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Cross Sectional Study of Lipid Peroxidation, Antioxidant Status and Serum Calcium in Benign Prostate Hypertrophy

Gururaj M. Udachankar[1], Shantala S. Herlekar[2], Savita S. Hiremath[3]

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ABSTRACT

Introduction: Oxidative stress, mediated by lipid peroxidation, contributes to cellular injury in BPH. Malondialdehyde (MDA) serves as a marker of oxidative stress, while the body's defence includes enzymatic antioxidants like Glutathione Reductase and non-enzymatic ones like Vitamin C. Calcium's role in prostate cancer is established, but its implications in BPH remain inconclusive.

Objectives: Assess oxidative stress by measuring MDA levels, Evaluate antioxidant status through Glutathione Reductase and Vitamin C levels, Compare serum calcium levels in BPH patients and healthy controls.

Methodology: Participants: 30 controls and 30 clinically diagnosed BPH patients, aged 65–75 years. Ethical Clearance and informed consent was obtained. Sample Collection: 10 ml of heparinized blood analyzed using:

- **MDA**: Thiobarbituric acid method.
- **Glutathione Reductase**: Beutler E method.
- **Vitamin C**: Evelyn and Melloy method.
- **Serum Calcium**: Modified O-Cresolphthalein Complexone method.

Results:

Demographics: No significant age difference between groups (p=0.117). ***Oxidative Stress and Antioxidants:*** MDA: Significantly increased in BPH (p <0.001). Glutathione Reductase: Significantly decreased in BPH (p <0.001).Vitamin C:

AUTHOR'S AFFILIATION:

[1] Consultant Pulmonologist, Belagavi, Karnataka India.

[2] Associate Professor, Department of Physiology, MSRUAS, MSR Medical College, Bangalore, India.

[3] Department of Physiology, SSPM Medical College & Lifetime Hospital, Affiliated to Maharashtra University of Health Sciences, Sindhudurg, Maharashtra, India.

CORRESPONDING AUTHOR:

Shantala S. Herlekar, Associate Professor, Department of Physiology, MSRUAS, MSR Medical College, Bangalore, India.

E-mail: shantalaherlekar.rmc@msruas.ac.in

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Significantly reduced in BPH ($p < 0.001$). ***Serum Calcium:*** Higher normal range in BPH patients, with a statistically significant difference compared to controls ($p < 0.05$). ***Correlations:*** MDA positively correlated with BPH (+0.2). Inverse correlations with Glutathione Reductase (-0.28) and Vitamin C (-0.20). Weak correlation between MDA and Glutathione Reductase ($r = -0.100$, $p = 0.6$). Low correlation between MDA and Vitamin C ($r = 0.41$, $p = 0.831$).

Conclusion: Increased oxidative stress and diminished antioxidant defenses in BPH patients.Serum calcium levels in the higher normal range, significantly differing from controls.

Implications: Potential benefits of antioxidant supplementation and calcium-lowering strategies in managing BPH. This study underscores the importance of addressing oxidative stress and maintaining optimal calcium levels in managing BPH. Adding specific recommendations for antioxidant-rich diets or pharmacological interventions could further enhance clinical outcomes.

KEYWORDS

• Benign Prostate Hypertrophy • Lipid peroxidation • Malondialdehyde • Glutathione Reductase • Vitamin C • Serum Calcium

INTRODUCTION

In contrast to middle-aged women, who experience an abrupt cessation of ovarian activity, middle-aged men do not face a sudden halt in gonadal function. Male fertility endures into later stages of life, although aging in men is marked by a gradual decline in both hormonal (endocrine) and reproductive (exocrine) functions of the testes. This gradual transition, known as andropause, adversely affects quality of life and contributes to the development of Benign Prostatic Hyperplasia (BPH). Ultimately, BPH can become a critical condition due to urethral obstruction, potentially progressing to uremia, a life-threatening complication. BPH is primarily associated with advanced age and involves hypertrophic changes across all four primary cell types in the prostate: smooth muscle cells, fibroblasts, acinar cells, and basal epithelial cells.[1,2]

