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Oxidative Stress Biomarker, Protein Carbonyl, Malondialdehyde and Antioxidants Superoxide Dismutase, Catalase on Prostate Cancer in Kurdistan Region of Iraq

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ABSTRACT

Prostate cancer (PCa) has notably ascended to the position of the second most commonly diagnosed cancer among men. It constitutes a considerable public health issue on a global scale; typically, prostate cancer causes urological issues in aging men. This study aimed to investigate biomarkers of oxidative stress and antioxidants associated with PCa pathogenesis and their potential implications for diagnosis and treatment. The study included 100 PCa patients and 50 healthy controls and used ELISA (Enzyme-Linked Immunosorbent Assay) kits for comprehensive analysis. Statistical analysis revealed significant differences in biomarker levels between PCa patients and controls. This report shows significantly lower serum antioxidant levels in PCa patients compared to healthy controls, suggesting a potential association between antioxidant deficiency and PCa risk. Moreover, alterations in antioxidant receptors may influence prostate physiology and pathology. Elevated levels of the oxidative stress biomarker Malondialdehyde (MDA). Protein carbonyl (PC) levels were observed in PCa patients, indicating their potential role in disease progression and poorer outcomes. Furthermore, we investigated the impact of enzymatic antioxidants, including superoxide dismutase (SOD) and Catalase (CAT), on PCa risk. This may inhibit tumor formation. Antioxidants can be classified into first, second, third, and even fourth-line defense categories. In conclusion, our study provides valuable insights into the complex interplay between biomarkers, oxidative stress, and antioxidant biomarkers and PCa pathogenesis. Understanding these relationships may improve diagnostic strategies and targeted therapies for PCa patients.

Keywords: Antioxidants, Oxidative stress, Prostate cancer, Protein carbonyl, SOD.

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مؤشر الإجهاد التأكسدي بروتين الكاربونيل، مالونديالدهيد ومضادات الأكسدة (سوبر أكسيد ديسميوتاز، كاتالاز) على سرطان البروستاتا في إقليم كردستان العراق

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قسم الكيمياء، كلية العلوم والصحة، جامعة كويه، كويه، إقليم كردستان، العراق

الملخص

لقد صنف سرطان البروستاتا بشكل ملحوظ الى المرتبة الثانية كأثر انواع السرطان تشخيصاً بين الرجال. يشكل سرطان البروستاتا مشكلة صحية عامة كبيرة على نطاق عالمي، وعادة ما يسبب مشاكل في المسالك البولية لدى الرجال المسنين. هدفت هذه الدراسة إلى التحقيق في المؤشرات الحيوية للإجهاد التأكسدي ومضادات الأكسدة المرتبطة بتطور سرطان البروستاتا وتأثيراتها المحتملة على التشخيص والعلاج. شملت الدراسة 100 مريض بسرطان البروستاتا و50 من المجموعة الضابطة، مع تحليل شامل باستخدام مجموعات ELISA. كشف التحليل الإحصائي عن اختلافات كبيرة في مستويات المؤشرات الحيوية بين مرضى سرطان البروستاتا والمجموعة الضابطة. انخفاض مستويات مضادات الاكسدة في مصل الدم بشكل كبير لدى مرضى سرطان البروستاتا مقارنةً بالضوابط الصحية، مما يشير إلى وجود ارتباط محتمل بين نقص مضادات الأكسدة وخطر الإصابة بسرطان البروستاتا. علاوة على ذلك، قد تؤثر التغيرات في مستقبلات مضادات الأكسدة على فسيولوجيا البروستاتا وعلم الأمراض. لوحظت مستويات مرتفعة من المؤشر الحيوي للاجهاد التأكسدي مالونديالدهيد (MDA) وبروتين الكاربونيل (PC) لدى مرضى سرطان البروستاتا، مما يشير الى دورها المحتمل في تطور المرض. علاوةً على ذلك، تناولت الدراسة تأثير مضادات الاكسدة الانزيمية، بما في ذلك سوبر أكسيد ديسميوتاز (SOD) وكاتالاز (CAT)، في تقليل خطر الإصابة بسرطان البروستاتا. وبالتالي قد تسهم هذه الانزيمات في تثبيط تكون الاورام. يمكن تصنيف مضادات الأكسدة إلى فئات خط الدفاع الأول، والثاني، والثالث، وحتى الرابع. وفي الختام ، توفر دراستنا رؤى قيّمة حول التفاعل المُعقد بين المؤشرات الحيوية، والإجهاد التأكسدي، والمؤشرات الحيوية المضادة للأكسدة، ومسببات سرطان البروستاتا. قد يسهم فهم هذه العلاقات استراتيجيات التشخيص والعلاجات المُستهدفة لمرضى سرطان البروستاتا.

