DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL BIOCHEMISTRY

TITLE

A STUDY ON MALONDIALDEHYDE (MDA) AND FERRIC REDUCING ABILITY OF PLASMA (FRAP) IN BENIGN PROSTATIC HYPERPLASIA PATIENTS AND CONTROL SUBJECTS

SUBMITTED

BY

ARNOLD RATEMO MULUHYA

2023

DEPARTMENT OF BIOCHEMISTRY

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

FACULTY OF HEALTH AND MEDICAL SCIENCES

INTEGRAL UNIVERSITY, LUCKNOW-226026,

(U.P) INDIA.

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A
DISSERTATION
SUBMITTED

In partial fulfillment of the requirements for the award of Degree of
Master of Science
In
Medical Biochemistry

By

ARNOLD RATEMO MULUHYA
Enrollment Number: 2000102773

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CERTIFICATE

This is to certify that **Mr. Arnold Ratemo Muluhya,** a student of **M.Sc. Medical Biochemistry**, Integral University has completed his dissertation titled **"A STUDY ON MALONDIALDEHYDE (MDA) AND FERRIC REDUCING ABILITY OF PLASMA (FRAP) IN BENIGN PROSTATIC HYPERPLASIA PATIENTS AND CONTROL SUBJECTS"** successfully. He has completed this work in the Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University under my guidance. The dissertation was a compulsory part of his M.Sc. degree.

I wish him good luck and a bright future.

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Declaration by the candidate

I hereby declare that Integral Institute of Medical Sciences & Research, Integral University, Lucknow shall have the right to preserve, use and disseminate this dissertation in print / electronic format for academic/ research purposes.

I will publish the research paper related to my dissertation only with the consent of my guide.

Date: **Arnold Ratemo Muluhya**

Place: Lucknow

Acknowledgement

It goes without saying that many hands make work light. The reason to which I wish to express my sincere gratitude to the people that surrounded me with support and encouragement enabling the success of this project.

First and foremost, I would like to extend my heartfelt gratitude to the ***Hon'ble Chancellor****, Integral University,* ***Prof. S.W. Akhtar*** *for providing the necessary resources and fostering an environment conducive for academic excellence.*

I am extremely grateful to the ***Hon'ble Pro Chancellor****, Integral University,* ***Dr. Syed Nadeem Akhtar****, for his valuable insights, constant motivation, and continuous support throughout my research journey. His dedication to fostering educational growth has been truly inspiring.*

My heartfelt thanks go to the ***Hon'ble Vice Chancellor****, Integral University,* ***Prof. Javed Musarrat****, for his vision and leadership in steering our university towards excellence. His valuable guidance and encouragement have been invaluable in shaping my academic pursuits.*

I am deeply indebted to ***Mr. Syed Mohammad Fauzan, Executive Director*** *(IIMSR, IIAHSR & IINSR) for his exceptional administrative support and for his efforts in creating a conducive research environment.*

With immense pride I acknowledge my guide, ***Dr. Roshan Alam****, Professor and Head, Department of Biochemistry, Integral Institute of Medical Science and Research, whose invaluable knowledge, time and encouragement were instrumental in facilitating the success of this project. I am extremely grateful, for in spite of his busy schedule, he always spared time to help whenever needed. His expertise and feedback have shaped the direction this project has taken.*

I would like to express my sincere appreciation to ***Prof. Dr. Abha Chandra****,* ***Dean****, IIMS&R for her insightful guidance and expertise. Her support and encouragement have been instrumental in shaping my research work.*

I wish to recognize the invaluable addition of my co-guide, ***Dr. Pankaj Kumar****, Professor and Head, Department of Surgery, Integral Hospital. His many years' experience and keen eye for patient's medical conditions proved an asset in understanding the project. I appreciate his contribution and the facilities availed for this project.*

*A ton of gratitude goes to **Dr. Saba Khan**, Associate Professor, Department of Biochemistry, for the critical suggestions, expertise and healthy criticism shown towards this project. I am grateful for her dedication and time to go through the segments of this project elucidating the essentials and making needful corrections as she saw best.*