Lipid peroxidation is a well-documented mechanism of cellular damage. This process results in the generation of lipid peroxides and related by-products, which compromise membrane integrity and functionality. It is widely recognized as a contributing factor in the progression of various human diseases. Malondialdehyde (MDA), a terminal product of polyunsaturated fatty acid (PUFA) oxidation, is a critical marker of lipid peroxidation. Unlike unstable free radicals, aldehydes such as MDA are relatively stable and can diffuse within or outside cells, targeting sites far from the original oxidative event. Furthermore, MDA reflects not only lipid peroxidation but also cyclooxygenase activity in platelets, with persistent platelet activation being a common feature in numerous clinical syndromes linked to elevated lipid peroxidation. Hence, measuring MDA levels provides a practical, in-vivo indicator of oxidative stress and is often used as a biomarker in studying radical-induced physiological and pathological conditions.[3]

Oxidative stress refers to an imbalance where pro-oxidants outweigh antioxidants, leading to cellular damage. This imbalance results in the oxidation of lipids, proteins, carbohydrates, and nucleic acids, which can ultimately cause cell death. Antioxidants are substances capable of delaying or preventing the oxidation of other molecules by competing with oxidizable substrates. Reactive oxygen species (ROS), which are highly reactive derivatives of oxygen, play a key role in oxidative damage. To counteract these harmful radicals, the body utilizes a scavenging system comprising enzymatic antioxidants like superoxide dismutase, glutathione reductase, glutathione peroxidase, and catalase, as well as non-enzymatic antioxidants such as vitamin E

(alpha-tocopherol) and vitamin C (ascorbate). Oxidative damage to DNA may result in mutations, deletions, or rearrangements, disrupting normal apoptotic regulation and leading to hyperplasia or precancerous alterations. The prostate gland is particularly susceptible to oxidative stress due to its high cellular turnover and limited DNA repair capacity.[4-9]

Substantial evidence indicates that men consuming diets rich in calcium or using calcium supplements are at an increased risk of developing prostate cancer. Serum calcium levels, which serve as an indirect measure of Vitamin D function, are often evaluated during routine blood tests. While dietary calcium intake does not directly influence serum calcium, pre-diagnostic serum calcium levels have been shown to strongly predict the likelihood of fatal prostate cancer. Men with serum calcium levels at the higher end of the normal spectrum are three times more likely to develop fatal prostate cancer.[10] This study was conducted to evaluate oxidative stress in BPH through MDA quantification, to assess the protective roles of antioxidants such as glutathione reductase and vitamin C against this oxidative stress, and to compare serum calcium levels in BPH patients with those of healthy controls.

OBJECTIVES

- To assess lipid peroxidation by estimating the levels of Malondialdehyde in BPH patients and comparing it with control group.
- To assess antioxidant defense, by measuring enzymatic antioxidant Glutathione Reductase levelsand non-enzymatic antioxidant Vitamine C in BPH patients and comparing it with control group.
- To estimate the Serum Calcium levels in BPH and controls.

MATERIALS AND METHODS

Study Design

This was a **cross-sectional study** conducted to investigate the biochemical parameters associated with Benign Prostatic Hypertrophy (BPH). The study involved **30 clinically diagnosed cases of BPH** admitted to or attending KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. These were compared to an equal number of **age-matched healthy controls** who were either patient attendants or hospital staff members that consented to participate.

Sample Size

The sample size was determined based on the **minimum effect size** and with the following statistical considerations:**α error = 0.05, β error = 0.2**. These parameters ensured a reliable study design with sufficient power to detect significant differences between groups.

Ethical Approval and Consent

The study received ethical clearance from the following authorities:

1. **Institutional Ethics Committee on Human Subject Research** of KLE's Jawaharlal Nehru Medical College, Belgaum.
2. **Head of the Department of Urology**, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Informed consent was obtained from all study participants, ensuring voluntary participation with full understanding of the study's nature and potential risks.

Data Collection

A **structured proforma** was used to gather **socio-demographic** and **clinical information** about all participants. This allowed for systematic data recording for subsequent analysis.The study was carried out over a **1-year period**, ensuring sufficient time for data collection and participant follow-up. This detailed design ensures the study's scientific rigor and ethical compliance while aiming to uncover important insights into the biochemical changes in BPH.

