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A PREDICTIVE CLINICAL MARKERS TO MAKE PROSTATE CANCER AND BENIGN PROSTATE HYPERPLASIA EASY DIAGNOSIS

Adnan Jassim Mohammed Al-Fartosy* and Mohammed Hammad Ati

Department of Chemistry, College of Science, University of Basra, Basra, Iraq.
*e-mail : adnan.jassim@uobasrah.edu.iq

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ABSTRACT : Prostate diseases are one of the most common health problems that afflict men worldwide. Hence, in the present study, we sought to assess clinical markers to make prostate cancer (PCa) and benign prostate hyperplasia (BPH) easy to diagnose. The study included 63 patients (volunteers) aged between 50–78 years: 32 with PCa, 31 with BPH and 33 healthy controls. Samples were collected from Tumors Center' in Al-Sadr Teaching Hospital in Basrah province from October 2020 till April 2021. Compared with normal controls, patients with PCa and BPH had significantly (P<0.01) increased levels of serum insulin, prostatic specific antigen (PSA), kallikrein-10 (KlK-10), nitric oxide (NO), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ). In addition, there was a significant (p<0.01 in PCa and p<0.05 in BPH) increased in glucose, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Cortisol, sex hormone-binding globulin (SHBG) and mesothelin (MSLN). On the other hand, Luteinizing hormone (LH), stimulating follicle hormone (FSH) decreased significantly (p<0.01 in PCa and p<0.05 in BPH patients). At the same time, estradiol (E2) was significantly (P<0.01) lower in PCa and non-significantly changed (p>0.05) in BPH. Asymmetric dimethylarginine (ADMA) was significantly (P<0.01) higher in PCa and non-significantly changed (p>0.05) in BPH. The area under the curve (AUC) indicates that insulin, HOMA-IR, Cortisol, SHBG, PSA, KlK-10, MSLN, ADMA, NO, IL-6, TNF-α and IFN-γ could potentially be used as predictive biomarkers in PCa than in BPH. Considering the combination of obesity, insulin resistance, and some markers of tumor, pro-inflammatory and oxidative stress may demonstrate excellent indicators for risk of PCa and BPH among men.

Key words : Insulin resistance, prostate cancer, tumor markers, oxidative stress, pro-inflammatory.

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INTRODUCTION

Many prospective studies have reported that obesity or being overweight may be promoted cancerogenesis as well, as it is associated with benign prostate hyperplasia as a risk factor. However, there is no direct association between prostate cancer (PCa) and benign prostate hyperplasia (BPH) from the molecular aspect. Hence, these diseases may represent two separate pathways of pathophysiological (Siemińska *et al*, 2018).

The prostate cancer pathogenesis is likely multifactorial and very complex. Although various etiologic variables have been suggested, the genesis of this form of cancer could involve a wide range of molecular systems, pathways and mechanisms. Age, limited physical activity, eating habits, family history and obesity or being overweight are all recognized as risk factors for the development of PCa, according to scientific evidence from clinical research (Zazzo *et al*, 2018).

On the other hand, although the mechanisms behind those correlations are not fully clear or recognized, insulin resistance, inflammation processes and dysfunction of adipose tissue may lead to the cancerogenesis of the prostate (Al-Fartosy *et al*, 2021).

Furthermore, Another study suggested that an imbalance among reactive oxygen species (ROS) and antioxidant activity could lead to oxidative stress, which could have a role in the pathogenesis of various disorders, including PCa (Al-Fartosy *et al*, 2019).

Moreover, Several substances have been linked to cancer development and progression in the prostate and other organ systems. These include some hormones, markers of an immune response, adipokines, metalloproteinases, adhesion molecules and some growth

factors which proposed based on their levels which detected in different biospecimens as biomarkers for early predicting of cancer as well as for prognosis and follow-up in PCa (Jakobsen *et al*, 2017).

For an easy and early prostate cancer diagnosis, there is an urgent need for biomarkers for several reasons: improving detection and staging of cancer, identifying prostate cancer subclasses, outcome prediction after the treatment and selecting patients for different treatment options (Jakobsen *et al*, 2017). Hence, this study was designed to assess the effect of insulin resistance on some blood markers of tumor, pro-inflammatory and oxidative stress in prostate cancer patients in Basrah province (southern of Iraq).

