INVESTIGATION OF SOME BIOMARKERS ASSOCIATED WITH MALIGNANT AND BENIGN PROSTATE TUMORS IN IRAQI MALES

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Abstract. Prostate cancer (PCa), a challenging ailment, impacts a substantial number of men globally, primarily in prestigious regions. The study aimed to explore the functions of PD-1, PDL-1, PSA, and testosterone markers in detecting the pathogenesis of PCa. Medical City-Baghdad hosted the research from July to October 2021. After examination and diagnosis by the Medical City consultant expert, 40 blood samples (20 benign and 20 malignant) were collected from prostate cancer (PCa) patients. Eight healthy individuals were used as a control group and their blood samples were taken. Patients and controls ranged in age from 20-49. The ELISA technique was employed to assess the levels of program death-1 (PD-1), program death ligand-1 (PDL-1), and prostate specific antigen (PSA), and testosterone parameters. The study found substantial differences ($P < 0.05$) across age groups and study groups, with malignant patients scoring highest (70.0%) at 40-49 years and benign patients scoring highest (45.0%) at 30-39 years. Elevated levels of PD-1, PDL-1, and PSA are observed in both benign and malignant PCa compared to healthy. Neither benign nor malignant PCa had significantly lower testosterone levels than healthy PCa ($P > 0.05$). Both PD-1, PDL-1, and PSA show a remarkably high sensitivity (100%) when used to screen patients for prostate cancer. Finally, there is a negative correlation between PSA and PD-1, PDL-1 parameters. The PD-1, PDL-1, and PSA have been found to play significant roles in the development of prostate cancer and have shown high sensitivity in screening for PCa.

Keywords: prostate cancer (PCa), PD-1, PDL-1, PSA, testosterone.

List of Abbreviations

PCa – Prostate cancer

ELISA – Enzyme linked immunosorbent assay

PD-1 – Program death -1

PDL-1 – Program death ligand -1

PSA – Prostate specific antigen

CD8 – Cluster differentiation 8

CD4 – Cluster differentiation 4

mCRPC – Management of castrated resistant prostate cancer

BPH – Benign prostatic hyperplasia

DHT – Dihydrotestosterone

TRT – Testosterone replacement therapy

ROC – Receiver operating characteristic

AUC – Area under the curve

APC – Antigen presenting cells

Introduction

Prostate cancer (PCa), is one of the many types of cancer such as bladder cancer (Ismael *et al.*,2023; Rasha *et al.*, 2022; Sama *et al*., 2019), gastric cancer (Bresam *et al*., 2023a; Sultan *et al*., 2023), breast cancer (Hameedi *et al*., 2022; Rasheed *et al*., 2022; Chemia *et al*., 2019), Colorectal and lung cancer (Rasheed & Al-Abassi, 2021; Lateef *et al*., 2021), Peptic Ulcer (Bresam *et al*., 2023b), and Bladder Cancer (Al-Humairi & Ad'hiah, 2023). Prostate cancer ranks fifth in terms of mortality rates and accounts for 13.5% of all malignancies globally; it is the second most common disease among men (Barsouk *et al*., 2020). Prostate cancer was diagnosed in 1.3 million Medicaid patients in 2018, and 359,000 people died from it (Barsouk *et al*., 2020). There is a genetic component, and members of Afro-Caribbean ethnic minorities are at a higher risk than members of other ethnic groups; nevertheless, there is also a time component, with males in their 45s and 50s being at risk (Merriel *et al*., 2018). Along with surgery, radiation, and chemotherapy, immunotherapy using PD-1/PD-L1 blockers has recently evolved as an additional viable option for cancer management (Bodepudi *et al*., 2021). Restricting adaptive immunity and reestablishing the mechanism of cancer immune escape, PD-1/PD-L1 antagonists enable the immune system to regenerate and directly attack tumour cells by blocking the PD-1/PD-L1 signal pathway (Hadeel & Rana, 2023; Carosella *et al.*, 2015). These CD8+ T cells were unable to pro-