INTRODUCTION

Prostate cancer (PCa) has notably ascended to the position of the second most commonly diagnosed cancer among men and constitutes a considerable public health issue on a global scale[1]. PCa is classified as an adenocarcinoma. It begins when the prostate epithelial cells, which are responsible for semen production and secretion, undergo genetic mutations. These alterations transform normal cells into malignant ones, resulting in dysregulated prostate growth [2]. This malignancy is recognized as a heterogeneous condition, Prostate cancer ranks among the most commonly diagnosed malignancies in males residing in developed nations. Various factors, including a variety of genetic, epigenetic, lifestyle, age, race, a family history of prostate cancer, and environmental influences, have been identified as contributing to the process of prostate carcinogenesis[3, 4]. Many prostate cancer cases could be preventable by addressing risk factors; however, certain factors, like age, are unavoidable. Generally, unmodifiable risk factors, particularly age, increase the likelihood of developing the disease as men age [5]. It is widely accepted that the significance of oxidative stress in the pathophysiology of prostate cancer is highlighted by studies of age-related dysfunctions such as altered redox status, free radical buildup, lipid peroxidation, and prostate DNA damage. ROS are continuously produced within cells, predominantly originating from the mitochondria[6]. An imbalance between the

generation of reactive oxygen species (ROS) and the cellular antioxidant mechanism defines OS. Leading to the destruction of cellular macromolecules. Antioxidants serve as effective scavengers of free radicals and may be employed to safeguard cellular components against OS [7, 8]. The progressive deterioration of tissue and organ function over time is a hallmark of aging. Originally known as the OS theory of aging, the free radical theory posits that this decline is a result of assemblage oxidative damage to macromolecules, including lipids, DNA, and proteins, caused by reactive oxygen and nitrogen species (RONS)[9]. Among the most prevalent and crucial antioxidant enzymes are superoxide dismutase and catalase [10]. The expression and activity of SOD isoforms differ due to their localization and functions, as well as regulatory mechanisms. SOD catalyzes reactions that generate hydrogen peroxide and oxygen, enhancing its antioxidant capacity, reducing cellular mortality, and mitigating oxidative damage [11]. The vital role of superoxide dismutase (SOD) in the dismutation of molecular oxygen, reactive oxygen species (ROS) levels are regulated to prevent cellular damage [12, 13] . Malondialdehyde (MDA) is the lipid peroxidation process's primary and key end-product[14]. Protein oxidation and lipid peroxidation manifest as indicative markers of oxidative stress, represented by PC and MDA [15] . Given that lipid peroxidation is a consequence of oxidative stress, malondialdehyde is recognized as a more reliable indicator of lipid peroxidation [16, 17]. Protein carbonyl compounds are considerable indicators of oxidative stress in prostate cancer, reflecting the degree of oxidative injury to proteins. This factor is associated with cancer's advancement. Reactive carbonyl species (RCS), produced via lipid peroxidation and oxidative stress, modify proteins, resulting in dysfunction within essential signaling pathways such as MAPK and PI3K/AKT, which are linked to tumor proliferation and survival[18]. Research has demonstrated that individuals diagnosed with prostate cancer present heightened concentrations of malondialdehyde (MDA) and advanced oxidation protein products (AOPP), signifying an elevation in oxidative stress that correlates with tumor aggressiveness and increased Gleason scores[19] . Moreover, oxidative stress plays a role in initiating oncogenic signaling and developing resistance to therapeutic interventions, underscoring its dual function in facilitating cancer cell survival and proliferation [20]. Consequently, the strategic targeting of oxidative stress and its derivatives, including protein carbonyls, may provide potential therapeutic strategies for the management of prostate cancer[21]. Elevations in protein carbonyl levels signify increased oxidative damage to proteins, potentially contributing to the onset of various diseases. Thus, it has been recognized that protein oxidation may serve as a valuable biomarker. High-throughput methodologies for quantifying PC levels on a mass scale include the sandwich enzyme-linked immunosorbent assay for protein carbonyls [22, 23].

