Eurasian Journal of Molecular and Biochemical Sciences

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Effects of Chemohormonal Therapy on Oxidant and Antioxidant Systems in Patients with Metastatic Castration-Resistant Prostate Cancer

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Cite: *Yüce N, Kölpınar M, Laloğlu E, Özmen HK, Turhan A, Bayrami M. Effects of Chemohormonal Therapy on Oxidant and Antioxidant Systems in Patients with Metastatic Castration-Resistant Prostate Cancer. Eurasian Mol Biochem Sci 2024;3(2): 1-13*

Received: 23 December 2024, Accepted: 25 December 2024 *DOI: 10.5281/zenodo.14992081*

Abstract

Objective: The aim of this study was to investigate the effects of chemohormonal therapy on catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), arylesterase (ARE), paraoxonase (PON), myeloperoxidase (MPO), malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS) in patients with metastatic castration-resistant prostate cancer (MCCRPC). The aim of this study was to investigate the effect of chemohormonal treatment and treatment resistance on oxidant and antioxidant system in cancer and prostate cancer through oxidative stress index (OSI) parameters and to show that it may have a predictive value in the diagnosis, prognosis and treatment of the disease. Material and method: This study included 112 patients diagnosed with prostate cancer and 30 healthy individuals (control group) who were admitted to the Departments of Medical Oncology and Radiation Oncology of Atatürk University Medical Faculty Hospital. Patients were divided into four groups according to the treatment protocol. Patients received androgen suppression therapy, secondary hormone therapy and non-hormone therapy (chemoteropathic drugs) respectively (as resistance developed). The first group was sensitive to androgen suppression therapy (ADT), the second group was resistant to androgen suppression therapy and sensitive to chemotherapeutic agents. The third group was the group that developed resistance to chemotherapeutics. The fourth group was patients who were diagnosed with prostate cancer and did not receive treatment. CAT, SOD, tGSH, GPx, GPx, ARE, PON, MPO and MDA levels were analyzed by manual antioxidant methods in patient and control serum samples, while TAS, TOS and OSI levels were analyzed by commercially purchased kits and according to the Erel method. Results: CAT, ARE, MPO, TAS, TOS and OSI parameters showed significant differences between the groups ($p<0.05$), while SOD, tGSH, GPx, MDA and PON parameters showed no significant differences between the groups. Conclusion: This is a research study that will shed light on the effects of chemohormonal therapy and treatment resistance on oxidant and antioxidant systems in cancer and prostate cancer and will fill the gap in the literature.

Keywords: Prostate, cancer, oxidant, antioxidant, chemohormonal therapy

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Introduction

Uncontrolled proliferation of secreting alveolar cells in the prostate gland due to genetic or environmental conditions is called prostate cancer (1). It is the second most common type of cancer in men and the leading cause of death, and ranks first in the world, accounting for 7% of newly diagnosed cancers in men worldwide. The top five most common cancers in men in Turkey in 2020 are: lung cancer, prostate cancer, colorectal cancer, bladder cancer and stomach cancer. Prostate cancer ranks first in Turkey with 19444 new cases and a rate of 14.6%. It is estimated that at least 40% of cancers seen in men are related to smoking (2).

Castration is stopping the production or blocking the effects of androgens (testosterone, dihydrotestosterone and androstenedione) by hormonal therapy. Metastatic prostate cancer is when cancer cells appear in tissues, organs or lymph systems outside the prostate. Castration-resistant prostate cancer (CRPC) is when metastases continue to grow even if there are no androgens in the body. These cancer cells are called castration-resistant because they do not respond to hormonal castration treatment (3). These are patients with high serum prostate-specific antigen levels (PSA) that progress progress progressively even though serum testosterone levels are at castration levels. The serum castration level of testosterone is considered to be <50 ng/dL (<1.7 nmol/L). The main treatment approach for metastatic prostate cancer is androgen suppression. Simultaneous chemohormonal therapy and radiotherapy have a positive effect on patient survival in prostate cancer. Antiandrogens affecting androgen receptor (AR)-mediated signaling pathways are among the important treatment modalities today (4, 5).

The gold standard in Androgen Deprivation Therapy (ADT) for CSPC is bilateral orchiectomy if the patient's compliance with the treatment is problematic. Docetaxel used in treatment shows antimitotic effect by affecting cell division through microtubule stabilization. Enzalutamide blocks the dihydrotestosterone receptor in the membrane and nucleus. Abiraterone androgen biosynthesis inhibitor blocks 17α hydroxylase and lyase enzymes via cytochrome p450. Sipuleucel-T is an antibody response therapy in which a vaccine derived from dendritic cells of the patient's peripheral blood is given to the patient. Kabazitaxel is an antimitotic treatment applied to docetaxel-resistant patients (6). Reactive oxygen species (ROS) including nitrogen dioxide, hydroxyl radicals, nitric oxide, peroxynitrite and superoxide play an important role in the regulation of blood pressure, neurotransmission, cell movement, immune control, smooth muscle relaxation and protection against microorganisms. Antioxidants that keep ROS under control are catalase, superoxide dismutase, coenzyme Q10, albumin, uric acid, ascorbic acid, glutathione, vitamin E and vitamin A. Overproduction of ROS causes oxidative stress and increasing levels of ROS damage macromolecules such as carbohydrates, proteins, lipids and DNA. High levels of oxidative stress underlie various diseases that cause cell damage, such as stroke, Alzheimer's, Parkinson's, diabetes, rheumatoid arthritis, cardiovascular diseases and cancer (7).

Free radicals, which are continuously produced in the cell, are inhibited by antioxidant defense systems produced during normal metabolism in the body. Antioxidants basically prevent or delay cell damage by destroying free radicals in the cell. Antioxidants can be produced endogenously in the body or can be taken exogenously through food. Antioxidant defense systems have enzymatic and nonenzymatic complex systems. Therefore, we can talk about the first, second and third antioxidant defense mechanisms in the cell. The first defense mechanism is superoxide dismutase (SOD) which suppresses the formation of free radicals, catalase (CAT) in peroxisomes, glutathione peroxidase (GPx) which breaks down hydrogen peroxide into

water in mitochondria and sometimes in the cytosol are the main antioxidant defense systems (8). Glutathione (GSH) is a powerful, natural antioxidant molecule involved in cell differentiation, proliferation and apoptosis. Myeloperoxidase (MPO) is found at high levels in neutrophils and macrophages. MPO is the antioxidant that catalyzes the reaction between hydrogen peroxide and chlorine to produce hypochlorous acid (HOCl-), a potent antimicrobial oxidant agent that damages proteins, DNA and lipids (9). Malondialdehyde (MDA) is the end product of lipid peroxidation resulting from the reaction of fatty acids with free radicals and its level is expected to increase in prostate cancer (8).