vide an adequate anticancer response because their PD-1 levels were elevated in the prostate cancer habitat according to Sfanos *et al*., (2009). On the other hand, research conducted on animals demonstrated that the efficacy and duration of immunotherapy in prostate cancer were associated with the presence of PD-1/PD-L1 on crucial CD8+ T cells. The administration of a combination of PD-1/PD-L1 antagonists in the intervention group resulted in a significant increase in the duration of disease-free progression survival (He *et al*., 2021; Hameedi *et al*., 2022). The function of PD-1/PD-L1 blockers has garnered increased attention as tumour immunotherapy has advanced (Ahmad *et al.*, 2018). The combination of PD-1/PD-L1 inhibitors with androgen receptor blockers can enhance the efficacy and viability of tumour therapy in clinical trials (Bishop *et al.,* 2015; Hala *et al.,* 2021). Graff *et al.* (2016) used PD-1 blockers to carry out a phase II clinical trial management of castrated resistant prostate cancer (mCRPC). The PSA is naturally produced by the prostate epithelial tissue and is not cancer specific; thus, it can be diluted by factors such as body mass index (BMI), androgen levels, prostatitis, benign prostatic hyperplasia (BPH), androgen rates, as well as by prostatic trauma (biopsy), urinary incontinence, and ejaculatory within 24 hours (McDougal *et al.,* 2016). Values ranging from 4 to 10 ng/mL are considered to be in the «grey zone», indicating uncertainty. However, any value beyond 4 ng/mL is indicative of a potential presence of prostate cancer (PCa) and should be viewed with caution. The distinguishing factor of PSA lies in its propensity for erroneous diagnosis and superfluous treatments, which represent its most significant hazards (Nassir, 2020). Furthermore, androgens have a significant role in the pathophysiology of the prostate as well as the maturation of male sexuality. Dihydrotestosterone (DHT), which is produced by 5-reductase from testosterone in peripheral tissues, and testosterone, which is produced by testicular Leydig cells, are the two primary androgens that are found in males (Michaud *et al*., 2015). In people with prostate cancer, the levels of testosterone in their serum are lower than normal. Testosterone replacement therapy, sometimes known as TRT, is a treatment for treating testosterone deficiency in males. In light of the fact that testosterone has historically been considered to be the primary driver of prostate cancer (PC), the use of this drug raises concerns regarding the potential for PC (Xie *et al.,* 2021). Within the context of prostate cancer pathogenesis, the purpose of the study that was carried out was to investigate the predictive functions that PD-1, PDL-1, PSA, and testosterone parameters play.

Material and Methods

Source of Samples

The study was conducted in the Medical City/Baghdad area between July and October 2021. The expert doctor at Medical City examined 40 blood samples provided by individuals diagnosed with prostate cancer to identify and analyse. There were twenty benign samples, twenty malignant samples, and eight control samples. We collected 8 blood samples from individuals who were in good health to serve as our control group. Individuals with both benign and malignant diseases, along with healthy subjects, fell within the age range of 20 to 49.

Ethical approval

The study received ethical approval from authorised personnel to guarantee that the blood samples were appropriately authorised, with signed informed permission from the patients. Specimens were obtained subsequent to obtaining authorization from the Ethics committee. The experiment was successfully concluded following the acquisition of the requisite authorization from the Department of Basic Science, College of Nursing, and University of Baghdad.

Collection and preparation of blood samples

Regarding the blood, 5 millilitres were obtained in an aseptic manner using a 5 millilitre disposable syringe. Placing the blood in an unadorned tube, it was allowed at 4 °C for 15 minutes to clot. After that, it went through a 10 minute centrifugation run at 5,000 rpm. Before being examined for biochemical analysis, sera were separated and preserved in Ep-

pendorf tubes, which were thereafter placed in a deep freezer at -20 °C. Serum PD-1, PDL-1, PSA, and testosterone levels were measured using Sandwich Enzyme-linked immunosorbent assays (ELISA) (CUSABIO Company).

Statistical analysis

Initially, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of the PD-1, PDL-1, PSA, and testosterone parameters. The Mean ± St. Error was used to depict the results that were deemed normal (meaning there were no notable differences). For comparisons involving more than two groups, the significance of differences was evaluated using the F test. To find statistically significant differences in frequency, we used Pearson's chi-square test, which included expressing additional components as percentages. The Pearson correlation coefficient (r) was used to assess the intensity and degree of the link between the parameters. Parameter sensitivity and specificity, as well as the Area Under the Curve (AUC), were computed using the Receiver Operating Characteristic (ROC) curve. $P \leq 0.05$ was measured significant. Our data were analyzed using SPSS v. 21.0 statistical software.