METHODS

Subjects

The present study is a cross-sectional clinical experiment. Samples were collected from the "Tumors Center" in Al-Sadr Teaching Hospital in Barah-Iraq, during the period from October 2020 until April 2021. In this clinical study, 147 men volunteers have been consecutively and prospectively screened, with 96 men completing the survey. Three groups of participants, aged between 50–78 years were included in this study: (i) 32 patients who are suffering from prostate cancer; (ii) 31 patients who are suffering from benign prostate hyperplasia, and (iii) 33 healthy men who represented as control group, as illustrated in Fig. 1.

The study received ethical approval from Basrah University (No. 7/54/1083) and informed consent was obtained from each participant after explaining the procedures in full detail. In addition, the informed consent and ethical guidelines were followed, based on the declaration of Helsinki for the year 2000.

Inclusion and exclusion criteria

None of the patients had previously actinotherapy, chemotherapy, hormonal treatment, including androgen deprivation therapy or suffered from an acute illness. Patients with diabetes mellitus, heart, kidney, or liver failures, a prognosis of cancer apart from prostate cancer, a history of smoking that could have impacted our results were excluded from the study. And every other disease that could have affected our results and a related condition that can affect the level of free radicals, such as antioxidant

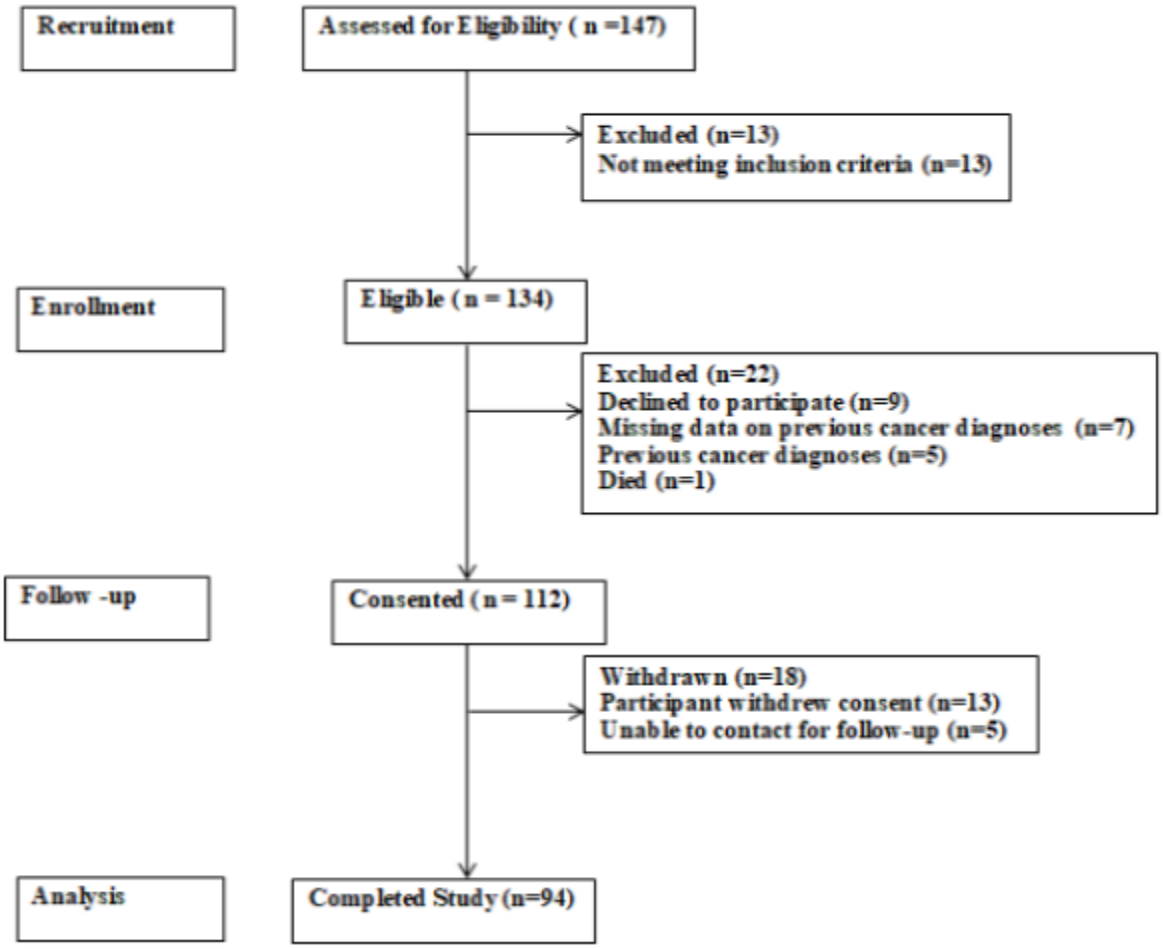

Fig. 1 : Participant recruitment flow diagram for the current study.

therapy were also excluded from the study.