Paraoxonase (PON) is an endogenous antioxidant anti-inflammatory enzyme in the structure of glycoprotein released from the liver into the circulation due to high density lipoprotein (HDL). This antioxidant role of PON stems from its protective effect on low-density lipoprotein (LDL) from oxidation (10). Arylesterase (ARE) is an esterase group antioxidant enzyme encoded by the same gene as PON1 and has similar active centers. The ratio of total oxidant status (TOS) and total antioxidant status (TAS) is accepted as oxidative stress index (OSI). Oxidative stress, which is an inevitable consequence of aerobic life, plays an important role in the mechanism of many diseases including cancer (11). The aim of this study was to investigate the effect of chemohormonal treatment and treatment resistance on oxidant and antioxidant system in cancer and prostate cancer, chemohormonal treatment and treatment resistance on oxidant and antioxidant system through CAT, SOD, GSH, GPx, ARE, PON, MPO, MDA, TAS, TOS and OSI parameters in patients with metastatic castration-resistant prostate cancer and to show that these parameters may have a predictive value in the diagnosis, prognosis and treatment of the disease.

Material and Method

Ethical Statement: The ethics committee approval of this study was obtained from Atatürk University Clinical Research Ethics Committee (Date:27.12.2024, No:2024/B.30.2.ATA.0.01.00/718).

Determination of Study and Control Groups: The patient group of this study consisted of 112 patients diagnosed with prostate cancer and receiving chemohormonal therapy at the Department of Medical Oncology and Radiation Oncology of Atatürk University Health Research and Application Center. The patients were divided into four groups according to the treatment protocol: Group I (n=41): ADT-sensitive patients. These patients received androgen deprivation therapy (ADT) (LHRH analog → Luprolid, Goserelin, Bicalutamide) as well as Docetaxel, Enzalutamide, Abiraterone. Patients who developed resistance while receiving any of the treatments in this group were included in group II. Group II (n=16): Patients who developed resistance while receiving Docetaxel, Enzalutamide, Abiraterone were included in this group. In summary, patients in this group were those who developed resistance to ADT. Group III (n=18): Cabazitaxel, Enzalutamide, Abiraterone treatment is started for patients who develop resistance to ADT. Group III included patients who developed resistance to this treatment protocol. Group IV (n=37): Patients who were diagnosed with prostate cancer and did not receive treatment.

The healthy control group (Group V, n=30), which was demographically similar to the patient group, was composed of people who came to the Central Laboratory, Blood Collection Unit of our hospital for routine control or among the relatives of the patients without any systemic disease. All volunteer participants were asked to complete and sign the "Informed Consent Form for Adult Patients".

Inclusion criteria: 1)Patients with no previous history of chemotherapy or radiotherapy, 2) male patients between the ages of 45-75, 3) Patients with no

systemic and chronic diseases, 4) Patients who have not been diagnosed with any other cancer other than prostate cancer, 5) Patients with no acute or chronic inflammation, 6) Patients who do not smoke or drink alcohol

Exclusion criteria: 1) Patients who have previously received chemotherapy or radiotherapy
2) Patients with systemic diseases such as diabetes, heart failure, hypertension 3) Presence of chronic or active infection.

Collection and Preparation of Samples: Blood samples taken from patients and healthy individuals in biochemistry tubes were kept at room temperature for 10-20 minutes and then centrifuged at +4°C, 4000 rpm for 10 minutes. The supernatants (sera) obtained after centrifugation were aliquoted and stored at -80˚C. Immediately before analysis on the study day, the frozen samples were thawed in stages. Repeated freezing and thawing was avoided. All standards, controls, kits and sera were brought to room temperature (18-26 °C) before use. Serum samples were analyzed on the same day and according to standard laboratory methods. CAT, SOD, tGSH, GPx, ARE, PON, MPO and MDA levels were analyzed by manual antioxidant methods, while TAS, TOS and OSI levels were analyzed by commercially purchased kits and according to the Erel method. Oxidant and antioxidant parameters of the samples were measured spectrophotometrically.

Measurement of Biochemical Parameters:

Determination of catalase (CAT) activity: Campo et al. (2004) method was used for determining the catalyze activity in the sera samples. Briefly, 20 µL of supernatant was incubated with 100 µL of substrate (65 mmol/L hydrogen peroxide in 60 mmol/L phosphate buffer solution, pH= 7.4) at 37 °C for 1 minute. Then, the enzymatic reaction was stopped with 100 µL of ammonium molybdate (32.4 mmol/L). The reaction is determined by measuring at 405 nm in the spectrophotometer (12). Standards were prepared from pure catalase enzyme as 16150 U/mL-126 U/mL. By drawing a standard graph, the catalase activity in the samples was calculated according to the equation of this curve. Results were expressed as U/mL for sera samples.

Determination of superoxide dismutase (SOD) enzyme activity measurement: The method is based on the principle of inhibiting the free radicals of the superoxide dismutase enzyme during the reduction of the free oxygen radicals released by the enzymatic reaction in the presence of nitro blue tetrazolium (NBT) in the sample. The color change observed as a result of the reaction is measured at 560 nm by a spectrophotometer (13).

Determination of total GSH (tGSH): The Sedlak and Lindsay (1965) method was used for tGSH measurement. After 30 min incubation at 37 °C, absorbance measurement was made at 412 nm. 2 mM, 1 mM, 0.5 mM, 0.250 mM, 0.125 mM, 0.0625 mM and 0.031 mM reduced glutathione were used as standards. A standard graph was plotted using standard concentrations and standard absorbance values. tGSH concentrations in the samples were calculated according to the equation of this curve. Results are expressed as mM for serum samples (14).

Determination of glutathione peroxidase (GPx) activity: The reduced glutathione (GSH) is oxidized by GPx to oxidized glutathione (GSSG). Then, in the opposite direction, NAPDH (nicotinamide adenine dinucleotide phosphate) is used during the reduction of oxidized GSSG by the enzyme glutathione reductase. GPx activity is calculated by measuring the decrease in absorbance during the oxidation of NADPH to NADP+ at 340 nm (15).

Determination of arylesterase (ARE): Phenylacetate (Sigma Co, UK), is used as a substrate for

determining arylesterase activity. ARE activity was determined by measuring the absorbance of the phenol formed at 270 nm (16). In the calculation of ARE activities, 1310 M−1 cm−1 molar absorption coefficient was used.

