Research Article

Cross sectional study of lipid peroxidation using enzymatic and non-enzymatic antioxidants in prostate cancer

[1] Gururaj.M.Udachankar, [2] Shantala.S.Herlekar

[1]Gururaj.M.Udachankar, Biochemistry, DTDC, Belagavi, Karnataka. India
[2] Shantala.S.Herlekar, Dept of Physiology, MSRUAS Ramaiah Medical College, Bangalore, Karnataka. India

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Corresponding Author: ***Shantala.S.Herlekar*** Email: Shantala.herlekar@gmail.com

ABSTRACT

Introduction: The present investigation is aimed to assess the magnitude of oxidative stress by quantifying malondialdehyde levels, and to evaluate the contribution of antioxidants glutathione reductase and vitamin C-in mitigating oxidative damage in prostate cancer patients.

Methodology: 30 healthy controls and 30 prostate cancer patients, aged 65 and 75 years, were enrolled. Venous blood samples (10 ml) were collected. 2 ml was allocated for MDA analysis, 2 ml for hemolysate preparation, and 6 ml was placed in plain tubes, centrifuged to separate serum, and stored at 4°C. Biochemical analyses were conducted within 24 hours using a colorimeter.

Results: There was no statistically significant difference in the mean age between the control and patient groups (p = 0.117). The biochemical findings are summarized as: Malondialdehyde (MDA): Controls: 6.17 ± 1.00 nmol/ml; Prostate Cancer Patients: 15.83 ± 3.63 nmol/ml ; $p < 0.00$; Glutathione Reductase: Controls: 8.96 ± 1.04 IU/g of Hb; Prostate Cancer Patients: 3.32 ± 0.76 IU/g of Hb; $p < 0.001$; and Vitamin C: Controls: 0.87 ± 0.01 mg/dl; Prostate Cancer Patients: 0.50 ± 0.15 mg/dl; $p < 0.001$.

Conclusion: There is marked elevation in oxidative stress and significant reduction in antioxidant defense mechanisms in patients with prostate cancer relative to age-matched healthy individuals. Supplementation with natural antioxidants could potentially retard the progression of such malignancies to some extent.

Keywords: Prostate Cancer, Malondialdehyde, Glutathione Reductase, Vitamin C, oxidant-antioxidant balance.

1. INTRODUCTION

Prostate cancer represents the second most prevalent malignancy in men, exhibiting an incidence of 25.3 per 100,000 males, a rate that is increasing due to improved early detection methods. The associated mortality rate is 8.1 per 100,000. Despite its prevalence, prostate carcinoma typically progresses slowly, leading to the observation that many patients may live with the disease rather than succumb to it. Diagnosis is most common in men over 60 years of age, and autopsy findings indicate that nearly three-quarters of men over 80 years old demonstrate histological evidence of the condition [1, 2].

Free radicals, characterized by their high reactivity, exhibit a localized impact (approximately 30 Å) and a short lifespan (milliseconds). Their interaction with stable molecules triggers a cascade of radical formation. Lipids, especially polyunsaturated fatty acids (PUFAs) within cell membranes, are highly vulnerable to oxidative damage. This lipid peroxidation compromises membrane integrity and disrupts critical cellular functions, including nutrient transport and secretion [3].

Lipid peroxidation, the oxidative degradation of polyunsaturated fatty acids (PUFAs), poses significant cellular harm due to its self-propagating characteristics, thereby amplifying cellular damage. Malondialdehyde (MDA), a critical byproduct of this process, modifies membrane proteins and lipids, consequently disrupting ion transport and enzymatic function. Considering these detrimental effects, serum

MDA levels are frequently utilized as an indicator of oxidative stress in patients diagnosed with prostate cancer [4].

Oxidative stress arises from an imbalance that Favors pro-oxidants over antioxidants. Antioxidants function by either delaying or preventing oxidation, frequently through competitive interaction with oxidizable molecules. The body's enzymatic antioxidant defences are significantly reliant on systems such as glutathione reductase, which necessitates riboflavin in its FAD form as a cofactor. Within erythrocytes, NADPH-crucial for glutathione reductase-is primarily generated via the hexose monophosphate pathway [5].

Ascorbic acid (Vit C), a water-soluble antioxidant, is essential for neutralizing peroxyl radicals in the aqueous environment, thus safeguarding plasma lipids. Furthermore, it facilitates the regeneration of oxidized vitamin E, thereby bolstering the overall antioxidant defence mechanism [6, 7].

OBJECTIVES

- To assess MDA levels as an estimate of lipid peroxidation in prostate cancer patients.
- To assess levels of enzymatic antioxidant like Glutathione Reductase and non-enzymatic antioxidants like Vitamin-C as an estimate of antioxidant defense in Prostate cancer patients.

2. MATERIALS AND METHODS

A cross-sectional study was conducted to assess the biochemical parameters associated with Benign Prostatic Hyperplasia (BPH). The study cohort comprised 30 clinically confirmed BPH cases, either admitted or receiving outpatient care at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. These cases were compared with 30 age-matched healthy controls, consisting of patient attendants or hospital staff who provided voluntary consent.

