Comparative study of antioxidant and nitric oxide level with PSA level in prostate cancer

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Abstract

Prostate cancer was the world's second most common malignancy after lung cancer accounting for 1,276,106 new cases and 358,989 fatalities (3.8% of all cancer-related deaths in males) in 2018. Prostate cancer incidence and fatality rates globally increase with age, with the average age at diagnosis being 66 years. According to reports, prostate cancer is commonly associated with a shift in the oxidant/antioxidant balance which results in increased oxidative stress. The present study aimed to check the levels of antioxidants and nitric oxide in prostate cancer. The Ethics Committee of Nitte (Deemed to be University) approved this study.

Blood samples were collected from 30 male patients with prostate cancer and 30 healthy volunteers. 5 ml blood samples were collected in a clean tube and 2.5 ml of serum was used for the estimation of total antioxidant (TAC), superoxide dismutase (SOD) and nitric oxide (NO) levels. The TAC was estimated by phosphomolybdenum method. SOD and NO in the sample were analyzed by spectrophotometric method. The current study found that greater levels of oxidative damage and changes in the antioxidant defence system in high-risk individuals may suggest a relationship between oxidative stress and prostate cancer.

Keywords: Prostate cancer, PSA, antioxidants, oxidative damage.

Introduction

Prostate cancer was the world's second most common malignancy after lung cancer accounting for 1,276,106 new cases and 358,989 fatalities (3.8% of all cancer-related deaths in males) in 2018[3,18]. Prostate cancer incidence and fatality rates globally increase with age, with the average age at diagnosis being 66 years.

Prostate cancer may be asymptomatic in its early stages, has an indolent course and requires little or no treatment. The most common complaint, however, is difficulty in urinating, increased frequency and nocturia, all of which can also be caused by prostate hypertrophy. The axis skeleton is the most prevalent site of bone metastatic illness, therefore more advanced stages may manifest with urine incontinence and back pain. Many prostate tumors are diagnosed based on increased plasmatic levels of PSA > 4 ng/mL, a glycoprotein usually expressed by prostate tissue. Men without cancer have also been shown to have increased PSA levels, therefore a tissue biopsy is the standard of care for confirming cancer[5]. Diet and physical activity influence prostate cancer growth and progression. Dietary variables are primarily related with documented global and ethnic disparities in the incidence rates of prostate cancer[5,12].

Antioxidants are compounds that can inhibit the generation of free radicals and the oxidation process; they can be classed based on their source. Endogenous sources include enzymes while exogenous sources include beta-carotene, lycopene and vitamins A, C and E. According to reports, prostate cancer is commonly associated with a shift in the oxidant/antioxidant balance which results in increased oxidative stress[16]. Increasing evidence suggests that intracellular synthesis of oxidative damage-causing chemicals has a significant role in aging and age-related illnesses such as prostate cancer [2]. Reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydrogen peroxides can cause lipid peroxidation and genomic deoxy ribonucleic acid (DNA) damage, affecting the activity of sulfhydryl (SH)-dependent enzymes.

According to reports, age-related molecular alterations in the prostate originate from hydroxyl radical-induced oxidative DNA damage. Prostate cancer clinical specimens exhibit progressive age-related DNA damage and a larger buildup of 8-oxo-2′-deoxyguanosine (8-OHdG) than benign tissue[10,16,17]. NO is a ubiquitous signaling molecule in the human body, having well-defined physiological activities across various organ systems. It is a free radical with a half-life of less than 5 seconds *in vivo*[15].

NO is produced as a byproduct during the process of converting L-arginine to L-citrulline, which requires oxygen and Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH). At low concentrations, NO is known to enhance cell growth and proliferation by acting as a signaling molecule[11]. At high quantities, the production of reactive species such peroxynitrite damages cell membranes and slows cancer cell proliferation[7]. Supraphysiologic levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) caused by iNOS overexpression are carcinogenic in the inflammatory state via a variety of

processes including cellular lipid alteration, angiogenesis and antiapoptosis[4]. NO can operate as either a pro or anticancer agent depending on several characteristics such as redox status, cell cycle, concentration and distribution.