Determination of paraoxonase (PON): The absorbance alteration of p-nitrophenol formation with paraoxon was taken into account at 37 °C, 412 nm spectrophotometrically (16, 17). In the calculation of PON activity, 17 100 M−1 cm−1 was used as molar absorption coefficient.

Determination of myeloperoxidase (MPO): It is based on the kinetic measurement of the absorbance of the yellowish-orange colored complex resulting from the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide at the wavelength of 460 nm (18).

Determination of lipid peroxidation (MDA): It is based on the spectrophotometric measurement of the absorbance of the pink colored complex formed by thiobarbituric acid and MDA at a wavelength of 532 nm after an incubation period of 60 minutes at 95°C (19). A stock standard solution was prepared with 1.1.3.3 tetraethoxypropane at a concentration of 200 µM. Standard solutions of different concentrations (100-1.56 µM) were obtained by serial dilution from the stock standard. A standard graph was drawn by using the concentration and absorbance values of the standard solutions. MDA concentrations in the samples were calculated according to the equation of this curve. Results were expressed in micromolar (µM) for serum samples.

Total antioxidant status (TAS) measurement: Total antioxidant level (TAS) was studied by Erel method (20). Serum TAS level was measured by spectrophotometry using Rel Assay Diagnostics commercial kit (Catalog no: RL0017, Gaziantep, Turkey). The principle of the test is based on the fact that antioxidants in the sample reduce the dark blue-green ABTS (2,2-azino-bis 3-ethylbenzothiazolin6-sulfonic acid) radical to the colorless reduced ABTS form. The change in absorbance at 660 nm, which can be measured spectrophotometrically, correlates with the total antioxidant level of the sample. The assay is calibrated with a balanced antioxidant standard solution called Trolox Equivalent, a vitamin E analog. Assay results were calculated using a standard of 1 mmol TroloxEquiv./L. The CV% values of the assay were reported by the manufacturer as ±10%, range 1.20-1.50 mmol/L. Results are given in mmol/L for serum.

Total oxidant status (TOS) measurement: Total oxidant levels of sera were measured using Rel Assay Diagnostics commercial kit (Catalog no: RL0024, Gaziantep, Turkey). Plasma TOS levels were determined using the automated measurement method developed by Erel (21). In this method, oxidants present in the sample oxidize the iron ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by the abundant glycerol molecules in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The CV% values of the assay are reported by the manufacturer as ±10%, range 4-6 umol/L. The assay was calibrated with hydrogen peroxide and results are given as µmol H2O2 Eq./L for serum.

Oxidative stress index (OSI): Calculated as total oxidant level divided by total antioxidant capacity (obtained by Erel method). For the calculation of the OSI value, the unit of TAS was converted to 50 µmol/l and the formula TOS (µmol H2O2 Eq/L) / (10*TAS (mmol/Trolox Eq/L) was used (22).

Statistical Analysis: Data were analyzed using SPSS 25.0 (Statistical Package for Social Science, IBM Corp

in Amonk, NY, USA) and GraphPad Prism software V.10.4.0 (Boston, MA, USA). The Shapiro Wilk test was used to determine whether the data fit the normal distribution. Descriptive statistics for numerical variables were expressed as mean ± standard error and median (Q1 and Q3 Quartiles) parameters. Comparison of means between two variables was evaluated by One Way Anova test in independent groups for normally distributed parameters and Kruskal Wallis test for non-normally distributed parameters. Median values were used in drawing the graphs. When evaluating the results $p<0.05$ was considered statistically significant.

Sample Calculation (G Power Analysis): The minimum number of participants required for the study was calculated in the G Power sample calculation program (version 3.1.9.4). The minimum sample size was calculated with Type I error (α) 0.05, Type II error (1-β) 0.95 and effect size 0.45 (23). Accordingly, the minimum sample size was calculated as 20 people for each group and 100 people in total (Cohen's f: 0.4) considering the independent groups (patient and control groups) (23).

Results

In this study, patients were divided into four groups according to the treatment protocol, and the sample number and average age of the groups were determined as follows: Group I (n=41; 70.73±10.69 years), Group II (n=16; 68.25±5.18 years), Group III (n=18; 73.00±8.00 years), Group IV (n=37; 71.86±8.42 years). The healthy control group (Group V, n=30; 69.18±12.25 years) without any systemic disease was formed. There was no statistically significant difference between the patient groups and the control group ($p>0.05$). Shapiro-Wilk tests were performed to determine whether there was a significant difference between the groups in our study parameters. As a result of these tests, the mean, standard error (SE), median, first and third quartile (Q1-Q3), minimum, maximum and p value results of the groups according to the parameters are shown in Table 1. According to this table, significant differences were found between the groups in CAT, ARE, MPO, TAS, TOS and OSI values ($p<0.05$). No significant difference was found between the groups in the measurement of SOD, tGSH, GPx, MDA, and PON levels ($p>0.05$).

Table 1. Oxidant/antioxidant measurement results of patient groups (Group I-IV) and control group (Group V)