Inclusion Criteria

Clinically and diagnostically confirmed cases of BPH which includes 2 or more of the following.[11]

- Patients with signs and symptoms of bladder outlet obstruction.
- Positive digital rectal examination.
- Confirmed by histological examination on biopsy samples.

- Prostate specific antigen (PSA) levels 4-10 ng/mL in cases of BPH.

Exclusion Criteria

Cancer prostate, Hepatocellular damage, Renal failure, Diabetes mellitus,Chronic smokers, Chronic alcoholics, Other systemic disorders, drug abuse, interventions and treatment affecting prostatic tissue were excluded from the study.

The age group ranges from 65 to 75 years. The data was tabulated and statistical analysis was carried out by using unpaired student's t–test and comparison within groups was done using analysis of variance. Multiple comparisons were made using ANOVA. Data was entered in Microsoft excel and analyzed using SPSS (version 23). The mean difference is significant at p value < 0.05.

Sample collection:

10 ml of blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe in heparinized tubes. 2 ml separated for MDA estimation, 2 ml for Glutathione reductase analyses and 6 ml in plain tubes, centrifuged, serum separated and kept at 40C for further analyses of hemoglobin, Vit C, Serum calcium and PSA (details not included in here).

Methods of assay

Blood: Malondialdehyde – Thiobarbituric acid method.[12]

Hemolysate: Glutathione Reductase – Beutler E method.[13]

Vitamin C – Evelyn and Melloy method.[14]

Serum calcium – Modification of O-Cresolphthaliencomplexone method.[15]

ESTIMATION OF MALONDIALDEHYDE (MDA) IN WHOLE BLOOD: 12

Principle:

The process described is for the estimation of Malondialdehyde (MDA) in whole blood using the Thiobarbituric Acid (TBA) method, which is commonly used to measure lipid peroxidation.

Procedure for MDA Estimation Using Thiobarbituric Acid (TBA) Method:

1. **Reagent Preparation:** Thiobarbituric Acid (TBA) Reagent:75 mg of thiobarbituric acid+15 ml of trichloroacetic acid+2.08 ml of 0.2 N HCl. The above components are mixed and the total volume is made up to 100 ml with distilled water.
2. **Reaction:** The prepared TBA reagent is added to the sample (blood or plasma) to form a pink-colored complex with malondialdehyde (MDA). The reaction mixture is kept in a boiling water bath for 15 minutes. After heating, the solution is cooled to room temperature and centrifuged for 10 minutes at 10,000 rpm to separate the supernatant.
3. **Measurement:** The absorbance of the supernatant is measured immediately at 532 nm (wavelength) using a spectrophotometer. A blank (containing all reagents except the sample) is used for zeroing the spectrophotometer.
4. **Calculations:** The concentration of MDA in the sample is determined using the following formula:

$$\text{Malondialdehyde (nano moles / ml)} = \frac{\text{Absorbance of test} \times \text{total volume}}{\text{Nanomolar extension coefficient} \times \text{Sample volume} \times 100}$$

This method is effective in quantifying lipid peroxidation levels, as MDA is a major product of lipid peroxidation and serves as an indicator of oxidative stress in biological samples.

PREPARATION OF HEMOLYSATE

Isolation of Red Blood Cells: 16 Enzyme activities in red blood cells (RBCs) are generally lower than those in white blood cells (WBCs) and platelets. Therefore, it was crucial to remove virtually all WBCs and platelets during the isolation process. To achieve this, whole blood was filtered through a column containing a mixture of α-cellulose and microcrystalline cellulose.

Preparation of the Cellulose Column:

- A 1:1 (w/w) mixture of α-cellulose and microcrystalline cellulose was prepared using isotonic sodium chloride solution (9.0 g/L).
- A 5 mL disposable plastic syringe without a barrel was placed vertically, with the outlet pointing downwards.
- A small piece of filter paper was inserted

at the bottom of the syringe to prevent the cellulose mixture from leaking.

- The well-mixed cellulose slurry was poured into the syringe up to the 2 mL mark.