This study seeks to explore whether the activity of the aforementioned antioxidant enzymes (SOD and CAT) are modified in the context of prostate cancer. Additionally, this paper aims to assess the monitoring of oxidative stress in prostate cancer by measuring malondialdehyde and protein carbonyl compounds and the variations in these levels compared to normal tissues. Superoxide dismutase (SOD) comprises a category of metalloenzymes that facilitate the dismutation of superoxide radicals into hydrogen peroxide and dioxygen[24, 25]. Catalase (CAT) is also crucial as it reduces hydrogen peroxide to oxygen and water, minimizing its accumulation and alleviating oxidative stress. CAT mitigates potential pro-oxidant effects of hydrogen peroxide, supporting SOD's activity[25, 26], which contributes to reducing oxidative stress. SOD serves as a scavenger of O_2 and is vital in the cellular defence against oxidative stress, while CAT predominantly represents the cellular response to hydrogen peroxide and prevents hydroxyl radical generation and damage[27, 28].

MATERIALS AND METHODS

Subject

The study involved 150 participants: 50 controls (without any type of cancer) and 100 patients diagnosed and monitored. In the Oncology department / Rzgare Hospital /Hawler Kurdistan Region, the blood samples were collected during a period from July 2024 to November 2024, all participants were aged 55-80 years and had previously been diagnosed with prostate cancer (PCa), and all prostate cancer patients were under hormonal therapy. Serum Serum Prostate-Specific Antigen (PSA) levels were determined for both patients and controls; the study sample was evaluated through an inclusive medical history, and according to (IPSS) the International Prostate Symptom Score, a 6 ml blood sample was drawn from the vein of the participants and centrifuged at 5000 rpm for 10 minutes. The separated serum samples were used to measure the levels of superoxide dismutase (SOD) (SL3490Hu), catalase (CAT) (SL2050Hu), protein carbonyl (PC) (SL1473Hu), and malondialdehyde (MDA) (SL1135Hu) by ELISA kits from SUNLONG company china brand. An immunosorbent assay was used, and the absorbance was read at 450 nm using the Microplate reader (USA BioTek). All concentrations were measured by ng/ml for n=150 .

Statistical analysis

GraphPad Prism Statistics version 10.4.1 was used to analyze the data. The normality test was performed using Shapiro-Wilk and Kolmogorov-Smirnov test to assay the homogeneity of variance for variables. A T-test was used to compare patient and control groups. Pearson's correlation test was used to establish a relationship between the antioxidants and the biomarkers for oxidative stress. One sample and the Wilcoxon test were used to analyze the BMI and Age column analysis data. The results were displayed as the mean and standard error of the mean (SEM). Mann-Whitney U was used for nonparametric test. A p-value of less than 0.05 was deemed statistically significant.

Approval ethical

Approval from the ethical committee (KOUFSDHM2024-001) was obtained from the Koya University ethical committee for this research. Animals and human cystic echinococcus (CE) were collected during the period from July 2022 to June 2023.

RESULTS AND DISCUSSION

Age, economic position, marital status, body mass index, and participant family history are among the general characteristics of the study's groups, as shown in Table 1.

The PSA test showed a highly significant difference between PCa individuals and healthy individuals. The disparity in BMI between the two groups is statistically significant ($p < 0.05$). According to age group, older men are more susceptible to prostate cancer. A considerable percentage of prostate cancer patients possess a family history, indicating a possible genetic or hereditary factor. Most individuals have intermediate 7 GS or high-grade GS (8-10) prostate cancer, indicating a notably aggressive disease profile among this group.

Table 1: General characteristic data for all studied groups.

Characteristics	Study groups		P-Value
	Prostate cancer (n=100) Mean±SE	Control group (n=50) Mean±SE	
Prostate cancer antigen (PSA) at diagnostic of disease	99.64±21.71	0.440 ±0.139	(P<0.05)
BMI (kg/m^2)	27.85±0.5708	27.36 ±0.5976	(P<0.05)
Age (yrs.)	69.07±0.7915	62.43±1.448	
Age distribution NO (%)			
Age	PCa	Control	
50-69 yrs.	52%n=52	76%n=38	
70-80 yrs.	48%n=48	24%n=12	
Family history of PCa No. (%)			
40 % with Family history		60 % without family history	
Gelson grade distribution with prostate cancer patients aged 55-80 years			
GS	**Percentage**		
6	3.77%		
7	43.39%		
8	24.53%		
9	24.52%		
10	3.79%		