*I extend my heartfelt thanks to **Dr. Priyanka Thapa Manger**, Assistant Professor, Department of Biochemistry, for her rich knowledge and mentoring nature. Her deep understanding, invaluable skills and wealth of experience has greatly added to the overall knowledge and understanding of the research project undertaken.*

*I recognize and greatly appreciate **Dr. Mustufa Khan**, Professor and Head, Department of Biomedical Sciences, for his incredible assistance and direction. His depth of knowledge, meticulous attention to detail and critical thinking has been crucial in shaping this research project.*

*I am equally grateful to **Dr. Ausaf Ahmad**, Assistant Professor and Statistician, Department of Community Medicine, for his constructive criticism and hand in refining the methodology and analysis of the research data obtained from tests.*

*I wish to acknowledge the assistance provided by **Ms. Richa Awasthi**, a PhD Scholar, and the non-teaching staff, for the valuable academic suggestions and reliable technical help during the course of this project.*

*I wouldn't want to miss recognizing my friends and colleagues, **Ankita Verma, Shanti Gupta, Ramashish Kushwaha, Apoorva** and **Smita Singh Chauhan**. Who together, have greatly helped throughout the year and helped face every kind of barrier that existed in regards to life and education. I am grateful for the constructive discussions and intellectual support that better shaped the understanding of the project.*

*Last but not least, my deepest gratitude is to my beloved mother, **Janet Kedogo Muluhya**, for her unwavering support, patience, and encouragement throughout this research undertaking. Her belief in me and her constant encouragement have been a constant push to seek and pursue limitless goals.*

I thank The Almighty GOD, who bestows upon one from the rich abundance that He keeps. Faith in Him through all times has been a fresh breather through all situations.

Though it may not be possible to mention by name, I am extremely grateful to everyone that played a role in promoting the success of this project. Your input, however large or small, is exceedingly valued and appreciated.

Thank you once again to everyone that made it possible for this project to see its successful completion.

Date: ***ARNOLD RATEMO MULUHYA***

Place: Lucknow, Uttar Pradesh, India.

CONTENTS

LIST OF ABBREVIATIONS

MDA	Malondialdehyde
FRAP	Ferric Reducing Ability of Plasma
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Species
TCA	Trichloroacetic Acid
LPO	Lipid Peroxidation
LUTS	Lower Urinary Tract Symptoms
DHT	Dihydrotestostrone
PCa	Prostate Cancer
AR	Androgen Receptor
OS	Oxidative Stress

INTRODUCTION

Benign prostate hyperplasia (BPH) is a prevalent condition commonly diagnosed in aging males, leading to lower urinary tract symptoms (LUTS). The prevalence of BPH is increasing, and there are various risk factors, both modifiable and non-modifiable, that can contribute to its development and progression. The symptoms associated with BPH and LUTS can be classified as obstructive (causing difficulties in initiating urination, weak urine stream, straining, or prolonged voiding) or irritative (resulting in increased frequency and urgency of urination, nocturia, urge incontinence, and reduced volume of voiding). Additionally, post-micturition symptoms like post void dribble or incomplete emptying can also affect patients. **[Chughtai et al. 2016]**

In 2010, the American Urological Association (AUA) published an article that discloses BPH as most prevalent among aging males, with onset in the early 40s, increasing to 80% by the age of 80 **[Lerner LB et al. 2021]**. Analyses of multiple studies show that every year in the fourth decade, there are pathologically identifiable prostatic enlargements at autopsy **[Langan et al. 2019]**. In India, although not well performed, epidemiological research data from 2008 suggest that the incidence rate of prostate enlargement is about 92.97% to 93.3% **[Bid et al 2008]**. A well packaged research encouraged by urologists is needed to have a realistic and updated survey in India.

Histological diagnosis of patients with BPH reveals abnormal proliferation of epithelial cells, toning of the smooth muscle and increased connective tissue, all within the prostate transition zone **[Besiroglu et al 2017].**This proves fatal due to the anatomic relationship that exists between the prostate, the bladder neck and urethra. An enlargement of the prostate from

the cellular level leads to a disturbance in the normal functioning of these adjacent organs. Thus, BPH occurs due to prostate enlargement and that may lead to the more severe Benign Prostate Obstruction (BPO). The BPO affects urinary functions; this has become the target for therapeutic intervention as no clear treatment for the disease exists to date.