Filtration Process:

- The cellulose bed was washed with 5 mL of isotonic sodium chloride.
- Subsequently, 1 mL of whole blood was allowed to flow through the column. To ensure efficient removal of WBCs and platelets, the volume of the cellulose mixture used was at least twice the volume of the blood sample.

Post-Filtration Procedure:

- The effluent, containing the RBCs, was collected into a centrifuge tube.
- The saline-suspended RBCs were washed twice with at least 10 volumes of ice-cold isotonic sodium chloride to remove residual contaminants.
- After washing, the packed RBCs were re-suspended in isotonic sodium chloride to create an approximately 50% suspension (1:1 dilution).
- This suspension was then subjected to hemolysis for subsequent analysis.

LYSING OF THE RBCS[17]

Reagents: Stabilizing Solution: Composition: 2.7 mM EDTA (pH 7.0) and 0.7 mM β-mercaptoethanol.

Preparation: Dissolve 100 mg of disodium EDTA in distilled water (D/W).Add 5 µL of β-mercaptoethanol (Merck) to the solution. Adjust the final volume to 100 mL with D/W.

Procedure:

Preparation of Hemolysate: Mix 1 volume of RBC suspension with 9 volumes of the stabilizing solution. Rapidly freeze the mixture at –20°C to –25°C in a freezer. Thaw the frozen hemolysate in a water bath maintained at 20°C to 25°C. The thawed hemolysate is now ready for further analysis.

Hemoglobin Estimation: Perform hemoglobin estimation on the hemolysate using Drabkin's reagent to express enzyme activities in units per gram of hemoglobin.

Timing of Preparation: Both the red cell suspension and its hemolysate were freshly prepared on the day of the assay to ensure accuracy. The prepared hemolysate was utilized for the assay of glutathione reductase (GR) enzyme activity.

PERFORMANCE OF THE ENZYME ASSAYS FROM 1:20 HEMOLYSATE

GLUTATHIONE REDUCTASE[13]

Principle:

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione(GSSG) by NADPH to reduced glutathione.

NADPH (NADH) + H^+ + GSSG GR $NADP^+$ (NAD^+) + 2GSH

Principle: Glutathione reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH as an electron donor.The oxidation of NADPH is tracked spectrophotometrically at **340 nm**, as NADPH absorbs strongly at this wavelength, whereas its oxidized form ($NADP^+$) does not.

FAD Activation: Glutathione reductase, a flavoprotein, is not fully active without sufficient FAD. To ensure maximum enzyme activity, the apoenzyme must be preincubated with FAD **before** adding GSSG or NADPH to the reaction, as the latter interfere with FAD activation.

Reagents Preparation: Tris-HCl (1 M, EDTA 5 mM, pH 8.0): Prepared as described in prior protocols. FAD (10 µM): Dissolved by adding 0.8 mg FAD sodium salt (MW = 829.52) to 100 ml distilled water (D/W). GSSG (0.033 M): Dissolved by adding 20.2 mg GSSG (MW = 612.7) in 1 ml D/W. NADPH (2 mM): Prepared as described earlier in the protocol.

Assay Procedure: Include a reagent blank, working standard, test sample, and quality control in each run. Monitor the decrease in absorbance at 340 nm, indicative of NADPH oxidation.

Steps for the Assay:

Preincubation: Preincubate the enzyme with FAD to ensure complete activation.

Reaction Mixture Setup: In a cuvette, mix: Tris-HCl buffer (pH 8.0) + Preincubated enzyme with FAD + GSSG + NADPH

Measurement: Start the reaction by adding the enzyme-FAD mixture to the cuvette. Measure the absorbance at **340 nm** immediately and record at regular intervals (e.g., every 30 seconds) to observe the rate of NADPH oxidation.

Controls: Use reagent blanks and working standards to calibrate and verify the assay.

Calculation: The rate of decrease in absorbance at 340 nm is directly proportional to the enzyme activity. Use the molar extinction coefficient of NADPH at 340 nm ($\mathbf{6.22 \times 10^3\ M^{-1}\ cm^{-1}}$) to calculate the rate of NADPH oxidation:

This method is a reliable approach for evaluating the activity of glutathione reductase in biological samples. Ensure accurate pipetting and consistent incubation times for reproducibility.