Parameters	Group I (n=41)	Group II (n=16)	Group III (n=18)	Group IV (n=37)	Group V (n=30)	P Value
	Mean ± SE Median (Q1-Q3) Min-Max	Mean ± SE Median (Q1-Q3) Min-Max	Mean ± SE Median (Q1-Q3) Min-Max	Mean ± SE Median (Q1-Q3) Min-Max	Mean ± SE Median (Q1-Q3) Min-Max	
CAT (U/mL)	61818.15±1484.37 61701.58 (57587.73-69702.39) 33721.41-79820.64	51649.59±3024.06 49579.29 **(**44779.33-62631.73**)** 20026.81-68477.32	48812.48±1495.79 48608.13 **(**44815.42-54169.62**)** 35803.26-59069.93	62706.15±1757.17 65878.20 (59069.93-67529.71**)** 9436.82-80032.38	57422.00±1886.68 **(**53523.74-64902.93**)** 33570.16-71791.70	**<0.0001***
SOD (U/mL)	91.51±4.66 100.48 (58.04-119.75**)** 40.00-134.15	82.43±5.48 82.68 (64.02-98.65**)** 51.22-123.90	92.35±8.44 86.34 **(**59.02-128.29**)** 38.54-151.22	100.83±3.58 102.92 **(**92.92-113.90**)** 42.93-144.39	89.47±3.39 91.21 **(**76.58-103.53**)** 45.85-125.37	0.159
tGSH (mM)	0.57±0.03 0.54 (0.43-0.67) 0.24-1.36	0.62±0.03 0.62 (0.55-0.67) 0.40-0.92	0.67±0.04 0.65 (0.54-0.73) 0.40-1.18	0.57±0.03 0.51 (0.42-0.59) 0.32-1.17	0.64±0.04 0.62 (0.48-0.74) 0.31-1.43	0.305
GPx (IU/L)	1.66±0.74 0.60 (0.25-1.57) 0.14-31.00	1.07±0.30 0.57 (0.33-1.42) 0.11-5.08	0.76±0.12 0.67 (0.35-1.07) 0.18-2.03	1.55±0.53 0.68 (0.45-1.47) 0.10-19.85	0.86±0.14 0.58 (0.35-0.95) 0.14-3.09	0.772
ARE (U/mL)	755±49.40 738.93 (584.73-980.15) 30.53-1264.12	512.97±58.88 598.47 (262.59-698.47) 134.35-836.64	495.50±69.58 404.58 (269.46-749.61) 54.96-1004.58	496.63±50.03 439.69 (273.28-745.03) 6.11-1200.00	435.72±55.13 415.26 (164.12-663.35) 12.21-1111.45	**<0.0001***
PON (U/mL)	4.27±0.38 3.77 (2.30-5.61) 0.23-10.87	3.66±0.48 3.89 (2.46-5.07) 0.11-7.04	4.57±0.73 4.22 (2.83-5.15) 0.72-14.35	3.96±0.38 3.49 (2.01-5.36) 0.33-9.05	6.05±0.65 5.03 (3.13-7.30) 1.82-17.56	0.0713
MPO (U/mL)	24.92± 1.60 24.94 (19.94-32.65) 3.61-53.56	18.66±2.47 18.20 (9.92-28.31) 1.18-34.27	18.11±2.20 16.59 (9.53-27.23) 3.19-34.34	10.87±0.86 9.90 (8.48-11.42) 4.69-29.20	8.66±1.15 6.75 (5.59-9.03) 2.98-35.06	**<0.0001***
MDA (µM)	10.31±0.37 9.61 (8.80-10.78) 7.99-18.99	11.61±0.58 10.42 (9.66-13.62) 8.35-19.35	10.52±0.33 10.15 (9.07-11.68) 8.53-15.57	11.40±0.93 11.40 (8.89-11.20) 8.35-22.06	12.94±0.96 12.94 (9.38-15.47) 8.53-23.14	0.410
TAS (mmol/L)	0.52±0.04 0.54 (0.36-0.64) 3.61-53.56	0.40±0.06 0.37 (0.17-0.52) 0.11-1.19	0.52±0.03 0.51 (0.40-0.62) 0.33-0.93	0.96±0.02 0.98 (0.93-1.05) 0.16-1.18	0.85±0.06 1.02 (0.68-1.10) 0.01-1.14	**<0.0001***
TOS (micromol/L)	25.92±1.00 20.90 (17.95-27.27) 8.64-89.09	15.16±1.62 13.72 (8.70-20.90) 7.42-31.82	14.79±0.47 15.68 (8.95-19.54) 1.61-31.94	15.10±0.20 10.90 (7.73-20.00) 2.38-40.00	18.41±0.42 11.07 (8.33-27.72) 2.27-63.18	**0.0001***
OSI ratio TOS/(10*TAS)	6.99±1.00 4.03 (2.97-8.58) 1.58-24.32	6.28±1.62 3.56 (1.79-8.84) 1.19-21.28	3.11±0.47 2.55 (1.99-3.99) 0.32-7.98	1.69±0.20 1.25 (0.74-2.28) 0.22-5.69	2.61±0.42 1.46 (0.90-4.18) 0.22-9.77	**<0.0001***

***p<0.05** was considered statistically significant in the analysis of variables.

Post-hoc analysis to determine between which groups our study parameters (CAT, SOD, tGSH, GPx, ARE, PON, MPO, MDA, TAS, TOS and OSI) differed and the results of the p values obtained are shown in Table 2.

Table 2. Comparison of patient groups (Group I-IV) in terms of oxidant and antioxidant measurement results (Post-hoc analysis)

	CAT (U/mL)	SOD (U/mL)	tGSH (mmol/L)	GPx (U/L)	ARE (U/mL)	PON (U/mL)	MPO (U/L)	MDA (µM)	TAS (mmol/L)	TOS (µmol/L)	OSI TOS/(10*TAS)
Adjusted P Value	P	P	P	P	P	P	P	P	P	P	P
Group I, II, III, VI and V	**<0.0001****	0.1592	0.305	0.7726	**<0.0001****	0.0713	**<0.0001****	0.410	**<0.0001****	**0.0001****	**<0.0001****
Group I vs. Group II	**0.0188***	0.7580	>0.9999	>0.9999	**0.0494***	>0.9999	>0.9999	0.1675	>0.9999	**0.0465***	>0.9999
Group I vs. Group III	**<0.0001****	>0.9999	0.2209	>0.9999	**0.0200***	>0.9999	0.6656	>0.9999	>0.9999	**0.0211***	0.1278
Group I vs. Group IV	>0.9999	0.5095	>0.9999	>0.9999	**0.0018***	>0.9999	**<0.0001****	>0.9999	**<0.0001****	**0.0003***	**<0.0001****
Group I vs. Group V	0.7360	0.9975	0.9283	>0.9999	**0.0002****	0.2631	**<0.0001****	0.0585	**0.0001****	**0.0054****	**0.0001****
Group II vs. Group III	>0.9999	0.7989	>0.9999	>0.9999	0.9998	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Group II vs. Group IV	**0.0031****	0.1293	0.6991	>0.9999	0.9997	>0.9999	0.1557	>0.9999	**<0.0001****	>0.9999	**0.0025****
Group II vs. Group V	>0.9999	0.9047	>0.9999	>0.9999	0.9188	0.3169	**0.0008****	>0.9999	**<0.0001****	>0.9999	0.1869
Group III vs. Group IV	**<0.0001****	0.7862	0.1344	>0.9999	>0.9999	>0.9999	0.2103	>0.9999	**<0.0001****	>0.9999	0.1337
Group III vs. Group V	**0.0227***	0.9959	>0.9999	>0.9999	0.9621	>0.9999	**0.0010****	0.6491	**0.0023****	>0.9999	>0.9999
Group IV vs. Group V	0.1596	0.3877	0.5733	>0.9999	0.9205	0.0792	0.4427	>0.9999	>0.9999	>0.9999	>0.9999

*p<0.05 **p<0.01

According to the results of serum CAT concentration measurements, there was a significant difference between all groups (p<0.05), but there was no significant difference between the groups in SOD concentration measurements (p>0.05). The graph of these measurement results is shown below (Figure 1).