Sample Size

The determination of the sample size was predicated on a minimum anticipated effect size, utilizing the following statistical parameters: an alpha (α) error of 0.05 and a beta (β) error of 0.2. These specifications were implemented to ensure adequate statistical power for identifying significant differences between the experimental and control samples [8].

Ethical Approval and Consent

The Institutional Ethics Committee on Human Subject Research is located at KLE's Jawaharlal Nehru Medical College, Belagavi. The Head of the Department of Urology is at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum..

Data Assembly

A structured proforma was employed to gather socio-demographic characteristics and pertinent clinical data, facilitating systematic recording for rigorous analysis. The study's one-year duration provided ample time for participant recruitment, data collection, and follow-up, thereby bolstering the scientific validity and comprehensiveness of the research.

Inclusion Criteria and Exclusion Criteria

Inclusion Criteria

The research encompassed cases of cancer of prostate that were clinically and diagnostically confirmed. Confirmation of the diagnosis was established upon the fulfillment of two or more of the subsequent criteria [9].

- Clinical manifestations and symptoms indicative of bladder outlet obstruction.
- Positive findings from digital rectal examination.
- Histopathological confirmation derived from prostate biopsy samples.
- Serum prostate-specific antigen (PSA) levels within the range of 4-10 ng/mL.

Exclusion Criteria The following conditions were excluded to minimize confounding variables: Hepatic dysfunction, Renal failure, Diabetes mellitus, Chronic smoking or alcoholism, Other systemic illnesses, History of drug abuse or medications known to affect prostatic tissue.

Study Population

All participants were males aged between 65 and 75 years.

Sample Collection

Overall 10 mL of venous blood was aseptically collected from each participant using disposable syringes and transferred into heparinized tubes. Blood was processed as follows: 2 ml for the estimation of malondialdehyde (MDA), 2 ml for

glutathione reductase analysis, 6 ml of venous blood was transferred n plain tubes, serum extracted by centrifugation, and saved at 4°C for subsequent biochemical testing, including hemoglobin, Vitamin C, serum calcium, and PSA (detailed methods for these analyses are not included here).

Statistical Analysis

Data were entered and analyzed using Microsoft Excel and IBM SPSS software (version 23) respectively. Quantitative variables were expressed as mean ± standard deviation. Unpaired Student's t-test and analysis of variance (ANOVA) were used for inter-group comparison and multi group analysis. A p-value of < 0.05 was contemplated as statistically significant.

Methods of assay

Blood: Malondialdehyde – Thiobarbituric acid method [10].

Hemolysate: Glutathione Reductase – Beutler E method [11].

Serum: Vitamin C – Evelyn and Melloy method [12], Serum calcium – Modification of O-Cresolphthalien complexone method [13].

3. RESULTS & DISCUSSION

Results

The study encompassed 30 clinically diagnosed prostate cancer cases and 30 age-matched healthy controls. Participants were male subjects aged 65 to 75 years. Data entry and tabulation were performed using Microsoft Excel, followed by statistical analysis utilizing SPSS (version 17). Data analysis employed an unpaired Student's t-test and ANOVA. The mean difference was considered statistically significant at a p-value less than 0.05.

Age (Table 1): The control group exhibited a mean age of 70.46 ± 3.58 years (range: 59-78 years), while the prostate cancer cases presented a mean age of 72.76 ± 4.98 years (range: 65-81 years). This difference did not reach statistical significance (p = 0.117).

Hemoglobin (Table 1): In the control group, the mean Hb% level was 10.49 ± 1.21 g/dL, while in the prostate cancer cases, it was 9.71 ± 1.74 g/dL (p = 0.55, not statistically significant).

Malondialdehyde (mda) (Table 1): In the control group, the mean MDA level was 6.17 ± 1 nmol/ml, whereas in prostate cancer cases, it was 15.83 ± 3.63 nmol/ml. A statistically significant increase in MDA levels was observed in prostate cancer patients (P < 0.001).

Glutathione reductase (Table 1): In the control group, the mean glutathione reductase level was 8.96 ± 1.04 IU/g of Hb, whereas in prostate cancer cases, it was 3.32 ± 0.76 IU/g of Hb. A statistically significant decrease (P < 0.001) in the level was observed in prostate cancer patients.

Vitamin C (Table 1): In the control group, the mean vitamin C level was 0.87 ± 0.01 mg/dL, while in the prostate cancer cases, it was 0.50 ± 0.15 mg/dL. A statistically significant decrease in vitamin C levels was observed in the prostate cancer group (p < 0.001).

Table 1: The analysis presents comparative anthropometric and biochemical data, including p-values, for both control subjects and patients diagnosed with prostate cancer.