ESTIMATIONS FROM SERUM[18]

Vitamin C[14]

The described method outlines the determination of ascorbic acid (Vitamin C) concentration using 2,6-dichlorophenol indophenol (DCPIP) as a redox indicator.

Here's a concise breakdown of the procedure:

Principle:

- 2,6-Dichlorophenol indophenol (DCPIP) acts as an oxidizing agent. Ascorbic acid reduces DCPIP to its colorless reduced form, while being oxidized to dehydroascorbic acid.
- The reaction leads to a decrease in the red color intensity of DCPIP, measurable spectrophotometrically at 520 nm.
- The decrease in optical density (OD) is proportional to the ascorbic acid concentration in the sample.

Reaction:

DCPIP (Oxidized)+Ascorbic acid→DCPIP (Reduced, colorless)+Dehydroascorbic acid\ text{DCPIP (Oxidized)} + \text{Ascorbic acid} \rightarrow \text{DCPIP (Reduced, colorless)} + \text{Dehydroascorbicacid} DCPIP (Oxidized)+Ascorbic acid→DCPIP (Reduced, colorless)+Dehydroascorbic acid

Reagents Preparation:

10% Sodium Tungstate: Dissolve 10 g sodium tungstate in distilled water and make up the volume to 100 mL.

2/3 N Sulfuric Acid: Mix 18.7 mL concentrated H_2SO_4 with distilled water and dilute to 1 L.

5% Metaphosphoric Acid: Dissolve 5 g metaphosphoric acid in 100 mL distilled water. Do not heat. Prepare weekly and store in the refrigerator.

2,6-Dichlorophenol Indophenol Solution: Dissolve 13 mg DCPIP and 3 g sodium acetate trihydrate in 1 L distilled water.Adjust pH to 3.5–3.6 by varying sodium acetate, if necessary.

Ascorbic Acid Standards: Stock Standard (100 mg%): Dissolve 100 mg L-ascorbic acid in 100 mL metaphosphoric acid.Working Standard (1 mg%): Dilute 1 mL stock standard to 100 mL with metaphosphoric acid.

Procedure:

Sample Preparation: In a clean, dry test tube, mix the following:1 mL plasma + 3 mL 5% metaphosphoric acid + 0.5 mL 10% sodium tungstate + 0.5 mL 2/3 N sulfuric acid. Mix well and let sit for 5 minutes.

Filtration: Filter the mixture to remove precipitates and obtain a clear filtrate.

Measurement: Add DCPIP reagent to the filtrate and immediately measure the decrease in OD at 520 nm.

Calculation: Prepare a calibration curve using ascorbic acid standards treated similarly. Plot the decrease in absorbance (ΔOD) against the concentration of standards. Use the standard curve to determine the ascorbic acid concentration in the test sample. This method is widely used due to its simplicity and specificity for ascorbic acid. Ensure reagents are freshly prepared and pH adjustments are accurate for consistent results.

SERUM CALCIUM[15]

Principle

O-Cresolphthalein Complexone (OCPC) reacts with calcium in alkaline medium to form a purple-coloured complex. The intensity of the purple colour formed is proportional to the calcium concentration and is measured photometrically between 540nm and 600 nm with maximum absorbance at 575nm.

Procedure

Reagent 1 (2-Amino-2-methyl-1-propanol – 505mmol/L) and reagent 2 (OCPC – 0.06mmol/L, 8-hydroxy Quinolone – 6.9mmol/L, HCl – 45mmol/L) are mixed in equal proportions. Serum calcium is calculated using standard formula.

RESULTS

Study Overview: The present study includes **30 clinically diagnosed cases of Benign Prostate Hypertrophy (BPH)** and **30 age-matched healthy controls**. The age range of participants was **65 to 75 years**. Data were tabulated and analyzed using **unpaired Student's t-test** and **analysis of variance (ANOVA)** for group comparisons. Multiple comparisons were conducted using ANOVA. Data entry was performed using Microsoft Excel and analyzed with **SPSS (version 17)**. A **p-value < 0.05** was considered statistically significant.