SOD is a class of antioxidant enzymes playing an important role in oxidative stress in cells. Manganese superoxide dismutase (MnSOD) is a major SOD antioxidant enzyme found in mitochondria that helps to detoxify ROS. MnSOD is frequently reduced or absent in cancer cells. In several studies, lower MnSOD activity is associated with increased cancer cell growth, migration and invasion[6]. With this background we aimed to check the levels of TAC, SOD and NO in the patients with prostate cancer.

Material and Methods

Sample Collections: Approval for the study was taken from the Institutional Ethics Committee (ISC/KSHEMA/10/ 2016-17) dated July 15, 2016.

Samples collections and processing were done according to the Helsinki's declaration. Written informed consent was obtained by all the subjects. Patients were categorized using PSA level as follows:

Normal level → 0-4 ng/ml

Slightly Elevated →4-10 ng/ml

Moderately Elevated 10-20 ng/ml

Highly Elevated → more than 20 ng/ml

5 ml blood samples were collected is clean tubes from 30 male patients with the cases of prostate cancer and 30 individuals not having the disease. 2.5 ml of serum was used for the estimation of TAC, SOD and NO levels.

Estimation of TAC by Phosphomolybdenum method: The assay is based on the principle of conversion of molybdenum (Mo VI) by reducing agents like antioxidants to molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695nm. 100µL of the sample was pipetted into a clean test tube. 5% TCA was added to precipitate out the proteins in the sample. The mixture was allowed to stand for five minutes and was centrifuged. Transfer 100µL of the clear supernatant into a clean test tube with 1mL of TAC reagent added to it and incubate the mixture in a water bath at 90^0C for 90 minutes.

Simultaneously, a blank is also maintained by substituting 100µL of water instead of sample in the reaction mixture. Cool and read the optical density of the greenish to bluish colour formed at 695nm against blank. The concentration of the total antioxidants in the serum is obtained by plotting the absorbance of the test against the standard graph and the concentration is expressed as µg/mL.

Spectrophotometric analysis for SOD: The substrate used for the assay consists of nitro blue tetrazolium chloride (NBT) which reacts with superoxide anions produced upon illumination of riboflavin in the presence of methionine as an electron donor to produce formazan which is a blue coloured complex. The SOD present in the sample will act on the superoxide anions produced by riboflavin and thereby will reduce the net superoxide anions in the substrate leading to decreased production of formazan manifested by decreased intensity of the blue color formed. The decrease in the formation of formazan is directly proportional to the amount of SOD in the sample, 50% decrease in the formation of formazan is taken as one unit of SOD. Centrifuge 500µL of heparinized blood at 1800 rpm for 10 min.

Separate the upper plasma layer, add 500 µL of normal saline to the erythrocyte layer, mix well and centrifuge, Discard the upper layer and add fresh normal saline to the erythrocytes, repeat this step two more times to wash the erythrocytes. Control for each sample analyzed has to be maintained. Common standard and blanks for each set of illumination are maintained. 100 µL of Red Blood cells (RBC) lysate is diluted further by the addition of 400 µL of 0.05 M phosphate buffer to get a final erythrocyte dilution of 1:20.

(i) Test: 0.3 mL Riboflavin, 2.5 mL Methionine, 0.1 mL NBT, 0.1 mL RBC lysate.
(ii) Control: 2.5mL Methionine, 0.3 mL Riboflavin, 0.1mL 0.05 M phosphate buffer, 0.1mL RBC lysate.
(iii) Standard: 2.5 mL Methionine, 0.3 mL Riboflavin, 0.1 mL NBT, 0.1 mL 0.05 M phosphate buffer
(iv) Blank: 2.5 mL Methionine, 0.3 mL Riboflavin, 0.2 mL 0.05 M phosphate buffer

Following the illumination, immediately read the optical density of all the reaction mixtures at 560nm. Calculate the units of enzyme present in the sample using the formula expressed as U/mg Hb:

SOD present/mg Hb = SOD activity/Hb/20 Dilution factor = 20

$$\text{SOD activity/mg Hb} = \frac{\text{SOD activity} * 20}{\text{Hb}} = \text{U/mg Hb}$$

Estimation of NO: This assay determines NO concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acidified NO produces a nitrosating agent which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-

naphthyl) ethylenediamine to form the chromophoric azo-derivative.