Samples preparation

The samples were taken at 08.00 or 09.00 am after 12 hours of fasting, 30 minutes of rest in the supine position. First, fresh peripheral venous blood (5 mL) was collected from patients with prostate cancer (before the surgery), vein punching healthy volunteers and benign prostate hyperplasia patients. They were then injected into a gel containing a polypropylene tube (without anticoagulant) that allowed clotting for 20 minutes at room temperature. Next, the blood was centrifuged at 402 x g for 20 minutes after it had clotted to obtain the serum. The obtained serum was immediately used in the study's variable estimation, while the rest was preserved in deep freezing at (-70°C) until it was needed again.

Methods of biochemical estimation

The biochemical characteristics of control and PCa and BPH patients' blood samples were evaluated using standard procedures as follows: The conventional BMI equation used to compute BMI (Al-Fartosy, 2021): BMI (kg/m²) = weight (kg)/height (m²). Serum glucose was assayed on "UV-Vis Spectrophotometer (UV-EMC-LAB, Duisburg, Germany)" by using the kit (Abnova-KA0831/Taiwan). Human ELISA kits are used to measure the levels of all serum indicators. The level of every biomarker was evaluated using a standard curve and a sandwich ELISA method. The level of SPA was determined using the (BT-Lab, Shanghai- E1713Hu / China) kit, Klk-10 was determined using the (BT-Lab, Shanghai- E0783Hu / China) kit, and MSLN was determined using the (BT-Lab, Shanghai- E2191Hu / China) kit.

The level of TNF-α was determined using the (BT-Lab, Shanghai- E0082Hu / China) kit, IFN-γ was estimated by kit (BT-Lab, Shanghai- E0105Hu / China) and IL-6 was determined using the (BT-Lab, Shanghai-E0090Hu / China) kit. The level of NO was determined using the (BT-Lab, Shanghai- E1510Hu/ China) kit, and ADMA was estimated by kit (BT-Lab, Shanghai-E1887Hu / China). Insulin was assessed by kit (BT-Lab, Shanghai- E0010Hu / China), SHBG was determined using the (BT-Lab, Shanghai- E1011Hu / China) kit. Cortisol was determined using the (BT-Lab, Shanghai-E1003Hu / China) kit, FSH was calculated by "kit (Abnova-KA0213/Taiwan"), LH was calculated by "kit (Abnova-KA0214/Taiwan)," and E2 was calculated by kit (Abnova-KA0234/Taiwan). "Insulin resistance (IR) was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation (Al-Fartosy *et al*, 2020): HOMA-IR = Fasting insulin (ìIU/mL) x Fasting glucose (mg/dL) / 405".

Statistical analysis

SPSS software version 21 was used for statistical analysis (IBM Corporation, New York, USA). We used a student t-test to find the statistical significance. The ROC curve was used to quantify the sensitivities and specificities, as well as the 95 percent confidence interval, by plotting sensitivity (y-axis) against 1-specificity (x-axis); the AUC (area under the curve) was calculated as well. P 0.05 was regarded statistically significant, p 0.01 was deemed highly important, and an AUC value near 0 (or 1) suggests a solid diagnostic value since the values of one group are usually higher (or lower) than the other group's values.

RESULTS

Table 1 shows the overall demographic features of all men who volunteered for this study.

As compared to the healthy control group, the results (as shown in Tables II & III) revealed that patients men with PCa and BPH had significantly (P<0.01) increased levels of serum insulin, PSA, KlK-10, NO, IL-6, TNF-α, and IFN-γ. Also, the results indicated that there were significantly (p<0.01 in patients with PCa and p<0.05 in patients with BPH) increased levels of glucose, HOMA-IR, Cortisol, SFGB, and MSLN. On the other hand, data obtained in a study reported that LH and FSH decreased significantly (p<0.01 in patients with PCa and p<0.05 in patients with BPH). At the same time, the level of E2 was seriously (P<0.01) lower in patients with PCa and non-significantly changed (p>0.05) in patients with BPH. Furthermore, the level of ADMA was significantly (P<0.01) higher in patients with PCa and non-significantly changed (p>0.05) in patients with BPH as compared to the control group. Moreover, our data indicated a non-significant change (p>0.05) in BMI level in patients with PCa and BPH compared to the control group.