Results

Age and study groups

The results indicate a notable difference in the percentages of malignant and benign patients based on age and study groups ($p < 0.05$). In the malignant group, the highest percentage was 70.0% for patients aged 40-49, while the lowest percentage was 0.0% for patients aged 20-29. In the benign group, the highest percentage was 45.0% for patients aged 30-39, and the lowest percentage was 15.0% for patients aged 40-49. Finally, both the 30-39 and 40-49 age groups received the same percentage of points from the control groups (50.0%) (Table 1).

The study found that malignant patients had the greatest mean levels of PDL-1 and PSA parameters (79.507 ± 3.653, 2.320 ± 0.171) respectively, whereas healthy individuals had the lowest mean values (12.509 ± 3.549, 0.860 ± ± 0.068) respectively. There was a significant difference among the three groups ($p < 0.05$). The PD_1 parameter exhibited the greatest mean level in malignant patients (169.603 ± ± 9.965) and the lowest mean level in benign patients (35.858 ± 2.044), with a significant difference seen among the three groups ($p < 0.05$). The testosterone levels did not exhibit any significant differences among the three groups ($p > 0.05$) (Table 2 and Fig. 1).

The study results showed that PD_1 (AUC = = 1.000 and Sn = 100%), PDL_1 (AUC = 1.000 and Sn = 100%), and PSA (AUC = 0.975 and Sn = 95%) shown great sensitivity in detecting prostate cancer, however testosterone exhibited poor sensitivity (AUC = 0.38 and Sn = 0.40%). The difference in the sensitivities of these markers was statistically significant ($P < 0.05$). According to specificity, testosterone and PD_1 exhibited the highest specificity rates (50%), surpassing PDL_1 (37%) and PSA (25%) with a statistically significant difference ($P < 0.05$) (Table 3 and Fig. 2).

Table1

Comparison between age groups and study groups; relation of PD_1, PDL_1, PSA and testosterone parameters within study groups

			Groups			Total	P value
			Malignant	Benign	Control		
Age	20-29	n	0	8	0	8	0.001***
		%	0.0%	40.0%	0.0%	16.7%	
	30-39	n	6	9	4	19	
		%	30.0%	45.0%	50.0%	39.6%	
	40-49	n	14	3	4	21	
		%	70.0%	15.0%	50.0%	43.8%	
Total		n	20	20	8	48	
		%	100.0%	100.0%	100.0%	100.0%	

Correlation studying among parameters

Our results show high positive significant correlation between PD_1 and PDL-1 (r = = 0.792** p < 0.05) and negative correlation among PSA vs. PDL-1, and PD-1 (r = –0. 016 p > 0.05 and r = –0. 294 p > 0.05) respectively.

Table 2

Mean levels of PD_1, PDL_1, PSA and testosterone parameters within study groups

		N	Mean	Std. Error	P value
PD_1	Malignant	20	169.603	9.965	0.001*** LSD = 39.21
	Benign	20	35.858	2.044	
	control	8	37.019	2.469	
PDL_1	Malignant	20	79.507	3.653	0.001*** LSD = 16.02
	Benign	20	46.936	1.855	
	control	8	12.509	3.549	
PSA	Malignant	20	2.320	0.171	0.001*** LSD = 1.35
	Benign	20	2.717	0.215	
	control	8	0.860	0.068	
Testosterone	Malignant	20	1.857	0.172	0.08
	Benign	20	1.569	0.097	
	control	8	2.141	0.184	