What stands out, however, is the role the testes plays in progression of BPH. Patients diagnosed with primary hypogonadism, while undergoing testosterone replacement, were found to develop normal prostatic growth as well as progression in prostate enlargement **[Bell MA et al 2018].**Furthermore, it was observed that patients with prostatic diseases (prostate cancer or BPH) who had bilateral orchiectomy performed, there was a significant decline in the disease progression as well as an eventual reversal in the prostate volume **[Nicholson et al 2011]**. This proves the androgen dependence of the prostate gland, without which prostate atrophy is likely. A constant supply and maintenance of circulating androgens is necessary for daily functioning of prostate cells and its overall role in the urogenital system.

The testis is the primary organ for androgen, and estrogen to a lesser extent, production in males. Testosterone serves as the chief androgen released from the testes. It is then transported to the prostate via circulation and once in the prostate, the androgen receptors (AR) located on prostatic cells are activated and lead to cellular growth. AR-dependent transcription in the cell nucleus produces more protein growth factors such as insulin-like growth factors, keratinocyte growth factors, epidermal growth factors, as well as fibroblast growth factors.**[Vignozzi et al. 2014]**. This leads to hyperplasia within the prostate gland.

It is worth mentioning though, that aging occurs, there is a decrease in circulating testosterone hormone levels. This casts a shadow over the idea that testosterone is the sole mitogen responsible for BPH progression as challenged by Liu et al. **[Liu et al 2007]**.

Before scientific advancements, surgery was considered the only viable option for patients. This, however, presents many complications in terms of morbidity and mortality during and after surgery. Recently, the understanding of the action of AR-dependent transcription has led to discovery of ***5 α-reductase Inhibitors*** that has been hailed as a pharmacological breakthrough in alleviating the symptoms resulting from BPH **[Vaselkiv et al 2022]**. The adrenergic inhibitors have proven selective in combating the disease and with varying results among patients **[Livingstone et al 2015]**.

Recent studies seem to suggest that oxidative stress may play a role in benign prostate hyperplasia **[Srivastava et al 2005]**. Oxidative stress can be defined as an increased concentration of free radicals in the body that causes oxidative damage to cells, and subsequent decline in the antioxidant systems responsible for combating these free radicals **[Minciullo et al 2015]**. This proves the active role that oxidative stress plays in BPH is not just a phenomenon. Reactive Oxygen Species (ROS) has been reported to promote autocatalytic formation of lipid peroxidation that leads to harmful genotoxic byproducts. These radicals eventually lead to formation of DNA adducts that damage DNA products **[Halliwell et al 2015].**

Understanding the cellular and biochemical changes that accompany BPH may prove useful in proper managing and treating the disease. This research study seeks to find the

correlation between oxidative stress status by measuring malondialdehyde, a marker for lipid peroxidation, and antioxidant profile through the blood serum's ability to perform the Fenton reaction, in patients diagnosed with clinical BPH **[Kawee-Ai et al 2016]**

REVIEW OF LITERATURE

Benign Prostate Hyperplasia accounts for approximately 40% of reported urological conditions in men above 60 years **[Aoun et al 2015]**. A study on sexually intact dogs indicated that 80% of them which are 5 years compared to 95% of them that are 9 years showed overall microscopic changes attributed to BPH **[Domoslawska et al 2022]**. This proves that the disease is age related and almost unavoidable as age progresses. When compared to other prostatic diseases, there has been some epidemiological similarity that makes it possible to understand the gland in terms of functional anatomy and disease. Their complex pathophysiology includes genetic instability, inflammation, endocrine disruptors, coronary artery disease hormones, and oxidative stress, among other aspects.

In the past, most men did not see the need to seek medical attention; accepting LUTS as part of the aging process. However, with the intense publicity on prostate cancer and the important awareness on men's health, more and more men are coming out to seek more insight and attention in regards to their health **[Taitt HE et al 2018].** The understanding of the molecular pathology and physiology of BPH is due to the advancements in molecular studies and imaging that occur within the prostate gland compared between patients with the disease and healthy individuals. Probable causes are being advanced. It was noted that there was an increased serum-insulin like growth factor 1 among patients with an enlarged prostate compared with those with normal prostate volumes **[Corrêa LL et al. 2015].**

The prostate is a small gland that forms part of the male genital system. Its main role is to provide fluid that goes into semen. This fluid is important for men's fertility. The gland surrounds the urethra, from the bladder neck downwards. The bladder and urethra form the *lower*

urinary tract **[Deters LA 2014]**. Most of the symptoms happen within this region and lead to LUTS. These symptoms are a result of blocked urethra or an overworked bladder.