This procedure is based on Greiss reagent method. Briefly, 100 µl of serum sample was taken in test tubes. To this, 0.9 ml distilled water, 2 ml of sulfanilamide solution (0.5 g of sulfanilamide in 100 ml of 20% V/V hydrochloric acid (HCl) and after 5 minutes 2 ml of N-(1-naphthylyl)-ethylenediamine HCl solution were added. The pH at this point was noted. The absorbance was read at 550 nm. The concentration of nitrite was calculated by plotting the graph from a series of standard nitrite solution.

Statistical Analysis: Values are expressed in Mean ± Standard Deviation (SD). Data was collected and statistically analyzed by the ANOVA for comparison between TAC, NO and SOD between the cases and control group. $P < 0.05$ is considered as significant. All statistical analysis was carried out using the statistical package for social science (SPSS 16.).

Results and Discussion

The present study aimed to check the levels of TAC, NO and SOD in prostate cancer. The Ethics Committee approved this study. Informed consent was taken before collecting the sample. Blood samples was collected from 30 male patients with prostate cancer and 30 healthy volunteers. Patients were categorized into different groups based on their PSA level. 5 ml blood samples were collected in a clean tube and 2.5 ml of serum was used for the estimation of TAC, NO and SOD levels.

The mean age of the study population in the control group was 56± 2.15 and in the case group, it was 60±1.34 (Table 1).

Mean TAC levels in the control sample were 18.76±1.6838, slightly elevated PSA was 11.22±0.7929, moderately elevated PSA was 9.78±0.7683 and highly elevated PSA was 7.36±0.7589. Mean NO levels in the control sample were 4.954±0.40, slightly elevated PSA was 3.875±0.2388, moderately elevated PSA was 3.253±0.16 and highly elevated PSA was 2.600±0.434. Mean SOD levels in the control sample were 17.791±4.21, slightly elevated PSA was 30.56±1.14, moderately elevated PSA was 45.87±12.56 and highly elevated PSA was 61.19±10.83 (Table 2).

Prostate cancer is the second most frequent disease in men, with an anticipated 1.4 million cases and 375,000 deaths. Prostate cancer may be asymptomatic in its early stages, have an indolent course and require little or no treatment. Many prostate tumors are identified based on elevated plasmatic levels of PSA > 4 ng/mL, a glycoprotein typically seen in prostate tissue. However, because men without cancer have been reported to have increased PSA, a tissue biopsy is the standard of care to confirm the existence of malignancy [3,5]. An effective prostate cancer prevention strategy would provide many benefits to men with a significant positive impact on public health, including the potential to reduce the high lifetime risks of prostate cancer development, the morbidities associated with cancer treatment, especially in newly diagnosed patients with biologically indolent prostate cancer who still undergo curative-intent therapy rather than active surveillance [13].

The present study aimed to check the levels of TAC, NO and SOD in prostate cancer. Oxidative stress and cumulative DNA damage raise the risk of prostate cancer[9]. Recent research suggests that natural plant-derived antioxidants may have therapeutic potential via modifying microRNAs (miRNAs), a kind of noncoding RNA implicated in inflammation and carcinogenesis that is unregulated in a variety of cancers including prostate cancer.