	CONTROLS	PROSTATE CANCER	p value
Age (years)	70.46 ±3.58	72.76±4.98	0.117
Hemaglobin (gm/dL)	10.49±1.21	9.71±1.74	0.55
PSA (ng/mL)	2.32±0.21	18.82±6.23	<0.001*
Malondialdehyde	6.17 ± 1	15.83± 3.63	<0.001*
Glutathione reductase	8.96 ± 1.04	3.32 ± 0.76	<0.001*
Vitamin C	0.87 ± 0.1	0.50 ± 0.15	<0.001*

* P value <0.001 and is statistically significant for difference in Hemoglobin, PSA, Malondialdehyde, Glutathione reductase, and Vitamin C values between prostate cancer patients and controls.

4. Discussion

Globally, prostate cancer remains the second most prevalent malignant tumor diagnosed in men. Incidence before the age of 50 is infrequent, accounting for less than 0.1% of all diagnoses [1, 2]. Chronic prostatitis is frequently characterized by macrophage infiltration within expressed prostatic secretions (EPS), a characteristic associated with elevated levels of reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide, within the prostate microenvironment . Prolonged inflammatory activity may induce oxidative stress, potentially causing protein dysfunction, genetic mutations, and DNA alterations that promote aberrant cellular

proliferation and the potential for tumor development [15].

Malondialdehyde (MDA)

The study revealed significantly elevated serum MDA concentrations in prostate cancer patients relative to healthy age-matched controls, consistent with prior research by Amar SA [16]. As a terminal product of polyunsaturated fatty acid oxidation, MDA functions as a reliable marker of lipid membrane damage. The observed increase suggests heightened lipid peroxidation, potentially resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, thereby reflecting the oxidative stress burden within malignant prostate tissue [17].

Glutathione Reductase (GR)

Prostate cancer patients exhibited a statistically significant reduction in erythrocyte glutathione reductase (GR) activity ($p < 0.001$), corroborating the findings of Freitas M [18]. GR plays a critical role in maintaining cellular redox balance by catalyzing the conversion of oxidized glutathione (GSSG) to its reduced form (GSH), utilizing NADPH as a cofactor. In prostate cancer cells, higher levels of ROS production and lower levels of GSH and Gl-Red activity contribute to oxidative stress resistance. Diminished GR activity suggests a compromised enzymatic antioxidant defense system, potentially leading to increased reactive oxygen species (ROS) accumulation and subsequent oxidative damage. Furthermore, the glutathione pathway, specifically involving glutathione S-transferase (GST), is often compromised in prostate cancer. Research by Gsur A et al. revealed that somatic mutations frequently inactivate the GSTP1 gene in nearly all prostate tumor samples examined, emphasizing the significance of impaired antioxidant mechanisms in the development of tumors [19, 24].

Vitamin C (Ascorbic Acid)

Vitamin C, a water-soluble antioxidant, is rapidly depleted under conditions of oxidative stress, acting as a direct neutralizer of reactive oxygen species (ROS) and aiding in the regeneration of oxidized vitamin E. The present study revealed significantly lower serum vitamin C levels ($p < 0.001$) in prostate cancer patients relative to controls, consistent with prior research [20]. This observed depletion likely indicates its accelerated utilization in mitigating elevated free radicals within cancerous prostate tissue. These findings suggest that vitamin C supplementation may offer therapeutic benefits in the management of prostate cancer [21].

Oxidative Stress and Hormonal Influence

Analysis of the provided data reveals a significant oxidative imbalance within prostate cancer cells, characterized by elevated levels of malondialdehyde (MDA) and diminished activity of glutathione reductase (GR) and vitamin C. While the precise mechanisms remain under investigation, androgens appear to influence the regulation of reactive oxygen species (ROS) dynamics within the prostate. Research suggests that androgen replacement therapy may alleviate oxidative stress by downregulating NADPH oxidase (Nox) expression, subsequently improving antioxidant capacity. Moreover, the transcription factor Nrf2, which controls the expression of antioxidant genes through the antioxidant response element (ARE), is critical for cellular protection. The observed downregulation of Nrf2 and its target genes in prostate cancer may contribute to disease progression and metastasis [22, 23].

Clinical Implications

The results emphasize the critical function of oxidative stress in the progression of prostate cancer. Nutritional approaches designed to enhance antioxidant capabilities, specifically through vitamin C supplementation, may provide therapeutic advantages. However, thorough clinical trials are essential to determine the efficacy of these interventions in either slowing disease advancement or enhancing patient results. The extent to which supplementation with natural antioxidants can retard the progression of prostate cancer is not fully established [25].

5. CONCLUSION

The research underscores a notable elevation in oxidative stress within the prostate cancer patient cohort, as evidenced by increased MDA concentrations and compromised antioxidant mechanisms, specifically GR and vitamin C.

This observed imbalance between oxidant and antioxidant species appears to be a contributing factor in the pathophysiology of prostate cancer. Therefore, therapeutic strategies that address this redox dysregulation warrant further exploration, particularly those involving antioxidant-based interventions.

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