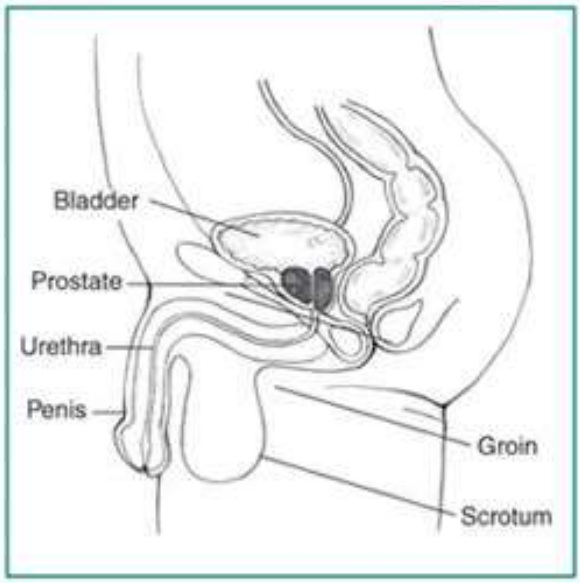


Fig 1. The anatomical position of the Prostate Gland **[Source: Deters LA 2014]**

Lower urinary tract symptoms of BPH include:- **[Deters LA 2014]**

- Increased urinary frequency; up to eight or more times a day
- The inability to delay urination leading to increased urgency
- Delayed micturition caused by trouble starting a urine stream
- An interrupted urine stream
- Dribbling towards the end of urination
- Increased nocturia
- Urinary retention.
- Urinary incontinence
- Painful ejaculation or urination

Diagnosis of LUTS

Diagnosis is based on taking patient history, physical and rectal examination, and urine volume examinations. Confirmation of BPH is normally done by ultrasound check of the volume of the prostate gland in addition to the above mentioned symptoms **[Nazarko et al 2023]**.

Free flow rates, symptoms scores (IPSS), frequency volume charts, and urodynamics are the other aspects of advanced assessments that are used in diagnosis.

Oxidative Stress and BPH

A homeostatic imbalance between the production of reactive oxygen species and their elimination from the body system through antioxidant mechanisms, defines oxidative stress. The body itself has mechanisms that kick in at the expulsion of free radicals into the environment. As discussed earlier, mounting evidence shows that these free radicals are responsible for the formation of DNA adducts that cause formation of protein and protein products outside the norm. These abnormal proteins mediate the formation of hyperplasia. Free radicals such as superoxide and hydrogen peroxide stand in the way of antioxidant systems **[Xiong et al 2020]**

The prostate has been described as an 'immunocompetent organ', referring to the rich immune cells that characterize its tissue **[De Nunzio et al 2016]**. These immune cells act as barriers against easy stimuli, e.g. bacterial infection, sexually transmitted organism or even autoimmune disorders, being easily activated resulting in chronic inflammation **[Gandaglia et al. 2017]**. Oxidative stress has also been tipped to mediate activation of these inflammation

systems leading to release of cytokines and growth factor proteins that trigger abnormal fibro muscular growth, characteristic of BPH.

Wilson, J. D. (1972), in his publication hypothesized the role testosterone plays in BPH. He postulated that while in the prostate, the testosterone hormone is activated to Dihydrotestostrone (DHT) by the ***5 α-reductase*** enzyme found on the stromal cells within the transitional zone **[Tong et al 2022]**. The enzyme exists in two isoforms; *5 α-reductase type 1 & type 2 (5AR1 & 5AR2).* The bio-activate DHT is responsible for activating the AR. One could argue then that BPH is an endocrine disorder based on this.