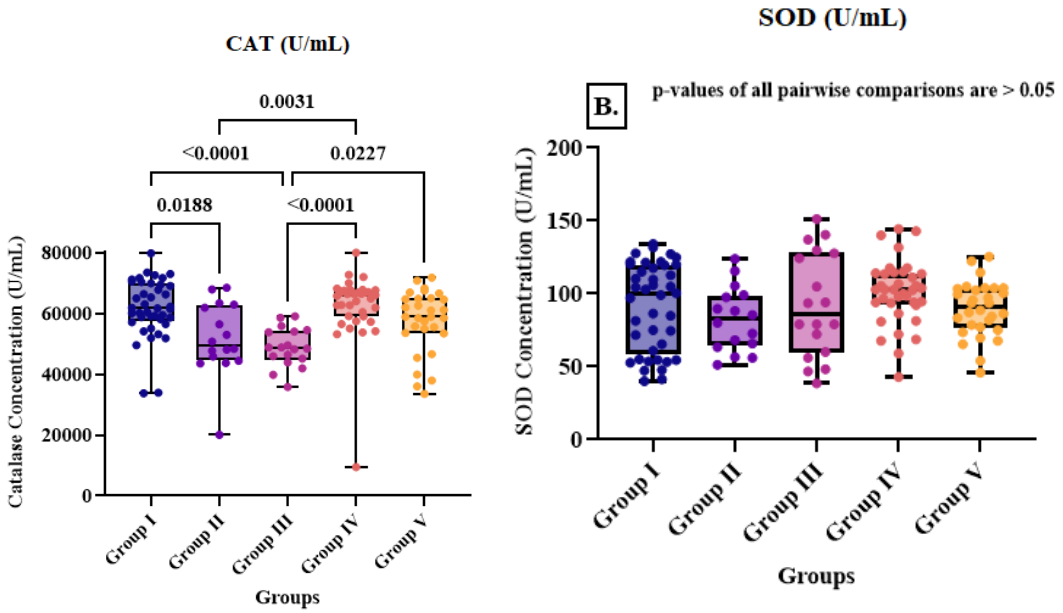

Figure 1. Serum CAT (A.) and SOD (B.) concentrations of patient and control groups

According to the results of tGSH and GPx measurements of the patients and controls, no significant difference was found between the groups in terms of these two parameters ($p>0.05$). The results of these measurements are shown in Figure 2.

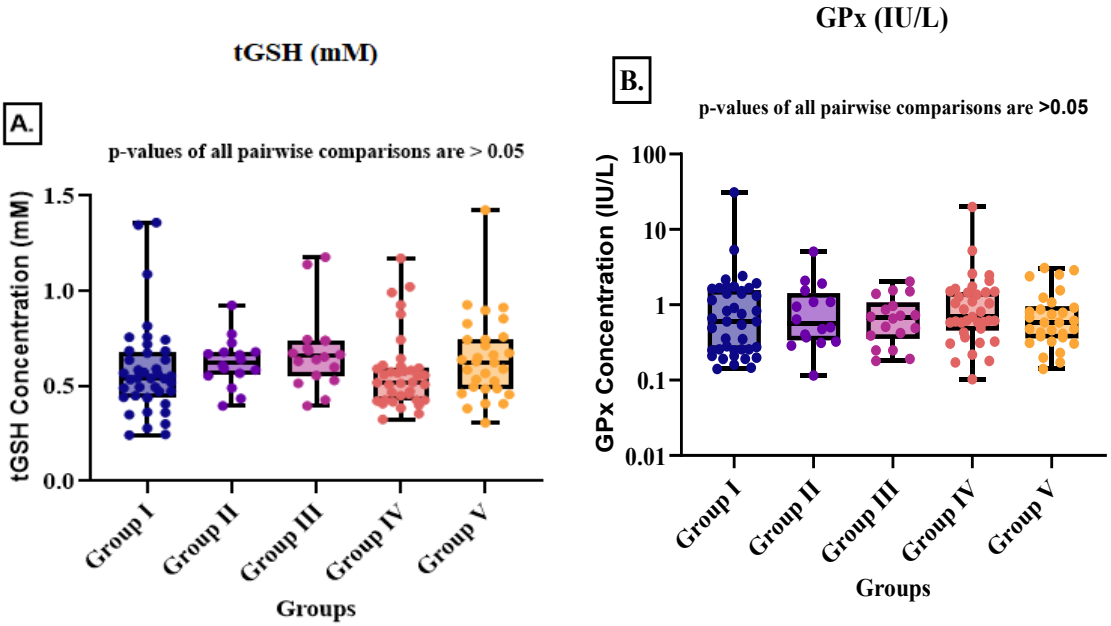

Figure 2. Serum tGSH (A.) and GPx (B.) concentrations of patient and control groups

Although there was a significant difference between the groups according to the results of serum ARE concentration measurements ($p<0.05$), there was no significant difference between the groups in PON concentration measurements ($p>0.05$). The graph of these measurement results is shown in Figure 3.

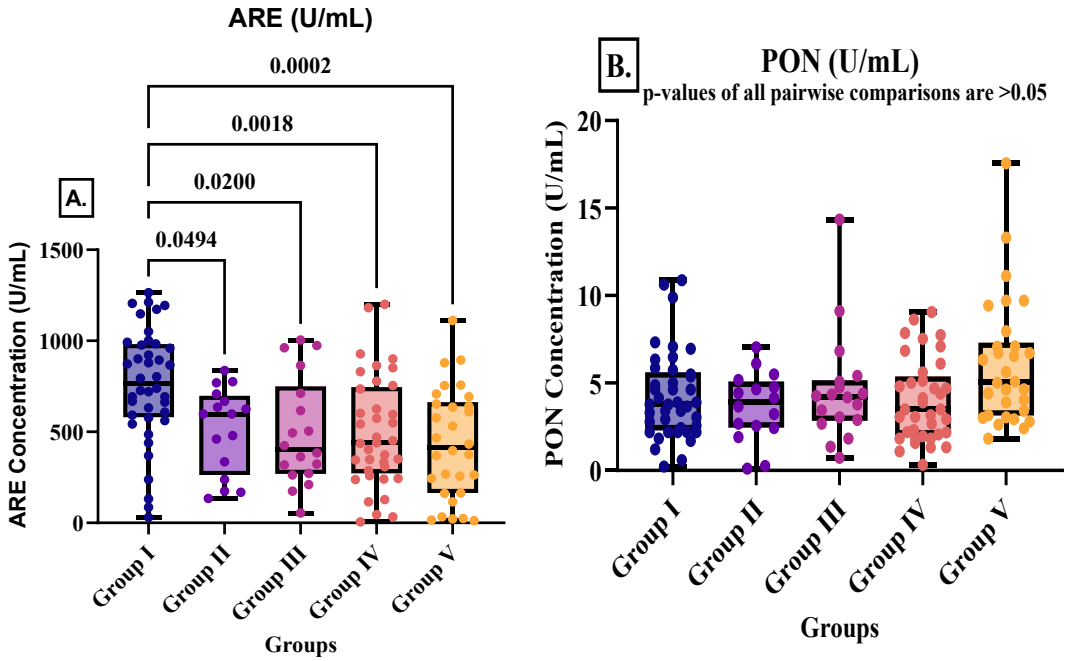

Figure 3. Serum ARE (A.) and PON (B.) concentrations of patient and control groups