The comprehensive results of the study indicated that patients diagnosed with prostate cancer exhibit diminished enzymatic antioxidant levels, specifically (SOD) and (CAT), in addition to reduced expression of their respective antioxidant genes. Both SOD and CAT may serve as viable therapeutic targets for prostate cancer contingent upon the cancer's grade. In this study, the value of SOD is significantly different between case and control groups with a rising mean with standard error of mean data seat of the control group (Mean ± SE) (5.484±0.263 > 3.844±0.067) as the case means have statistically significant difference with ($p < 0.0001$) as shown in (figure 1). When CAT activity was investigated (Table 2), the relevance between control individual levels (1.930±0.114) and PCa activities (1.628±0.028) showed an important statistical relevance ($p <0.0008$) as in (figure 2). Another study found that SOD and CAT with dimensioned levels in PCa patients compared to healthy controls suggesting the damage of antioxidants affected by oxidative stress as a potential factor in PCa development [29]. There is another study found a statistical decrease in SOD activity in PCa participants but no important differences in CAT levels compared to controls [28]. The study further elucidated that the protein carbonyls compound (PC) response showed the relevance between healthy activities (18.8±0.745) and PCa with arising activities (23.18 ± 0.959) was found to have a statistically important relevance ($p <0.001$) as in (figure 3).

Malondialdehyde (MDA) has a role as a biomarker of OS within the context of PCa; by inspecting MDA in healthy individuals the relation of healthy levels (200.9±11.80) and PCa activities (247.2±12.40) was found to have a statistically important relevance ($p =0.030$) with increasing the case MDA level as in (figure 4). Another study confirmed increased MDA levels in PCa participants compared to healthy ones. And suggested that MDA could be a potential biomarker for oxidative injury in prostate cancer [3, 30]. Found that MDA levels were significantly higher in PCa patients compared to controls. Concluded that MDA, alongside other oxidative markers, could aid

in diagnosing PCa. In the current stages of the disease, these biomarkers may play a pivotal role in evaluating oxidative damage within patients' prostate tissues while also potentially reflecting the status of the patient's treatment.

Table 2: Oxidants and antioxidants Concentration in control and PCa patient group.

Parameters	Control group mean±SE	Patient group mean±SE	P value (p<0.05)
SOD (ng/ml)	5.484 ± 0.263	3.844 ± 0.067	0.0001
CAT (ng/ml)	1.930 ± 0.114	1.628 ± 0.028	0.0008
PC (ng/ml)	18.8 ± 0.745	23.18 ± 0.959	0.0006
MDA (ng/ml)	200.9 ± 11.80	247.2 ± 12.40	0.030

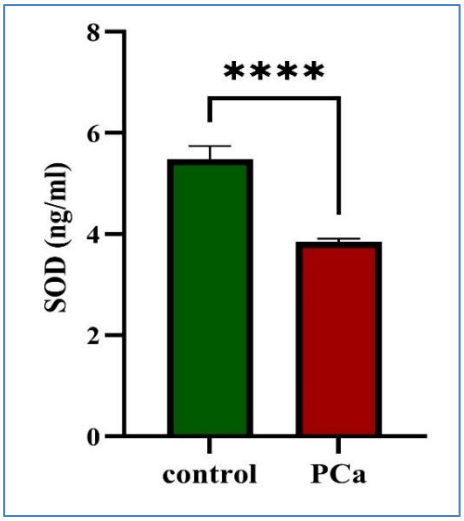

Fig. 1: SOD level in control and PCa.

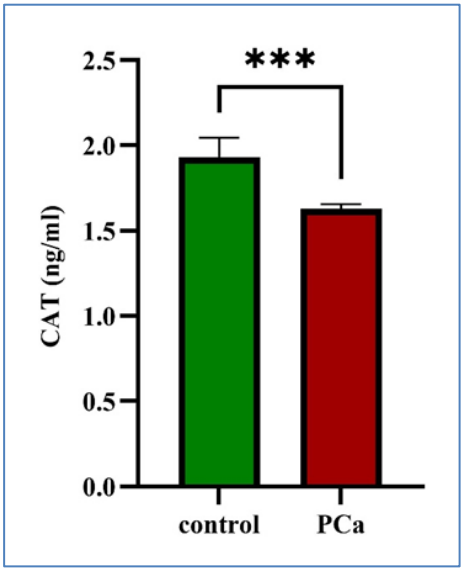

Fig. 2: Catalase level in control and PCa.