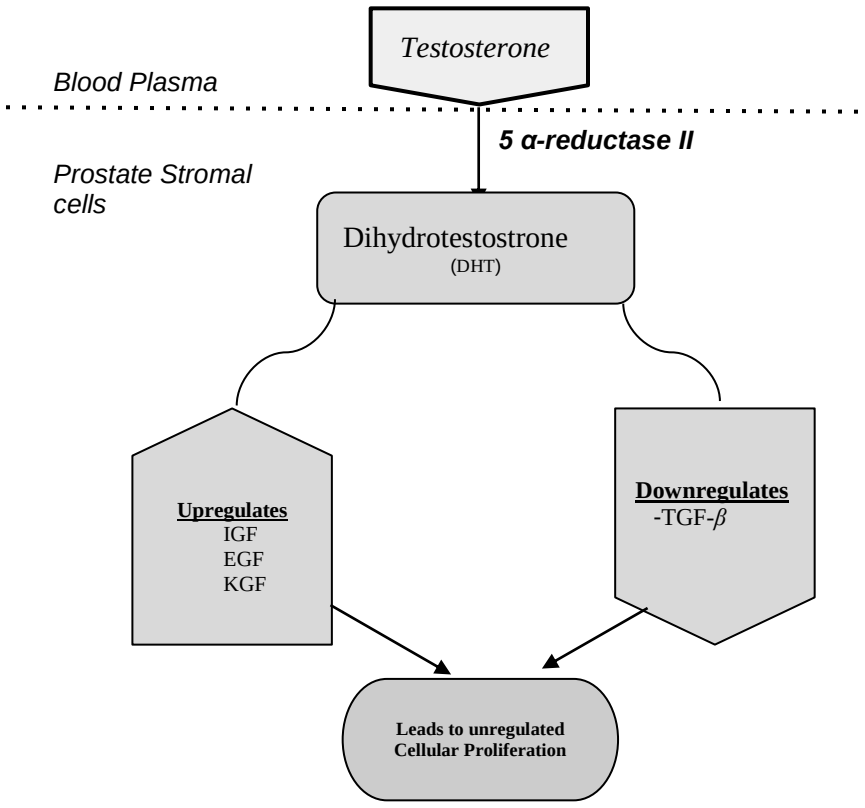


Fig. 2. Pathogenesis of Benign Prostate Hyperplasia [Tong et. al, 2022]

This knowledge has been used therapeutically in the treatment of BPH symptoms and complications. **5 *α-reductase inhibitors*** have been used to inhibit the activation of testosterone in patients with BPH **[Reynard et al 2004]**.

Effects of Oxidative Stress on BPH

Chronic prostatitis has been linked with production of ROS through its effective stromal cellular proliferation leading to oxidative damage and injury. These ROS had been thought to only affect protein products, but studies show that damage is incurred even to the DNA level through mutations and post transcriptional modifications. The mutagenic effect of ROS causes deletion or point mutations that cause a defect from the default operation or a cell. This departure could mean the prostate cells evade cell growth regulation signals leading to hyperplasia and cancerous transformations **[Hamid et al 2011]**.

Similarly, ROS has been found to activate the immune response pathway that causes CP **[Wong et al 2009]**. ROS activates NF-*κ*B inducing kinase (NIK) that stimulates the NF-*κ*B; a transcription factor involved in the regulation of cellular immune response. Activation of NIK has been studied and proven to induce signal transduction that leads to formation of CP. Alternatively; NIK may also interfere with the translation process leading to abnormal growth factor proteins.

Indirectly, ROS can contribute to generation of more OS through autocatalytic lipid peroxidation. The result of which is continual generation of genotoxic breakdown byproducts that are free radicals. These byproducts such as peroxyl radicals and aldehydes have been

estimated and analyzed in blood serum **[Vieira et al 2017]**. One of these byproducts, Malondialdehyde, formed the basis of our research. Examining the serum levels of malondialdehyde serves as a non-invasive diagnostic mechanism of estimating oxidative stress levels and determining extent of lipid peroxidation in damaged tissue.

Enhanced lipid peroxidation triggers the immune response protocol and activates platelets. Platelet activation induces cyclooxygenase activity, an enzyme whose byproduct is MDA. Thus, MDA does not just measure lipid peroxidation but also the level of immune response that are triggered due to clinical symptoms arising from lipid peroxidation in tissue **[Merendino et al. 2003]**.