Results:

Age (Table 1): The mean age of controls was 70.46 ± 3.58 years (range: 59–78 years). The mean age of BPH cases was 72.26 ± 4.70 years (range: 65–81 years). There was no statistically significant difference between the mean ages of the two groups (p = 0.117).

Malondialdehyde (MDA) Levels (Table 2): The mean MDA level in controls was 6.17 ± 1.0 nmol/mL, while in BPH cases, it was significantly elevated to 11.44 ± 0.82 nmol/mL (p < 0.001).

Table 1: Age distribution in Controls and BPH

	Controls	BPH	p value
Age (years)	70.46±3.58	72.26±4.70	0.117

No significant difference was seen between controls and BPH patients.

Glutathione Reductase Levels (Table 2): The mean glutathione reductase level in controls was 8.96 ± 1.04 IU/g Hb, compared to 3.39 ± 0.74 IU/g Hb in BPH cases. This represents a significant decrease in glutathione reductase levels in BPH cases (p < 0.001).

Vitamin C Levels (Table 2): The mean vitamin C level in controls was 0.87 ± 0.01 mg/dL, whereas in BPH cases, it decreased significantly to 0.42 ± 0.14 mg/dL (p < 0.001).

Table 2: Concentration of Malondialdehyde (MDA), Glutathione Reductase, Vitamin C and Serum Calcium levels in BPH in comparison with Controls. (p<0.05 is taken as statistically significant)

Variables	Controls	BPH	't' value	df	'p' value
PSA (ng/mL)	1.68±0.76	5.33±0.96	11.726	58	<0.001
Malondialdehyde	6.17 ±1	11.44 ± 0.82	-6.467	98	<0.001
Glutathione reductase	8.96 ± 1.04	3.39± 0.74	0.350	98	<0.001
Vitamin C	0.87 ± 0.1	0.42± 0.14	-2.168	98	<0.001
Serum calcium	9.08 ± 0.37	9.92± 0.97	1.238	98	<0.05

There was a statistically significant difference between Malondialdehyde (MDA) Glutathione Reductase, Vitamin C and Serum Calcium levels in BPH in comparison with Controls. MDA and Serum Calcium was higher in BPH patients. While Glutathionereductase and Vitamin C was higher in controls

Serum Calcium Levels (Table 2): The mean serum calcium level in controls was 9.08 ± 0.37 mg/dL, compared to 9.92 ± 0.97 mg/dL in BPH cases. Serum calcium levels were significantly increased in BPH cases (p < 0.05).

Correlation Analysis:

Lipid Peroxidation (MDA) and Antioxidant Levels: In BPH cases, MDA levels showed a positive correlation with themselves (r = +0.20) but were inversely correlated with glutathione reductase (r = -0.28) and vitamin C (r = -0.20). A strong negative correlation was observed between plasma MDA and glutathione reductase (r = -0.10, p = 0.6). The correlation between plasma MDA and vitamin C was low (r = 0.41, p = 0.831).

DISCUSSION

Involvement of Oxidative Stress in BPH Pathophysiology

Oxidative stress, driven by reactive oxygen species (ROS), has been implicated in the inflammation and chronic diseases affecting various organs and tissues. In

Benign Prostatic Hypertrophy (BPH), ROS generation is evidenced by elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation. The study also assessed antioxidant defenses, including the enzymatic activity of glutathione reductase and non-enzymatic antioxidant levels of vitamin C, alongside serum calcium concentrations in BPH patients. The involvement of oxygen free radicals in the pathophysiology of inflammation and chronic diseases affecting various organs and tissues is well-documented in the literature.[19,20] Evidence of reactive oxygen species (ROS) generation in patients with Benign Prostate Hypertrophy (BPH) has been demonstrated through elevated levels of malondialdehyde (MDA), a lipid peroxidation product. The enzymatic antioxidant defense was assessed by measuring glutathione reductase, and the non-enzymatic antioxidant defense was evaluated through Vitamin C levels. Serum calcium levels were also examined in BPH cases.