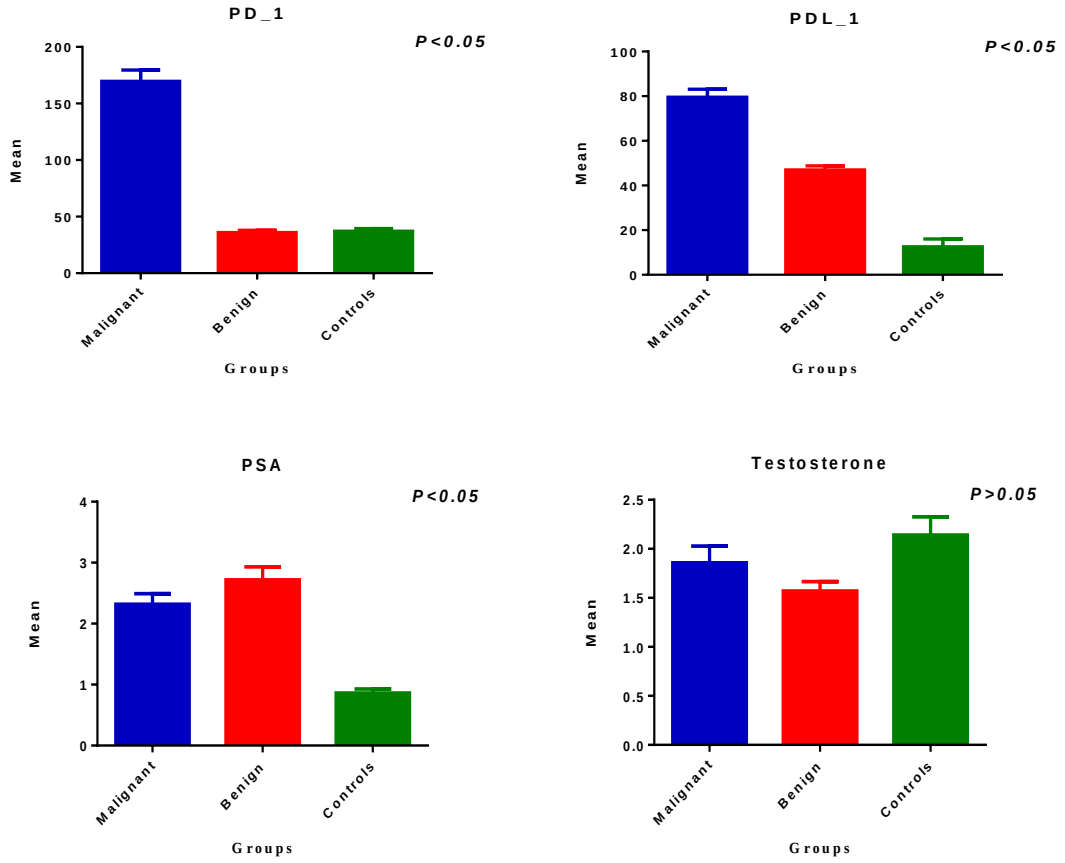

Fig. 1. Mean levels of PD_1, PDL_1, PSA and testosterone parameters within study groups

Table 3

ROC curve, sensitivity and specificity of variable in malignant and control

Parameters	AUC	St. Error	Sig.	C.I (95%)		Sensitivity	Specificity
				Lower	Upper		
PD_1	1.000	.000	.000	1.000	1.000	100%	50%
PDL_1	1.000	.000	.000	1.000	1.000	100%	37%
PSA	.975	.025	.000	.926	1.000	95%	25%
Testosterone	.381	.106	.334	.173	.590	40%	50%

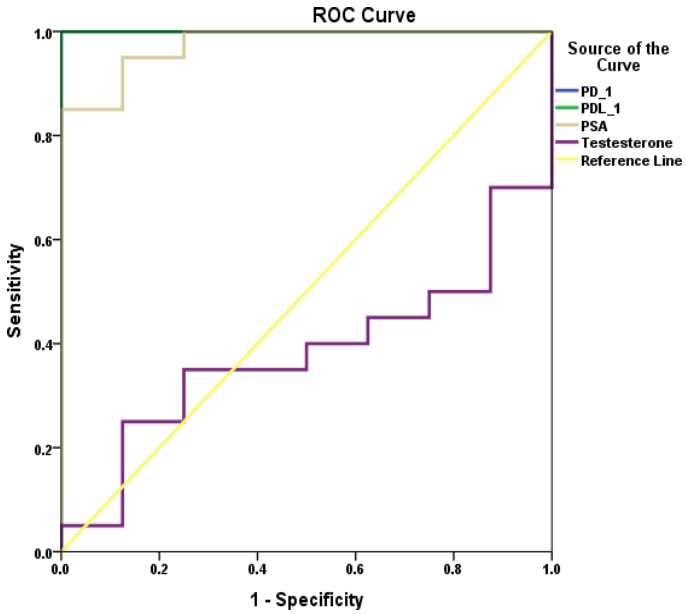

Fig. 2. ROC curve, sensitivity and specificity of parameters in malignant and control