As discussed, ROS caused damage to DNA leading to adducts that are mutant in nature. Cellular component oxidation affects carbohydrate, lipids and protein as well. As is the case with proteins, post translational modification influenced by ROS results in direct oxidation of amino acids residues. The oxidation of methionine and cysteine has been shown to be a frequent phenomenon **[Grimsrud et al 2008]**. Aldehydes contain the most common of these ROS-induced post translational modifications in the form of protein carbonylation.

Antioxidant Systems

In normal systems, Oxidative stress is met by a host of mechanisms that counter the high ingress of free radicals. These systems may be enzymatic with enzymes such as Superoxide Dismutase, Glutathione Peroxidase and Catalase, as well as vitaminic antioxidants such as $\boldsymbol{\alpha}$-

tocopherol and ascorbate. ROS-induced oxidative damage occurs when an imbalance exists between the presence of free radicals and the mechanisms responsible for keeping them at bay. Data from numerous studies show an elevated OS level with a concomitant decrease in antioxidant defense system efficiency **[Khandrika et al 2009]**.

Comparative study between patients with BPH and control groups showed a rise in DNA adduct products compared to activities of SOD in the serum collected **[Javed et al 2017]**. These results signaled a possible association between antioxidant enzymatic activity and DNA base lesions. However, the data is open to debate as some other articles show no significant change in total antioxidant capacity in some studies. Other small-molecular weight molecules, mostly non-enzymatic, scavenge the circulatory system for free radicals. These include vitamins and trace metals **[Rahal et al 2014]**.

Previous studies indicated that oxidative stress and anti-oxidant levels play a major role in etiology of disease. In view of these findings, the present study sought to assess the role played by oxidative stress and antioxidant status in the etiopathogenesis of BPH.

AIM & OBJECTIVES

Aim

To find out the association of malondialdehyde and ferric reducing ability of plasma in benign prostate hyperplasia patients and control subjects.

Objectives

1. To determine the concentration of malondialdehyde (MDA) in benign prostate hyperplasia patients and control subjects.
2. To determine the ferric reducing ability of plasma (FRAP) in benign prostate hyperplasia patients and control subjects.
3. To find out the correlation between malondialdehyde and ferric reducing ability of plasma in benign prostate hyperplasia patients, if any.

MATERIALS &

METHOD

RESEARCH QUESTION

Is there any association between malondialdehyde (MDA) and ferric reducing ability of plasma (FRAP) in BPH patients and control subjects?

Hypothesis

Null Hypothesis, H_0

There is no significant association between MDA and FRAP in BPH patients and control subjects.

Alternate Hypothesis, H_1

There is significant association between MDA and FRAP in BPH patients and control subjects.

METHODOLOGY

Type of Study: - A case-control Study.

Study design: - Prospective

Place of Study: - Department of Biochemistry, Integral Institute of Medical Science and Research, Lucknow U.P.

Collaborating Department: - Department of General Surgery, IIMSR, Integral Hospital, Lucknow.

Subject Selection.

Selection of Controls

- Apparently healthy subjects.
- Age 45 years and above.
- Individuals who have agreed to sign the consent form.

Selection of Cases

Inclusion Criteria:-

- Diagnosed patients of BPH on the basis of clinical findings and ultrasound.
- Age 45 years and above.
- Individuals who have agreed to sign the consent form.

Exclusion Criteria:-

- Patients with a history of chronic liver disease
- Patients with history of acute renal disease

Enrollment of participants: - Cases were enrolled from the diagnosed benign prostate hyperplasia patients attending the Integral Hospital.

Sampling Method:-Non-probability, Purposive sampling

Sample Collection and Storage.

Under aseptic conditions, **4 ml** of venous blood were obtained in plain and EDTA vials for the determination of MDA and FRAP assay respectively. The samples were refrigerated at -20°C at the Central Clinical Laboratory to preserve and store throughout the sampling period.

Sample Size.

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

[Charan, J., & Biswas, T. 2013]

n = sample size in the case group.

r = ratio of controls to cases.

σ = standard deviation of the outcome variables.

difference = effect size (the difference in means of cases and controls).

$Z\beta$ = Represent the derived power (typically for 80% power).