Malondialdehyde (MDA):

In this study, the mean level of MDA was significantly increased in BPH cases compared to controls. These findings align with the study by Aryal M *et al*.[4] MDA, a decomposition product of lipid peroxidation of polyunsaturated fatty acids, serves as an index of oxidative damage. Elevated MDA levels indicate increased membrane lipid peroxidation, which may result from an imbalance between free radical generation and antioxidant scavenging mechanisms.[5,21]

Programmed cell death, or apoptosis, protects the body by eliminating defective cells. However, senescent cells show reduced susceptibility to apoptosis under oxidative stress. Reactive aldehydes, such as 4-hydroxynonenal and MDA, produced during lipid peroxidation, can modify DNA and proteins, leading to mutagenic, genotoxic, and cytotoxic effects. High MDA levels may contribute to DNA base modifications observed not only in prostate cancer but also in the BPH epithelium.[22]

Glutathione Reductase:

Glutathione reductase, a 44 kDa enzyme found in the liver, kidney, pancreas, heart, thyroid, and erythrocytes, catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH. This reaction maintains a high GSH/GSSG ratio in normal cells.[24] At its active site, glutathione reductase contains FAD, which is reduced by NADPH. The FAD then transfers electrons to a disulfide bridge within the enzyme, regenerating GSH from GSSG.[25]

In this study, glutathione reductase levels were significantly decreased in BPH cases ($p < 0.001$) compared to controls, consistent with the findings of Hayes *et al*.[23] This decrease may reflect the enzyme's role in counteracting oxidative stress, with lower levels indicating an enhanced protective mechanism in BPH.

Vitamin C:

Vitamin C (ascorbic acid) is a water-soluble, non-enzymatic antioxidant known for its free radical scavenging properties. It reacts directly with superoxide, hydroxyl radicals, and lipid hydroperoxides. Additionally, it regenerates oxidized Vitamin E, underscoring its role in antioxidant recycling. Vitamin C is abundant in mammalian tissues, particularly the adrenal and pituitary glands, with smaller amounts found in the liver, spleen, pancreas, and brain.

Although Vitamin C primarily acts as an antioxidant, it may function as a pro-oxidant at high concentrations (≥ mM) in the presence of transition metals like Fe^{3+} or Cu^{2+}, facilitating lipid peroxidation.[9,26]

In this study, Vitamin C levels were significantly decreased in BPH cases ($p < 0.001$) compared to controls, aligning with previous research.[19] The observed depletion may result from the body's attempt to neutralize the excess free radicals in BPH patients. Supplementation with antioxidants such as Vitamin C could be beneficial in managing BPH.

Serum Calcium:

Elevated serum calcium levels have been associated with an increased risk of fatal prostate cancer, with some studies reporting a two- to three-fold higher risk in men with the highest serum calcium levels.[28,29] In this study, serum calcium levels were significantly increased ($p < 0.05$) in BPH cases compared to controls.

The active fraction of serum calcium, ionized calcium, is tightly regulated by parathyroid hormone (PTH) and typically remains within a narrow range. Prostate cells express calcium-sensing receptors and calcium-dependent channels, which regulate cell proliferation. [31] Elevated calcium levels may correlate with

serum PSA levels, although PSA itself is unlikely to influence calcium homeostasis.[32]

Importantly, calcium levels within the "high end of the normal range" (9.9–10.5 mg/dL) are modifiable through lifestyle and pharmacological interventions, offering potential avenues for reducing BPH risk.

CONCLUSION

The findings of this study highlight a significant increase in oxidative stress in patients with Benign Prostatic Hypertrophy (BPH), as evidenced by elevated malondialdehyde (MDA) levels. This increase in oxidative stress was coupled with a marked reduction in antioxidant defenses, including both the enzymatic antioxidant erythrocyte glutathione reductase and the non-enzymatic antioxidant vitamin C. These results suggest a strong association between oxidative stress and impaired antioxidant mechanisms in BPH, offering valuable insights into the pathophysiology of the condition.

These findings support the potential for developing preventive and therapeutic strategies aimed at mitigating oxidative damage. The possible therapeutic benefits of exogenous antioxidants, such as vitamin C, merit further investigation through rigorously controlled clinical trials with larger sample sizes. Additionally, the observed elevation in serum calcium levels in BPH patients may play a role in the disease process. Follow-up studies are necessary to further explore this association and establish a clearer understanding of its relationship with BPH.

Conflict of interest: None

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