The AUC results obtained indicate that Insulin, HOMA-IR, Cortisol, SHBG, PSA, KlK-10, MSLN, ADMA, NO, IL-6, TNF-α and IFN-γ could potentially be used as greater predictive biomarkers in PCa (AUC=0.891, 0.984, 0.809, 0.828, 0.992, 0.887, 0.801, 0.9610.836, 0.930, 0.938, 0.867, respectively) than in BPH patients (AUC= 0.477, 0.391, 0.508, 0.594, 0.516, 0.582, 0.602, 0.445, 0.572, 0.516, 0.523, 0.641, respectively). While, LH, FSH, Estradiol, could not be used as predictive biomarkers in PCa patients (AUC= 0.00, 0.289, 0.023, respectively) and BPH patients (AUC= 0.734, 0.480, 0.578, respectively) as illustrated in Fig. 2.

Table 1: The demographic characteristics of the present study (n=96).

The characteristics		Prostate patients		Healthy controls
		PCa	BPH	
Total Subjects no.		33	31	32
Age (Mean ± SD)		68.33±7.24	65.2±7.73	54.9±5.08
Prostate Disease duration(Mean ± SD)		1.5±0.047	1.166±0.034	0
Prostate Cancer duration (Mean ± SD)		1.333±0.051	0	0
Family history		6	3	0
Demographic area	**Urban**	24	21	26
	Rural	9	10	6
Educational background	**Learned**	26	28	27
	Illiterate	7	3	5
Smoking habits	**Positive**	0	0	0
	Negative	33	31	32
Food habits	**Vegetarian**	2	0	0
	Non-Vegetarian	31	31	32
Employment status	**Employed**	27	26	28
	Not- Employed	6	5	4

Table 2 : Levels of total parameters measured in the present study for Prostate Cancer (PCa) patients and healthy control.

Biomarkes	Prostate cancer patients (PCa) n=33						Healthy controls n=32
	95% CI						
	Mean ± SD	Median	SE	Range	Lower	Upper	Mean ±SD
BMI (Kg/m^2)	32.52±2.82	30.2	0.49	28.50-37.2	24.69	40.34	34.63±1.14
F. Glucose (mg/dL)	126 ±3.04**	113.2	0.53	105-180	117.56	134.43	90.9±2.05
F. Insulin (mIU/L)	22.30±1.08**	17.4	0.19	4.79-42.28	19.30	25.29	7.47±1.06
HOMA-IR	13.74±1.13**	11.44	0.19	9.11-33.93	10.6	16.87	2.33±0.78
F. LH (mIU/mL)	1.82±0.09**	1.02	0.01	0.30-6.31	1.57	2.06	6.36±0.12
F. FSH (mIU/mL)	4.03±0.47**	3.07	0.08	2.02-6.52	2.72	5.33	7.29±0.05
F. Cortisol (ng/mL)	13.06±0.46**	11.19	0.08	8.64-18.50	11.78	14.33	6.84±0.42
FEstradiol (pg/mL)	27.0±1.6**	23.23	0.28	14.56-46.62	22.55	31.44	46.3±1.41
F. SHBG (nmol/L)	28.80±2.08**	18.66	0.36	17.59-46.70	23.02	34.57	15.16±0.95
F. PSA (ng/mL)	73.4±2.06**	94.83	0.36	8.05-122.52	67.68	79.11	0.57±0.04
F. KLK-10 (ng/mL)	6.45 ±0.17**	4.33	0.03	3.54-11.14	5.97	6.92	2.79±0.07
F. MSLN (ng/mL)	2.24±0.05**	1.60	0.008	1.32-3.84	2.10	2.37	1.17±0.03
F. ADMA (ng/L)	27.61±1.07**	19.45	0.18	15.67-47.18	24.63	30.58	12.46±1.15
F. NO (µmol/L)	73.22±2.04**	41.13	0.36	38.81-129.74	67.55	78.88	32.35±1.16
F. IL-6 (ng/L)	83.02±3.21**	53.24	0.56	43.20-122.28	74.10	91.93	27.98±1.37
F. TNF-α (ng/L)	92.4±3.09**	84.34	0.54	54.35-137.92	83.82	100.97	37.18±1.09
F. INF-γ (ng/mL)	49.73±2.06**	47.20	0.36	25.11-78.60	44.01	55.44	20.41±1.73

Data are presented as mean ± SD, SD: Standard Deviation, SE: Standard Error, Range: is the difference between the highest and lowest values in the set, 95% CI: Confidence Intervals (Lower and Upper), F.: Fasting, n: No. of subjects, p-value (Non-Significant [p>0.05], A * indicated Significant [p<0.05], A ** indicated High Significant [p<0.01]) stated the level of significance in comparison with the corresponding control value.