There was a significant difference between the groups according to the results of serum MPO concentration measurements ($p<0.05$); however, there was no significant difference between the groups in MDA concentration measurements ($p>0.05$). The graph of these measurement results is shown in Figure 4.

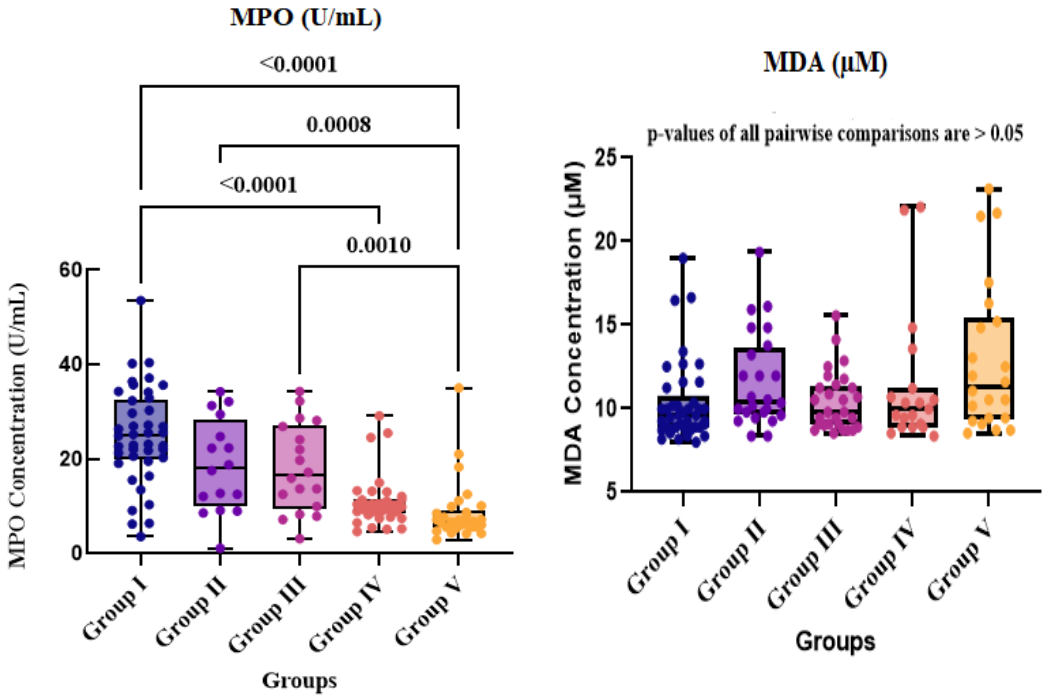

Figure 4. Serum MPO (A.) and MDA (B.) concentrations in patient and control groups.

The graphs showing that there were significant differences ($p<0.05$) between the groups in the measurement of total oxidant and antioxidant parameters TAS, TOS and OSI concentrations in serum are shown in Figure 5.

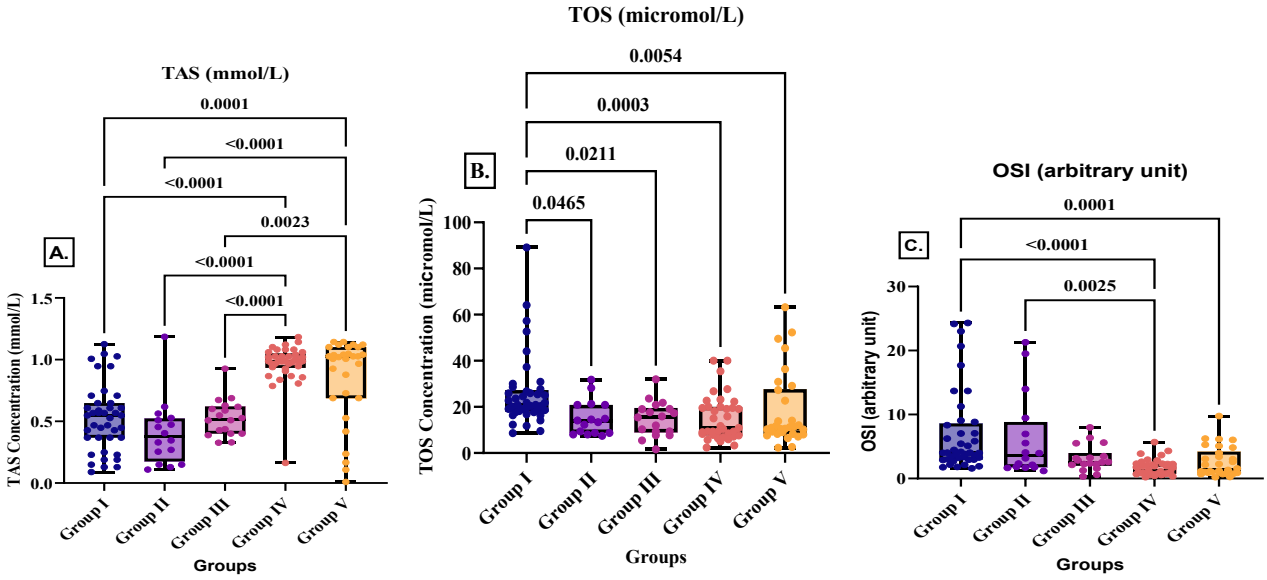

Figure 5. Serum TAS (A.), TOS (B.) concentrations and OSI values (C.) of patient groups and control group

Discussion

Yüksel et al. found that the activities of total antioxidant enzymes decreased because the antioxidant balance was disrupted as a result of oxidative stress caused by ROS in prostate cancer cells, and SOD antioxidant enzyme level was high to balance this level (24). In our study, the fact that the SOD level was low in the 2nd group (although not statistically significant) may be an indication that a resistance to SOD also developed in patients resistant to ADT. Again, the fact that TAS level was significantly ($p<0.0001$) lower in patients receiving ADT treatment (Groups 1, 2 and 3) compared to healthy control and untreated groups is an indicator of increased ROS in accordance with the literature.