Table 4

Correlation relationship study among parameters

r = Pearson correlation p = probability		PD_1	PDL_1	PSA	Testosterone
PD_1	r	1	.792**	-.294	.098
	P		p < 0.05	p > 0.05	p > 0.05
PDL_1	r	.792**	1	-.016	.240
	P	p < 0.05		p > 0.05	p > 0.05
PSA	r	-.294	-.016	1	.095
	P	p > 0.05	p > 0.05		p > 0.05
Testosterone	r	.098	.240	.095	1
	P	p > 0.05	p > 0.05	p > 0.05	

Discussion

According to results, the most common kind of cancer discovered in males over the age of 65 is prostate cancer (Bray *et al.*, 2018). The frequency of prostate cancer and the number of deaths caused by it both increase with age around the world, with the average age of diagnosis reaching 66 years (Rawla, 2019). The risk

of prostate cancer rises with age (Ferlay *et al.*, 2019). Although only one out of every 350 people under the age of 50 would be diagnosed with testicular cancer, the prevalence of the disease increases to one out of every 52 males between the ages of 50 and 59 (Perdana *et al*., 2017). Adults over the age of 65 have a nearly 60% chance of developing prostate cancer (Rawla, 2019). A number of factors, including weakened immune systems, malfunctioning organs, chronic illnesses, underdiagnoses, disparities in screening technologies, and healthcare access disparities, may exacerbate the age-related rise in prostate cancer cases. We found that instances of malignant prostate cancer were more common in men aged 40–49 than benign prostate cancer, which was less common in this age group. The results of this study are in line with previous research showing that PD-1 and PDL-1 levels are elevated in prostate cancer patients compared to healthy males (He *et al*., 2021). Research into cancer genetic targeted treatment has recently emerged as a hot topic in the cancer interest area. Increased mortality of effector T cells due to the PD-1/PD-L1 cascade (Verze & Lorenzetti, 2016) limits T cell activation, and reduces the body's anti-tumor immune reaction, is linked in the development and progression of malignancies (Kimberly *et al*., 2016). Many different kinds of tumours have PD-1/PD-L1, which are crucial members of the B7 group. Evidence suggests that overexpressed PD-L1 in tumour tissues downregulates anti-tumor activities via binding to its receptor PD-1. When PD-L1 is overexpressed on APC cells, it can promote tumour cell growth and lead to the killing of related T lymphocytes in a prostate cancer setting, hence reducing the likelihood of malignancy. T cells are unable to proliferate and release anti-tumor chemicals due to the PD-1 and PD-L1 link (Modena *et al.*, 2016). T cell reactivation, proliferation, and anti-tumor resistance are enhanced by antibodies that bind to PD-1 or PD-L1, blocking the PD-1 pathway. Multiple clinical trials demonstrated that inhibitors of the PD-1/PD-L1 signalling pathways, namely anti-PD-1 and anti-PD-L1, have substantial anticancer benefits (Massari *et al*., 2016). PD-1/PD-L1 production has been found to be elevated in a variety of tumors, including breast, ovary, and esophagus cancers, according to many studies (Fajgenbaum, 2020; Soliman *et al.*, 2014). The use of PD-1/PD-L1 antagonists in clinical trials involving prostate cancer patients need to be encouraged. Although the long-term efficacy of immune checkpoint monotherapies is debatable in most patients, several subsets of patients have shown encouraging early results. Members of these subgroups may include those with extremely high levels of PD-L1 overexpression (Isaacsson Velho & Antonarakis, 2018). PD-1/PD-L1 production in tissues was found to be ineffective in forecasting tumor growth in prostate cancer patients who had undergone radical prostatectomy, according to Sharma *et al.*, (2019). According to the studies, PD-L1 status, rather than PD-1 status, is linked with clinical characteristics in primary human prostate cancers (Xian *et al*., 2019). The results of the study reveal that individuals with prostate cancer have elevated amounts of prostate- specific antigen (PSA) than healthy individuals, which is consistent with Yusim *et al*. (2020). Yusim *et al*. (2020) report high PSA sensitivity (AUC = 0.75 and sensitivity 77%) in screening individuals with prostate cancer, which is consistent with our findings of high PSA sensitivity (AUC= 0.97 and sensitivity 100%). PSA screening has also revolutionized therapy response tracking and illness recurrence diagnosis (Pezaro *et al*., 2014). According to research, up to 25% of individuals with normal PSA levels may have hidden prostate cancer (Merriel *et al*., 2018). Each of these findings suggest indicated PSA is not in itself a strong predictor of biopsy outcomes, and should be used in conjunction with other indicators to improve the overall quality (Vickers *et al*., 2010). Despite the fact that just using the PSA level for prostate cancer diagnosis is not advised, it would be the most valuable tool for follow-up following active therapy to date (Nassir, 2020). Since it has consequences in angiogenesis, penetration, metastases, and cancer signals, it'll still continue to have been in the limelight for prostate cancer (Moradi *et al.*, 2019). Prostate-specific antigen (PSA) has been suggested as a potential target molecule for the