DISCUSSION

In industrialized or often war-stricken countries, we find that the most common diseases affecting men are benign and malignant disorders of the prostate. PC is one of the most frequently diagnosed diseases and the most prevalent form of cancer, ranking third after lung

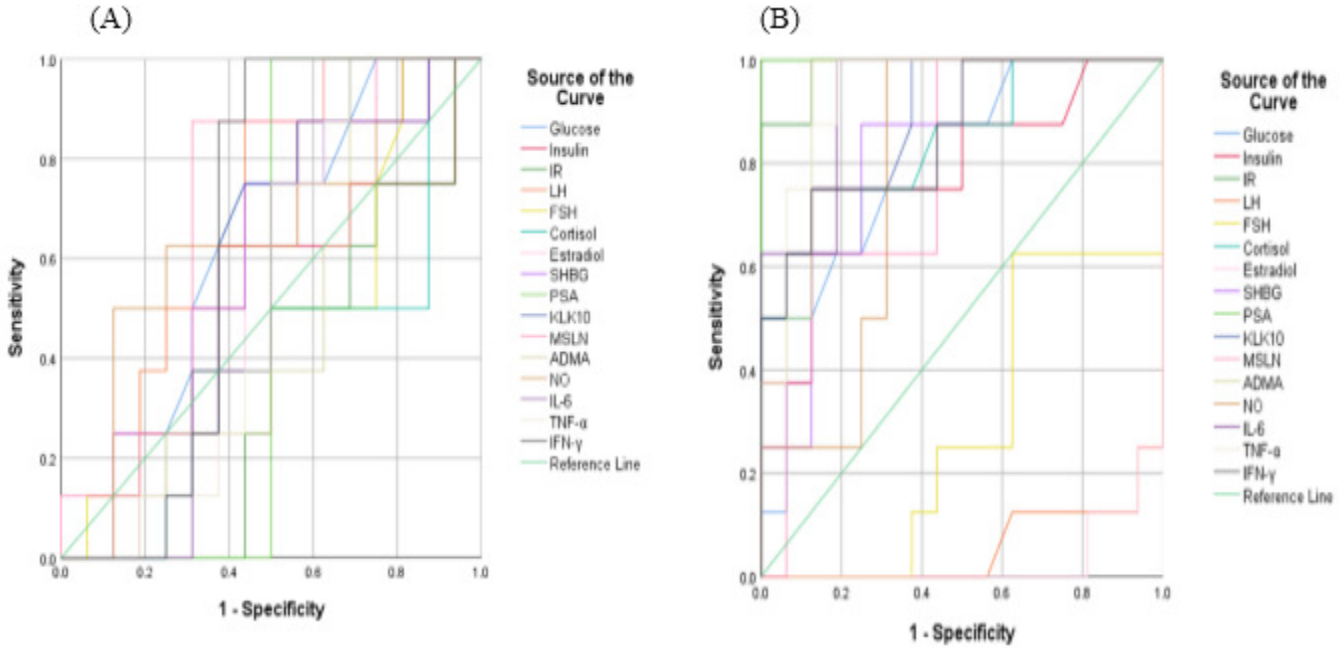

Fig. 2 : Receiver operating characteristic curve (ROC) for the levels of various clinical parameters in patients with PCa (A) and BPH (B).

Table 3 : Levels of total parameters measured in the present study for Benign Prostate hyperplasia Patients (BPH) patients and healthy control.