Seçkin et al. reported that MDA, which is a lipid peroxidation product and a biomarker of oxidative stress, increased in the plasma of 64 patients with prostate cancer with an increase in vascular endothelial growth factor (VEGF) (25). The fact that MDA was lower in our study compared to the control group ($p>0.05$) may be due to inhibition-related error at the laboratory analysis stage.

In a study in which 40 participants with prostate cancer were included, OSI level in the serum of patients was found to be statistically significantly lower in the control group than in the patient group; serum PON and ARE levels were found to be statistically significantly higher in the control group than in the patient group (26). PON and ARE enzyme activity was found to be low in trauma patients due to oxidative stress by Yıldırım et al. (27). The fact that OSI was significantly ($p<0.0001$) lower in the healthy control group in our study and in accordance with the literature is an indication that cancer cells are exposed to oxidative stress as an inevitable result of aerobic life. The fact that PON concentration was higher in the control group compared to the patient groups (although not statistically significant) is an indication of suppression of antioxidant levels in cancer cells (as an indicator of oxidative stress) in accordance with the literature. The fact that ARE was significantly higher ($p<0.0001$) in the patient group (especially in ADT-sensitive Group 1 patients) compared to the control group (mean 755±49.40) may indicate a positive response to the treatment protocol.

Sajjaboontawee et al. showed that GSH and GPx levels were significantly lower in the serum of patients with prostate cancer compared to the control group (28). In our study, no significant difference was found between the groups in terms of total GSH and GPx parameters.

Arsova-Sarafinovska et al. showed higher MDA concentrations and lower GPx, SOD and CAT activities in prostate cancer patients compared to control and benign prostatic hyperplasia (BPH) groups (29). In our study, the low level of SOD in Group 2 may be an indicator of ADT resistance. Oxidoreductase, which reduces hydrogen peroxide to water and oxygen, and antioxidant CAT enzyme activity were found to be significantly higher between the groups ($p<0.0001$) in Group 1 (mean 61818.15±1484.37) in Group 1 (mean 61818.15±1484.37) may be indicative of a favorable response to ADT, whereas lower levels in Groups 2 (mean 51649.59±3024.06) and 3 (mean 48812.48±1495.79) may be indicative of a biomarker of resistance to ADT, cabazitaxel and other chemohormonal treatments.

Xiang et al. found that serum TOS and OSI values were significantly higher and serum TAS values were significantly lower in patients with lung cancer than in the control group (30). In our study, TAS measurement was higher in those who did not receive treatment (Group 4) and lower in those who received chemohormonal treatment (Groups 1, 2, 3) with a significance level of $p<0.0001$, which may be a biomarker for cancer diagnosis due to increased oxidative stress. The fact that TOS and OSI measurements, which are oxidant parameters, were higher in patients with $p<0.05$ significance level in

accordance with the literature is evidence that they are biomarkers that help cancer diagnosis.

Sincan et al. 40 acute myeloid leukemia patients and 18 healthy individuals, serum MPO level was found to be higher and PON level was found to be lower (31). MPO level, which acts as an antimicrobial agent to prevent DNA damage in cancer cells, was found to be high with a significance level of $p<0.0001$ in accordance with the literature. Although PON activity was not statistically significant ($p>0.05$), it was found to be lower in patients compared to the control group in accordance with the literature.

Blatt et al. Enzalutamide (Enz)-resistant patients have higher ROS levels than Enz-sensitive patients. AR is positively correlated with oxidative stress pathways as it decreases glutamine metabolism and antioxidants. However, in the relationship between oxidative stress and treatment resistance in patients with untreated primary prostate cancer, ROS levels were found to be lower than those who received treatment (32). In our study, contrary to the literature, TOS, one of the important indicators of ROS, was significantly higher in Enz-sensitive patients with a significance level of $p=0.0001$. The fact that TOS measurement was lower in patients with prostate cancer who did not receive treatment compared to those who received treatment in accordance with the literature may be associated with chemohormonal treatment resistance.

Mondal et al. androgen deprivation may increase oxidative stress and the resulting ROS may activate both AR and non-AR signaling. Nrf2 gene activators and antioxidants may eliminate this cycle and suppress the development of endocrine-resistant prostate cancer (33). In our study, since androgen suppression or androgen castration was the treatment target in patients with prostate cancer, oxidative stress-related oxidant and antioxidant increases were observed. This is an indication of the effect of chemohormonal treatment on patient survival.

Taxane-based chemotherapeutics such as docetaxel or cabazitaxel have been used effectively in the treatment of MDRCC. However, this treatment is inadequate in later stages. The main reason for this failure is acquired drug resistance in cancer cells. Cabazitaxel shows a significantly higher cytotoxic effect than docetaxel in MDRCC cells and increases ROS production more. This suggests that cabazitaxel is a more pro-oxidant agent than docetaxel (by inducing apoptosis through mitochondrial damage). The lack of difference in total GSH between docetaxel-resistant and sensitive prostate cancer cells suggests that taxane activity does not affect GSH metabolism (34). The fact that there was no significant difference between the groups in total GSH measurement in our study supports the literature. There was a difference between cabazitaxel-resistant (mean 14.79±0.47) (Group 3) and docetaxel-sensitive (25.92±1.00) (Group 1) in the measurement of TOS, one of the ROS parameters, with a significance level of $p=0.0001$. According to our study, the fact that docetaxel increased the TOS parameter more may be an indication that the resistance to this drug is higher.

Conclusions

As a result of our study, CAT, ARE, MPO, TAS, TOS and OSI parameters were significantly different between the groups ($p<0.05$), whereas SOD, tGSH, GPx, MDA and PON parameters were not significantly different between the groups. However, our study is a comprehensive research study that will shed light on the effects of chemohormonal treatment and treatment resistance on oxidative stress levels and antioxidant enzyme activities in cancer and prostate cancer as predictive parameters in patient follow-up, prognosis and treatment and will fill the gap in the literature.