$Z\alpha_{/2}$ = Represents the derived level of statistical For 95% CI.

$Z\alpha_{/2}$ = 1.96, For 80% power $Z\beta$ = 0.84.

Mother Article:[Aydin, A.et al (2006).]

Expected mean difference between cases and controls is **4.21**

Standard Deviation (**σ**) is **6.09**

n1 (Cases) = ***32***

n2 (Control) = ***32***

LABORATORY INVESTIGATION

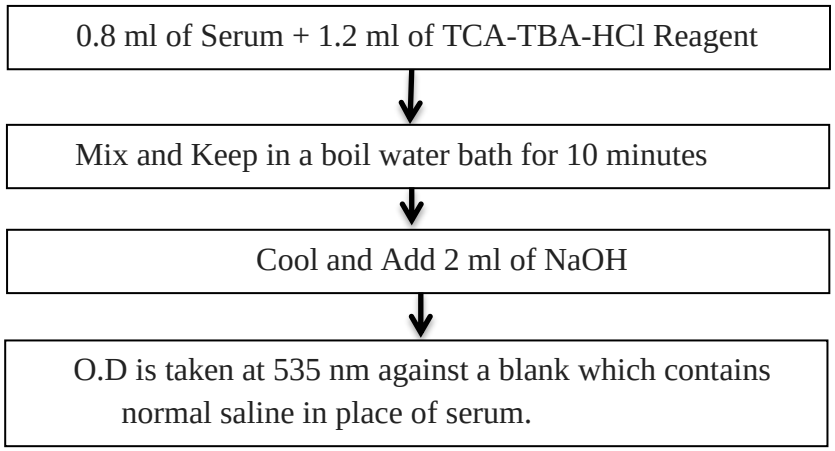
Determination of Malondialdehyde, MDA, by Satoh K. (1978) method.

Principle: - Deproteinized serum is treated with Thiobarbituric acid (TBA) at 90°c for about 10 minutes giving a pink color product. This gives a determination of the TBA reactive substance measured at 535 nm on a spectrophotometer.

Reagents

1. Trichloroacetic Acid (TCA)
2. Thiobarbituric Acid (TBA)
3. 0.25N Hydrochloric Acid
4. 1N Sodium Hydroxide
5. Tetramethoxypropane

MDA Procedure



Calculations

$$\text{Sample (nmol/ml)} = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times 10$$

Determination of Antioxidant Status of plasma by FRAP assay. [**Griffin et al 2004**]

Principle: - In FRAP assay, antioxidants are used as reductants using colorimetric method where Ferric Tripyridyltriazine is reduced to Ferrous Tripyridyltriazine producing a blue color observed through a spectrophotometer at 593 nm.

Reagents.

1. Tripyridyltriazine
2. Ferric Chloride
3. Dilute Hydrochloric acid
4. Acetate Buffer
5. Standard used Iron *II* Sulphate

Procedure-

1. Add the above reagents into the glass tube (standard is added last)
2. For blank: 2ml FRAP reagent + 1 ml Distilled Water + 2ml FRAP reagent.
3. Into a cuvette add 100µL sample + 900µL Distilled water + 2mL FRAP reagent
4. Invert tubes to mix properly.
5. Transfer the cuvettes for the spectrophotometer.
6. Zero the spectrophotometer using blank at 593 nm and then read the O.D.

Calculations-

The FRAP equation is:-

$$\text{FRAP Value of sample (µM)} = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times \textit{FRAP value of standard (µM)}$$

ETHICS REVIEW

Permission from the Institutional Ethics Committee was taken (**IEC/IIMS&R/2023/65**)

DATA COLLECTION

Details from the subjects were obtained using data collection proforma after taking written consent.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Software and Microsoft –Excel (Version 2010). All the data was expressed as mean and standard deviation. An unpaired *t*-test was performed to compare the study parameters between cases and controls. Karl Pearson's correlation analysis was employed to determine the relationship between variables. *p-value* < 0.05 was considered statistically significant.

OBSERVATIONS & RESULTS

RESULTS

Mean of age and weight have not shown any significant differences between cases and controls ($p = 0.76$, $p = 0.77$, respectively). Mean of MDA levels was found significantly elevated in cases compared to controls ($p < 0.001$). However, mean of FRAP levels was found significantly low in case compared to controls ($p < 0.001$), shown in **Table No. 1, Figure 3 & 4,** respectively.

