer Rep. 2025; 8(12): e70407. doi: 10.1002/cnr2.70407.
26. Shafique K, Proctor MJ, McMillan DC, Qureshi K, Leung H, Morrison DS. Systemic inflammation and survival of patients with prostate cancer: evidence from the Glasgow Inflammation Outcome Study. Prostate Cancer Prostatic Dis. 2012; 15(2): 195-201.
27. Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, *et al.* Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. J Hepatol. 2013; 58(1): 58-64.
28. Liang CL, Li WD, Wang J. Preoperative immune-inflammation index in predicting the diagnosis and adverse pathological features of prostate cancer. Front Oncol. 2025; 15: 1537542. doi: 10.3389/fonc.2025.1537542.
29. Coradduzza D, Sibono L, Tedde A, Marra S, De Miglio MR, Zinellu A, *et al.* Diagnostic stratification of prostate cancer through blood-based biochemical and inflammatory markers. Diagnostics. 2025; 15(11): 1385. doi: 10.3390/diagnostics15111385.
30. Liu J, Wang ZQ, Li M, Zhou MY, Yu YF, Zhan WW. Establishment of two new predictive models for prostate cancer to determine whether to require prostate biopsy when the PSA level is in the diagnostic gray zone (4-10 ng/mL). Asian J Androl. 2020; 22(2): 213-6.
31. Maynard JP, Ertunc O, Kulac I, Baena-Del Valle JA, De Marzo AM, Sfanos KS. IL8 expression is associated with prostate cancer aggressiveness and androgen receptor loss in primary and metastatic prostate cancer. Mol Cancer Res. 2020; 18(1): 153-65.
32. McClelland S, Maxwell PJ, Branco C, Barry ST, Eberlein C, LaBonte MJ. Targeting IL-8 and its receptors in prostate cancer: Inflammation, stress response, and treatment resistance. Cancers. 2024; 16(16): 2797. doi: 10.3390/cancers16162797.
33. Tufail M, Jiang CH, Li N. Immune evasion in cancer: Mechanisms and cutting-edge therapeutic approaches. Signal Transduct Target Ther. 2025; 10(1): 227. doi: 10.1038/s41392-025-02280-1.
34. Oseni SO, Naar C, Pavlović M, Asghar W, Hartmann JX, Fields GB, *et al.* The molecular basis and clinical consequences of chronic inflammation in prostatic diseases: prostatitis, benign prostatic hyperplasia, and prostate cancer. Cancers. 2023; 15(12): 3110. doi: 10.3390/cancers15123110.

35. Pratiwi SE, Wahyuningrum SN, Putri RP, Danarto D, Heriyanto DS, Arfian N*, et al*. ZEB1 is negatively correlated with E-cadherin in prostatic anomaly tissue. Mol Cell Biomed Sci. 2022; 6(1): 28-34.
36. Zhou C, Zhang J, Chen Y, Wang H, Hou J. Interleukin-35 as a predictor of prostate cancer in patients undergoing initial prostate biopsy. Onco Targets Ther. 2017; 10: 3485-91.
37. Chatrabnous N, Ghaderi A, Ariafar A, Razeghinia MS, Nemati M, Jafarzadeh A. Serum concentration of interleukin-35 and its association with tumor stages and FOXP3 gene polymorphism in patients with prostate cancer. Cytokine. 2019; 113: 221-7.
38. Li F, Cui L, Zhang G, Hao J. Predictive value of peripheral blood immune markers for castration-resistant prostate cancer development after endocrine therapy. Front Oncol. 2026; 16: 1696687. doi: 10.3389/fonc.2026.1696687.
39. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. Cell. 2015; 161(2): 205-14.
40. Lilja H, Ulmert D, Vickers AJ. Prostate-specific antigen and prostate cancer: Prediction, detection and monitoring. Nat Rev Cancer. 2008; 8(4): 268-78.