Biomarkes	Benign Prostate hyperplasia Patients (BPH) n=31						Healthy controls n=32
	95% CI						
	Mean± SD	Median	SE	Range	Lower	Upper	Mean± SD
BMI (Kg/m^2)	30.78±0.8	29.3	0.143	28.2-32.2	28.55	33.00	34.6±1.14
F. Glucose (mg/dL)	108.9±2.02*	107	0.36	88 -156	103.29	114.50	90.9±2.05
F. Insulin (mIU/L)	15.01±1.31**	13.2	0.23	4.04-21.06	11.37	18.64	7.47±1.06
HOMA-IR	5.59±0.19*	6.78	0.03	0.64-9.49	5.06	6.11	2.33±0.96
F. LH (mIU/mL)	4.59±0.03*	3.87	0.005	2.55-6.18	4.50	4.67	6.36±0.12
F. FSH (mIU/mL)	4.89±0.88*	4.36	0.15	3.47-8.36	2.44	7.33	7.29±0.05
F. Cortisol (ng/mL)	9.62 ±0.41*	10.80	0.07	5.60-13.56	8.48	10.75	6.84±0.42
F. Estradiol (pg/mL)	43.0±2.04	40.90	0.36	27.01-71.45	37.33	48.66	46.3±1.41
F. SHBG (nmol/L)	24.62±1.36*	20.88	0.24	13.94-44.93	20.84	28.39	15.16±0.95
F. PSA (ng/L)	7.00±0.52**	6.73	0.09	5.83-9.60	5.55	8.44	0.57±0.04
F. KLK-10 (ng/mL)	4.73±1.33**	3.97	0.23	2.91-11.10	1.03	8.42	2.79±0.07
F. MSLN (ng/mL)	1.74±0.42*	1.47	0.07	1.11-3.68	0.57	2.90	1.17±0.03
F. ADMA (ng/L)	16.52±0.19	13.56	0.18	12.72-36.34	15.99	17.04	12.46±1.15
F. NO (µmol/L)	50.05±1.45**	31.95	0.26	29.36-96.57	46.16	53.93	32.35±1.16
F. IL-6 (ng/L)	41.01±0.55**	41.75	0.09	25.37-51.55	39.48	42.53	27.98±1.37
F. TNF-α (ng/L)	51.37±2.03**	46.54	0.36	38.94-70.65	45.73	57.00	37.18±1.09
F. INF-γ (ng/mL)	33.61±1.63**	29.45	0.29	26.65-57.50	29.08	38.13	20.41 ±1.73

Data are presented as mean ± SD, SD: Standard Deviation, SE: Standard Error, Range: is the difference between the highest and lowest values in the set, 95% CI: Confidence Intervals (Lower and Upper), F.: Fasting, n: No. of subjects, p-value (Non-Significant [p>0.05], A * stated Significant [p<0.05], A ** indicated High Significant [p<0.01]) indicated the level of significance in comparison with the corresponding control value.

and colorectal cancers, contributing to increased mortality in men in the World. BPH is a non-malignant disease that causes prostate enlargement and affects men over the age of 50 and thus significantly affects the quality and harmony of patients' lives (WHO, 2019).

The urban area populations had some crucial differences with people of rural, such as the differences in pollution, environments, social, psychological, genetic,

food factors, and others, which are increasing dramatically in urban areas. On the other hand, the pressures of work and its requirements could affect the psychology of men volunteers. Furthermore, household and marital relationship factors represent tense factors and lead to an increase in the problem in oxidant/antioxidant status (Al-Fartosy *et al*, 2019).

The insulin hormone has a vital role in cancer diseases by increasing the production levels of free insulin-like growth factor I (IGF-I) and decreases IGF-I binding proteins, promoting carcinogenesis (Al-Fartosy and Mohammed, 2017). On the other hand, obesity and diet, which induced hyperinsulinemia, may significantly grow tumor and clinic pathological outcomes in a xenograft model. Therefore, many recent studies proved that obese or overweight men might have higher-state and pathologically more grade PCa (Langlais *et al*, 2019).

High cortisol levels may result from hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal system that may affect some psychological or behavioral factors, giving progression and a predictor of tumors through tumor stimulation or immunosuppression. In addition, cortisol stimulates the cellular P450 aromatase enzyme, which is involved in metabolizing androgens to estrogen and increased abdominal fat deposition, metabolic syndrome, and PCa (Fabrel *et al*, 2016). Some studies have recently suggested a synergistic relationship between cortisol, estradiol, insulin and some other hormones that stimulate P450 aromatase, changing the intracellular signaling chain and promoting mitogenic growth and endothelial growth damage (Ellem and Risbridger, 2010).