Author Contributions: HKÖ, AT, MB: contributed to the collection of samples; EL, NY and MK: contributed to the writing of the manuscript, the study concept, design, statistical analyses and laboratory

work. All authors read and approved the final manuscript. NY: Neslihan YÜCE, MK: Muhammed KÖKPINAR, EL: Esra LALOĞLU, HKÖ: Hilal KIZILTUNÇ ÖZMEN, AT: Aykut TURHAN, MB: Mahsa BAYRAMİ,

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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References

1. Erzurumlu Y. Prostat Kanseri: Androjen Reseptörü Sinyal Mekanizması. Medical Journal of Süleyman Demirel University. 2021;28(1):187-98.
2. Özdoğan M. Türkiye Kanser İstatistikleri 2020. Erişim adresi: https://www drozdogan com/turkiye-kanseristatistikleri-2020/ Erişim tarihi. 2021;13.
3. Yıldızhan M, Dündar M, Ertek MŞ. Metastatik Prostat Kanserinde Aralıklı veya Devamlı Androjen Baskılama Tedavisi: Derleme. Üroonkoloji Bülteni. 2015;14(2).
4. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. Journal of clinical oncology. 2005;23(32):8253-61.
5. Seçkiner İ. Kastrasyona Dirençli Prostat Kanseri Tedavisinde Son Gelişmeler. Journal of Reconstructive Urology. 2013;4(3):126-33.
6. Erbin A, Esen T. Kastrasyona Dirençli Prostat Kanserinde Tedavi 2024 [Available from: https://uroturk.org.tr/urolojiData/Books/520/kastrasyona-direncli-prostat-kanserinde-tedavi.pdf.
7. Ahmed Amar SA, Eryilmaz R, Demir H, Aykan S, Demir C. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. The Aging Male. 2019;22(3):198-206.
8. Aslankoç R, Demirci D, İnan Ü, Yıldız M, Öztürk A, Çetin M, et al. Oksidatif stres durumunda antioksidan enzimlerin rolü-Süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPX). Medical Journal of Süleyman Demirel University. 2019;26(3):362-9.
9. Özer ÖF, Güler EM, Selek Ş, Çoban G, Türk HM, Koçyiğit A. Akciğer, meme ve kolon kanserli hastalarda oksidatif stres parametrelerinin değişimi Variation of oxidative stress parameters in patients with lung, breast and colon cancer.
10. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS letters. 1991;286(1-2):152-4.
11. Eroglu M, Yilmaz N, Yalcinkaya S, Ay N, Aydin O, Sezer C. Enhanced HDL-cholesterol-associated anti-oxidant PON-1 activity in prostate cancer patients. The Kaohsiung journal of medical sciences. 2013;29(7):368-73.
12. Campo GM, Avenoso A, Campo S, Ferlazzo AM, Micali C, Zanghı` L, et al. Hyaluronic acid and chondroitin-4-sulphate treatment reduces damage in carbon tetrachloride-induced acute rat liver injury. Life sciences. 2004;74(10):1289-305.
13. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical chemistry. 1988;34(3):497-500.
14. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry. 1968;25:192-205.
15. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine. 1967;70(1):158-69.
16. Gülcü F, Gürsu MF. Paraoksonaz ve aril esteraz aktivite ölçümlerinin standardizasyonu. Türk Biyokimya Dergisi. 2003;28(2):45-9.
17. Kaya H, Polat F, Çetinkaya R, Taysi S. Kronik hemodiyaliz hastalarinda serum paraoksanaz aktivitesi. Türk Nefroloji, Diyaliz ve Transplantasyon Dergisi. 2000;9(3).
18. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Journal of investigative dermatology. 1982;78(3):206-9.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979;95(2):351-8.
20. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical biochemistry. 2004;37(2):112-9.
21. Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry. 2005;38(12):1103-11.
22. Goolsby MJ. National Kidney Foundation Guidelines for chronic kidney disease: evaluation, classification, and stratification. Journal of the American Academy of Nurse Practitioners. 2002;14(6):238-42.
23. Cohen J. Statistical power analysis. Current directions in psychological science. 1992;1(3):98-101.
24. Yüksel B, Özkan AD. The role of citrus nobiletin on oxidative stress levels and superoxide dismutase activities in metastatic castration-resistant prostate cancer. Commagene Journal of Biology. 2021;5(1):84-9.
25. Seçkin D, Onur R, İlhan N, Ardıçoğlu A, İihan N. Prostat kanserli ve bening prostat hiperplazili hastalarda plazma vasküler endotelyal büyüme faktörü, oksidatif stres ve nitrik oksit ilişkisi. Fırat Üniversitesi Sağlık Bilimleri Tıp Dergisi. 2005;19:277-81.
26. Orhan N. Prostat Kanserli Hastalarda Oksidatif Stres ve Paraksonaz Aktivite Azalması. Konuralp Medical Journal. 2015;7(2):113-7.
27. Yıldırım A, Aslan Ş, Ocak T, Yildirim S, Kara F, Sahin Y. Travmalı hastalarda serum paraoksonaz/arilesteraz aktiviteleri ve malondialdehit düzeyleri. Eurasian J Med. 2007;39(2):85-8.
28. Sajjaboontawee N, Supasitthumrong T, Tunvirachaisakul C, Nantachai K, Snabboon T, Reiche EMV, et al. Lower thiol, glutathione, and glutathione peroxidase levels in prostate cancer: a meta-analysis study. The Aging Male. 2020;23(5):1533-44.
29. Arsova-Sarafinovska Z, Eken A, Matevska N, Erdem O, Sayal A, Savaser A, et al. Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clinical biochemistry. 2009;42(12):1228-35.
30. Xiang M, Feng J, Geng L, Yang Y, Dai C, Li J, et al. Sera total oxidant/antioxidant status in lung cancer patients. Medicine. 2019;98(37):e17179.
31. Sincan S, Sincan G, Aşkın S, Kızıltunç A. Evaluation of Serum Paraoxonase, Myeloperoxidase, and HDL-Cholesterol Levels in Acute Myeloid Leukemia. Inflammation. 2023;46(6):2470-6.
32. Blatt EB, Parra K, Neeb A, Buroni L, Bogdan D, Yuan W, et al. Critical role of antioxidant programs in enzalutamide-resistant prostate cancer. Oncogene. 2023;42(30):2347-59.
33. Mondal D, Narwani D, Notta S, Ghaffar D, Mardhekar N, Quadri SS. Oxidative stress and redox signaling in CRPC progression: therapeutic potential of clinically-tested Nrf2-activators. Cancer Drug Resistance. 2021;4(1):96.
34. Eryilmaz IE, Egeli U, Cecener G. Association between the apoptotic effect of Cabazitaxel and its pro-oxidant efficacy on the redox adaptation mechanisms in prostate cancer cells with different resistance phenotypes. Cancer biology & therapy. 2024;25(1):2329368.