treatment of prostate cancer due to its functional presence in prostate tissue and significant involvement in prostate cancer signalling pathways including proliferative, invasive, metastatic, angiogenesis, apoptosis, immune function, and targeted tumour control (Moradi *et al*., 2019). According to Nordström *et al.* (2018), PSA density could help guide biopsy selections and save some men from the morbidity of a prostate biopsy and detection of limited prostate cancer. The link between PSA level and the occurrence of metastases, according to Singh *et al*., (2019), underlines the utility of bone scan spect in prostate cancer stages. Prostate diagnostic test, at best, results in a slight reduction in disorder death over ten years but it has no effect on overall death (Ilic *et al.*, 2018). In this study, patients exhibited reduced testosterone levels compared to healthy individuals, however no significant statistical difference was seen. Testosterone treatment provides substantial clinical and general medical advantages for individuals who have prostate cancer and are experiencing testosterone deficiency. Although further safety evidence is required, testosterone therapy is a feasible therapeutic choice for individuals with low prostate cancer who have received surgery or radiation (Morgentaler & Caliber, 2019). It is no longer true that high levels of testosterone in the blood are linked to a higher chance of prostate cancer. New research shows that androgen-stimulated prostate cancer grows fastest when blood testosterone levels are pretty low. This means that testosterone treatment might be a good choice for some men with prostate cancer and hypogonadism (Khera *et al.*, 2014). The testosterone levels of prostate cancer patients following radiotherapy were found to be significantly lower than pre-treatment values, according to the data (Mortezaee *et al*., 2020). Reason being, treatment-induced modifications might manifest in a variety of ways; for example, d-loop area genetic variations in breast cancer patients compared to those with other cancers (Buniya *et al.*, 2018; Hassoon *et al.*, 2017), As a result of this, several research have been conducted with the same objective, which is to discover an alternative medication for these purples. One example of this is the use of the Newcastle disease virus as a modified vector in gene therapy (Rasoul *et al.*, 2022a; Rasoul *et al.*, 2023b), utilized bacterial protease derived from nature to target the MCF-7 breast cancer cell line (Abdulrazaq *et al.*, 2022), and chitinase, which was found to have a cytotoxic impact on cancer liver cell lines during the study (Saleh *et al.*, 2020).

Conclusions and Recommendations

Age proved to be a risk factor for the development of malignant prostate cancer in males. Hypothesised to have a role in prostate cancer pathogenesis include PSA, PD-1, and PDL-1 variables. These measurements are highly sensitive when used for disease screening. Characteristics of PD-1 and PDL-1 are inversely associated to PSA. Additional comprehensive study is required to fully understand the crucial role of additional variables, such as IL-6, in the development and progression of prostate cancer.

Funding: self.

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