E2 levels are likely to be lower due to its high utilization rate, implying that this hormone could be employed to combat oxidative stress. Both the increase in free radicals and the disruption of the free radical scavenging system in PCa have been shown to cause oxidative stress. Hence, chronic oxidative stress may cause kinase activation such as c-Jun-N-Terminal Kinase (JNK) and "interleukin kappa B-kinase" β (IKKβ), which inhibit activation of the insulin signaling intermediates (Bonkhoff, 2018). As a byproduct, E2 may decrease oxidative stress through both nongenomic and genomic mechanisms, activating pathways that inhibit the production of reactive oxygen species (ROS) and boosting the efficiency by which ROS are scavenged. Therefore, some of the effects of E2 deficit on insulin action are likely attributable to the increased adiposity associated with estradiol deficit. On the other hand, low testosterone levels and, therefore, a shortage of substrate for aromatization to estradiol could be one of the plausible explanations for the reduction in E2 levels in PCa. (Al-Fartosy and Mohammed, 2017).

SHGB is produced and secreted from hepatic cells and had a significant role as a serum carrier of sex hormones. The response begins when the hormone-binding with their receptors that distributed in some target tissues such as prostate, lymph node carcinoma of the prostate (LNCaP) cells, testes, duodenum, ovary, placenta, proximal tubule epithelial cells (PTEC) and cerebral cortex and several cancers (Li *et al*, 2016). Therefore, increases in SHGB level in patients with PCa may be due to enhanced expression of this hormone in prostate cancer tissues in relationship with poor clinicopathological features. Hence, it may explain its affected role in prostate cancer progression, which perhaps results from the prolonged-expression of androgen-responsive genes. Moreover, SHBG may actively participate in signaling at the prostate cell membrane (Park *et al*, 2020).

All the recent studies reported that in oncology, new biomarkers are needed to optimize and regulate the decision-making, treatment, and therapy monitoring for individual patients of PCA. Hence, in response to the development and treatment of cancer, there are several cytokines, growth factors and all other proteins that may need to be systematically up-regulated (Vendrell *et al*, 2015). Furthermore, to monitor cancer, especially in patients on chemo and radiotherapy, the actual tumor mass cannot be reflected via a single biomarker such as PSA because cancer patients may exhibit a heterogeneous array of responses to therapy. In this regard, using a novel clinical marker may be very necessary to diagnose tumors and the response of normal tissue for treatment (Srinivasan *et al*, 2019).

The serine protease called KlK10, which proved interesting nearly three decades ago as a biomarker for prostate cancer, may be implicated in the growth and invasion of several human tumors. In the present study, a significantly high level of KlK-10 in patients of BPH and PCa may result from the fact that all may enhance the metabolism of glucose via several pathways such as:

Activation and vasodilation of endothelium, flow increases of blood, delivery of hormones and energy substrates to tissues, and facilitating glucose transport transcapillary (Fuhrman-Luck *et al*, 2014). On the other hand, due to the proteolytic activity of KlK, their role in tumor progression may be confused. Therefore, KlK-10 may hold promise for PCa diagnosis or its developments (Koistinen *et al*, 2014).

The differentiation antigen named MSLN is an essential clinical marker present on normal mesothelial

cells. It undergoes polymorphism in numerous human tumors such as mesothelioma, melanomas, renal cell cancer, thyroid cancer, breast cancer, prostate cancer and adenocarcinoma of ovarian and pancreatic (Jöhrens *et al*, 2019). The significantly higher serum MSLN may be due to insulin resistance and obesity, two modern drivers in PCa etiology (Hassan *et al*, 2016). Furthermore, some studies revealed that patients with advanced-stage malignant mesothelioma had significantly increased levels of MSLN as compared to those with stage I disease. Therefore, promoting cell mobility and tumor invasion via pathways activation of JNK and ERK and enhancing the activity of matrix metalloproteinase (MMP)-7 may represent one of the essential mechanisms that explain the correlation between cancer development in advanced-stage and MSLN level (Jöhrens *et al*, 2019).

Recent studies proved that helper T cells (Th) have significant involvement in anti-tumor immunity. Based on the pattern of cytokines secreted, the cell responses tend to be polarized phenotype either to the Th1-like or Th2-like. All types of (Th1) such as lymphotoxin, tumor necrosis factor α (TNF-α), and IFN-γ, have clearly shown its significant association with cellular immunity and ability to protect the cells against the development of certain types of intracellular pathogens and in eliciting anti-tumor responses (Fu *et al*, 2020).