Table 1
Baseline characteristics of patients with Prostate cancer

Parameter	Normal (control)	Slightly Elevated PSA	Moderately Elevated PSA	Highly Elevated PSA
Mean age	56± 2.15	60±1.34		
Number in %	100	33.3	33.3	33.3

PSA- Prostate Specific Antigen

Table 2
Comparison of the levels of Total antioxidant, Nitric oxide and SOD with normal control

Parameter	Normal (control)	Slightly Elevated PSA	Moderately Elevated PSA	Highly Elevated PSA
Total Antioxidant (µg/ml)	18.76±1.6838	11.22±0.7929	9.78±0.7683	7.36±0.7589***
NO (µg/ml)	4.954±0.40	3.875±0.2388	3.253±0.16	2.600±0.434***
SOD SODU/gm Hb	17.791±4.21	30.56±1.14	45.87±12.56	61.19±10.83***

***P<0.001 statistically significant, NO- Nitric Oxide, SOD- Superoxide Dismutase

These data suggest that the use of antioxidants could be an appealing miRNA-mediated chemopreventive and therapeutic strategy in prostate cancer[14].

NO is an important signaling molecule and can alter many cellular processes depending upon its production rate. In general, at very high levels of NO, cancer cell killing occurs and at very low levels there seems to be very little effect, however at more intermediate levels, the results clearly indicated that NO protects cancer cells from apoptosis. Therefore, NO has been shown to be both pro-apoptotic depending upon many factors, including not only the flux and dose of NO. Antioxidant markers were assessed in fifteen different investigations. SOD was evaluated in eight investigations using serum samples. Five studies have revealed decreased SOD levels in patients[1,8]. In the study[5], lipid hydroperoxide content was shown to be higher, although not significantly, in high-risk participants than in healthy controls.

Conclusion

PSA is a protein produced by the cells of the prostate gland. PSA test results show the level of PSA detected in the blood. The PSA level that is considered normal for an average man, ranges from 0 to 4 nanograms per milliliter (ng/ml). A PSA level of 4 to 10 ng/ml is considered slightly elevated; levels between 10 and 20 ng/ml are considered moderately elevated and anything greater is considered highly elevated. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells.

TAC, NO and SOD levels play a role in prostate cancer etiology by lowering oxidative stress. In conclusion, the current study found that greater levels of oxidative damage and changes in the antioxidant defense system in high-risk individuals may suggest a relationship between oxidative stress and prostate cancer. These findings could help with risk classification and the development of nomograms for prostate cancer prevention and therapy.

References

1. Bassey I.E., Emodi B.A., Akpan U.O., Iyakndue I.F., Anakebe E.A., Icha B.E., Efobi H.A., Ntinya A.J. and Udoh A.E., Impact of androgen depivation on oxidative stress and antioxidant status in Nigerian patients with prostate cancer undergoing androgen deprivation therapy, *JCO Global Oncology*, **6**, 1481-9, https://doi.org/10.1200/GO.20.00290 **(2020)**

2. Biesiadecki M., Mołoń M., Balawender K., Kobylińska Z. and Galiniak S., Shedding light on the shadows: oxidative stress and its pivotal role in prostate cancer progression, *Frontiers in Oncology*, **14**, https://doi.org/10.3389/fonc.2024.1393078 **(2024)**

3. Bray F., Laversanne M., Sung H., Ferlay J., Siegel R.L., Soerjomataram I. and Jemal A., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: A Cancer Journal for Clinicians*, **74(3)**, 229-63, https://doi.org/10.3322/caac.21834 **(2024)**

4. Burke A.J., McAuliffe J.D., Natoni A., Ridge S., Sullivan F.J. and Glynn S.A., Chronic nitric oxide exposure induces prostate cell carcinogenesis, involving genetic instability and a pro-tumorigenic secretory phenotype, *Nitric Oxide*, **127**, 44-53, https://doi.org/10.1016/j.niox.2022.07.005 **(2022)**

5. Giona S., The epidemiology of prostate cancer, *Exon Publications*, **27**, 1-5, https://doi.org/10.36255/exonpublications.prostatecancer.epidemiology.2021 **(2021)**

6. Ismy J., Sugandi S., Rachmadi D., Hardjowijoto S. and Mustafa A., The effect of exogenous superoxide dismutase (SOD) on caspase-3 activation and apoptosis induction in Pc-3 prostate cancer cells, *Research and Reports in Urology*, **12**, 503-8, https://doi.org/10.2147/RRU.S271203 **(2020)**