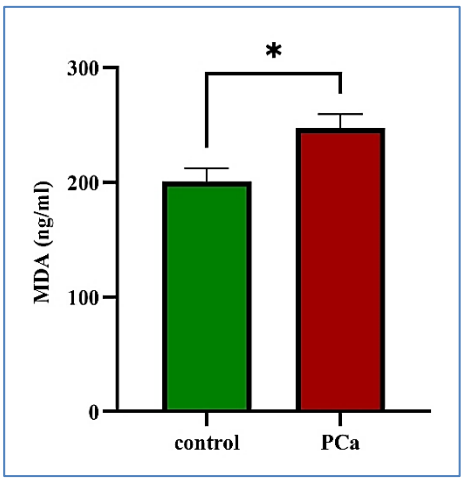

Fig. 3: PC level in control and PCa.

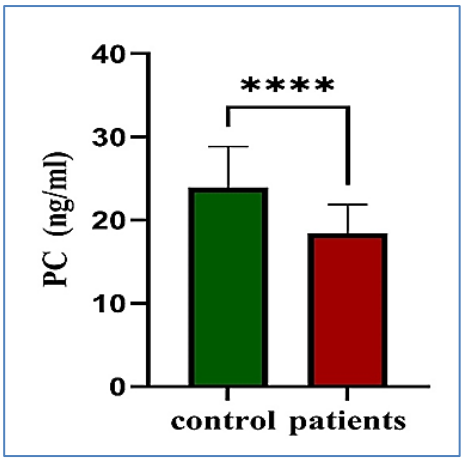

Fig. 4: MDA level in control and PCa.

Correlations between PC and antioxidant

In our unique study The correlation analysis presented in (Table.3) demonstrates PC exhibited a significant negative correlation with CAT (Figure.6) as oxidative stress biomarker protein carbonyl compound level raised with prostate cancer patients which decreased the CAT level, a significant positive correlation between PC and SOD of prostate cancer in participants as in (Figure 5) may the high Gelson score of patients with aggressive prostate cancer and long trips with treatment affected, as illustrated in (Table 3).

Table 3: Correlation between PC and antioxidants involved in this study.

Parameter	R	P value (P<0.05)
CAT	R= -0.203	0.2
SOD	R=0.532	0.0024
R= pearson's correlation coefficient		

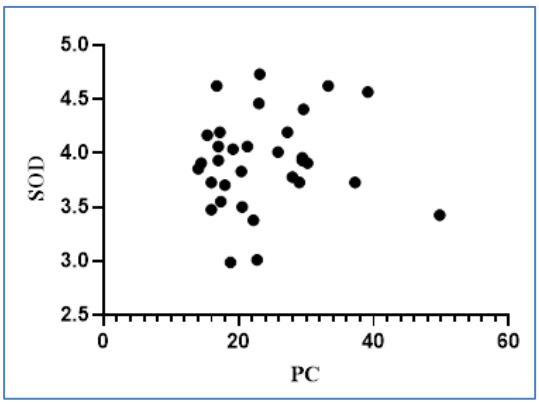

Fig. 5: correlation between PC and SOD.

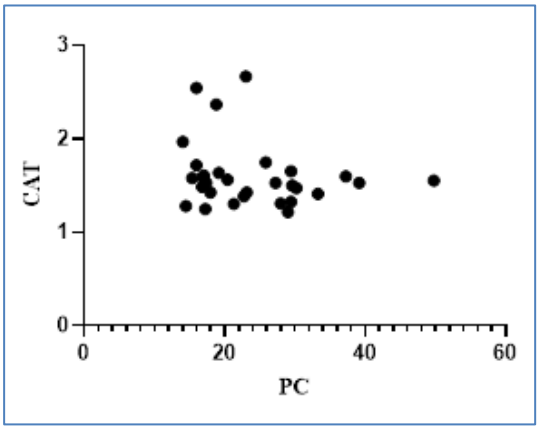

Fig. 6: correlation between PC and CAT.

CONCLUSION

In conclusion, this study highlights the intricate connection between OS, antioxidant biomarkers, and prostate cancer pathogenesis. Elevated levels of oxidative stress biomarkers, such as malondialdehyde and protein carbonyl compounds, were associated with disease progression. At the same time, enzymatic antioxidants like superoxide dismutase (SOD) and catalase (CAT) demonstrated protective roles against tumor formation. These findings provide valuable insights that could enhance diagnostic approaches and inform the development of targeted therapies for prostate cancer patients.

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Author contribution: Gulala, Methodology, Investigation, Data Curation, Review, Written. Data analysis. Musher: Methodology, Visualization, Investigation, Data Curation, Writing- Original Draft, Preparation.

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