An elevated level of IL-6 may be due to the result of a complex etiology of obesity such as diet, levels of physical activity and genetic factors interaction. Also, this etiology is influenced by social, environmental, economic, and behavioral factors, which are highly associated with an increased risk of insulin resistance and its adverse effects on cancer (Vendrell *et al*, 2015).

TNF was described early in its discovery as a circulating factor that causes tumor necrosis as the primary regulator of the inflammatory response and a multidirectional cytokine that plays a dual role in cancer biology, including prostate cancer (PCa) (Maolake *et al*, 2018). It is produced by cancer cells and the tumor microenvironment and has multi-capacity activities in tumor formation and development. In addition, many recent studies have indicated that TNF-α has a direct or indirect role in neoplasia through its participation in the maintenance and homeostasis of the immune system, inflammation, and host defense, in addition to pathological processes such as chronic inflammation, autoimmunity, and malignant diseases (Fu *et al*, 2020).

Hence, the pro-and anti-inflammatory response directly correlates with the promoter's polymorphism in the TNF-α gene that consequently affects the production of TNF-α, thereby creating differences in the immune response of individuals and influencing susceptibility to prostate cancer. Therefore, it can be considered an additional biomarker to PSA that could reflect the activity of PCa and BPH (Srinivasan *et al*, 2019).

IFN-γ is a cytokine whose biological activity is conventionally associated with cytostatic/cytotoxic and antitumor mechanisms during a cell-mediated adaptive immune response and its central role in the coordination of humoral immune responses (Jorgovanovic *et al*, 2020). Increased serum IFN-γ in PCa and BPH patients could represent an adaptive response to the collaboration with lymphocytes for regulation and development of tumors, and IFN-γ receptors developed tumors with greater frequency. It has been reported that the main effect of IFN is on the transformed cell itself and improving the immune system's ability to recognize those transformed cells. Moreover, it exhibits more than one face as pro-carcinogenic, cytostatic and cytotoxic under certain circumstances (Fu *et al*, 2020).

ADMA is a substance with a small molecular weight (202 Dalton) that inhibits nitric oxide (NO) production from L-arginine via the enzyme nitric oxide (eNOS). It plays a clear and vital role in the endothelial dysfunction process and represents a hallmark of oxidative stress (Abdel-Messeih *et al*, 2017). The following reasons may give a possible explanation for the higher levels of serum ADMA in PCa patients; first, it might be due to increased ADMA production under oxidative stress. The second is an elevation of degradation and turnover of proteins containing methylated arginine. Third, Increased level of insulin resistance and oxidative stress in PCa patients upregulates the expression of the protein arginine methyltransferase (PRMT) and decreases dimethylarginine activity dimethylaminohydrolase (DDAH) enzyme in ADMA catabolism. Finally, maybe due to reducing the renal excretion of ADMA (Reddy *et al*, 2018).

Nitric oxide (NO) is a free radical that is easily soluble in water and has a significant role in various physiological and pathological processes and its essential role in carcinogenesis and tumor growth development (Krzystek-Korpacka *et al*, 2020). In the present study, NO levels were significantly elevated in BPH ($p<0.05$) and ($p<0.01$) in PCa patients. In most tissues, it may represent a second messenger produced predominantly at a slight level via two expressed isoforms of the enzyme nitric oxide synthase (eNOS) found in tumors. Therefore, increased levels of NO, which is not associated with normal physiology may result from the correlation between malignancy and expression of a high level of

the inducible isoform of NOS. Hence, it can inhibit or promote carcinogenesis as a multifaceted molecule (Abdel-Messeih *et al*, 2017). Also, it can exhibit various activities, such as regulating vasodilatation and platelet aggregation, that may affect the outcome of progressive cancer disease and metastasis. Additionally, NO production is primarily associated with tumorigenic cell apoptosis (De Oliveira *et al*, 2017). Moreover, NO can increase the flow of tumor blood as well as angiogenesis promotion, which explains the association between lethal prostate cancer and increases the level of iNOS in the tumor epithelium (Reddy *et al*, 2018).

CONCLUSION

In conclusion, insulin resistance is strongly associated with obesity and has an essential role in the pathogenesis and increased complication of many human diseases, such as prostate cancer among men through various pathways such as inflammation, oxidative stress, and apoptosis. Hence, considering the combination of obesity, insulin resistance, and some markers of tumor, pro-inflammatory and oxidative stress may demonstrate an excellent indicator of PCa and BPH risk among men. However, further studies using larger sample sizes should be performed to establish the diagnostic value of other biomarkers to detect PCa and BPH.

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