7. Khan F.H., Dervan E., Bhattacharyya D.D., McAuliffe J.D., Miranda K.M. and Glynn S.A., The role of nitric oxide in cancer: master regulator or Not?, *International Journal of Molecular Sciences*, **21(24)**, 9393, https://doi.org/10.3390/ijms21249393 **(2020)**

8. Kumar S. and Clair D.S., Raioresistance in Prostate Cancer: Focus on the Interplay between NF-κB and SOD, *Antioxidants*, **10(12)**, 1925, https://doi.org/10.3390/antiox10121925 **(2021)**

9. Lantwin P., Kaczorowski A., Nientiedt C., Schwab C., Kirchner M., Schütz V., Görtz M., Hohenfellner M., Duensing A., Stenzinger A. and Duensing S., Deficiency in DNA Damage Repair Proteins Promotes Prostate Cancer Cell Migration through Oxidative Stress, *Onco*, **4(2)**, 56-67, https://doi.org/10.3390/onco4020005 **(2024)**

10. Li Y.S., Kawasaki Y., Watanabe S., Ootsuyama Y., Kasai H. and Kawai K., Diurnal and day-to-day variation of urinary oxidative stress marker 8-hydroxy-2'-deoxyguanosine, *Journal of Clinical Biochemistry and Nutrition*, **68(1)**, 18-22, https://doi.org/10.3164/jcbn.19-105 **(2021)**

11. Moretti C.H., Schiffer T.A., Montenegro M.F., Larsen F.J., Tsarouhas V., Carlström M., Samakovlis C., Weitzberg E. and Lundberg J.O., Dietary nitrite extends lifespan and prevents age-related locomotor decline in the fruit fly, *Free Radical Biology and Medicine*, **160**, 860-70, https://doi.org/10.1016/j.freeradbiomed.2020.09.018 **(2020)**

12. Oczkowski M., Dziendzikowska K., Pasternak-Winiarska A., Włodarek D. and Gromadzka-Ostrowska J., Dietary factors and prostate cancer development, progression and reduction, *Nutrients*, **13(2)**, 496, https://doi.org/10.3390/nu13020496 **(2021)**

13. Panigrahi G.K., Praharaj P.P., Kittaka H., Mridha A.R., Black O.M., Singh R., Mercer R., van Bokhoven A., Torkko K.C., Agarwal C. and Agarwal R., Exosome proteomic analyses identify inflammatory phenotype and novel biomarkers in African American prostate cancer patients, *Cancer Medicine*, **8(3)**, 1110-23, https://doi.org/10.1002/cam4.1885 **(2020)**

14. Rago V. and Di Agostino S., Novel insights into the role of the antioxidants in prostate pathology, *Antioxidants*, **12(2)**, 289, https://doi.org/10.3390/antiox12020289 **(2023)**

15. Schiffer T.A., Lundberg J.O., Weitzberg E. and Carlström M., Modulation of mitochondria and NADPH oxidase function by the nitrate-nitrite-NO pathway in metabolic disease with focus on type 2 diabetes, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, **1866(8)**, 165811, https://doi.org/10.1016/j.bbadis.2020.165811 **(2020)**

16. Shukla S., Srivastava J.K., Shankar E., Kanwal R., Nawab A., Sharma H., Bhaskaran N., Ponsky L.E., Fu P., MacLennan G.T. and Gupta S., Oxidative stress and antioxidant status in high-risk prostate cancer subjects, *Diagnostics*, **10(3)**, 126, https://doi.org/10.3390/diagnostics10030126 **(2020)**

17. Stracker T.H., Osagie O.I., Escorcia F.E. and Citrin D.E., Exploiting the DNA Damage Response for Prostate Cancer Therapy, *Cancers*, **16(1)**, 83, https://doi.org/10.3390/cancers16010083 **(2023)**

18. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A. and Bray F., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: A Cancer Journal for Clinicians*, **71(3)**, 209-49, https://doi.org/10.3322/caac.21660 **(2021)**.

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