Table 1: Baseline characteristics of cases and controls

Parameters	Cases (Mean ± SD) n=32	Controls (Mean ± SD) n=32	p-value	Significance
Age (years)	50.16±6.29	50.56±3.67	0.76	Not Significant
Weight (kg)	73.19±8.01	72.56±8.90	0.77	Not Significant
MDA (µmol/L)	2.80±0.45	2.15±0.30	**<0.001***	**Significant**
FRAP (µmol/L)	1046.66±75.15	1280.13±72.83	**<0.001***	**Significant**

Data represented as Mean ±SD.
**p-value < 0.05, considered as statistically significant.*
FRAP: Ferric reducing ability of plasma, MDA: Malondialdehyde

Table 2. Comparison of mean concentration for MDA (µmol/L)					
Groups	N	Mean	Standard Deviation	p-value	Significance
Cases	32	2.8	± 0.45	0.001	Statistically Significant
Controls	32	2.15	± 0.3		

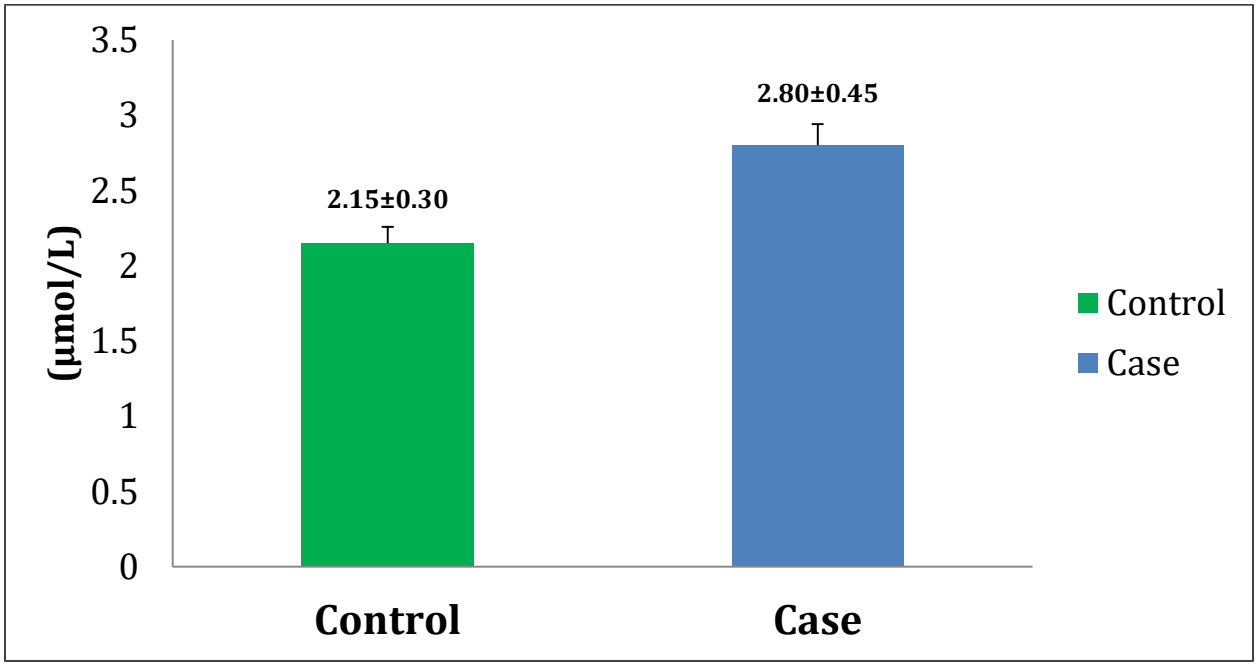


Fig 3. Mean of MDA compared between Cases and Control.

Table 3. Comparison of concentration of FRAP in Cases and Controls (µmol/L)					
Groups	N	Mean	Standard Deviation	p-value	Significance
Cases	32	1046.66	± 75.15	0.001	Statistically Significant
Controls	32	1280.13	± 72.83		

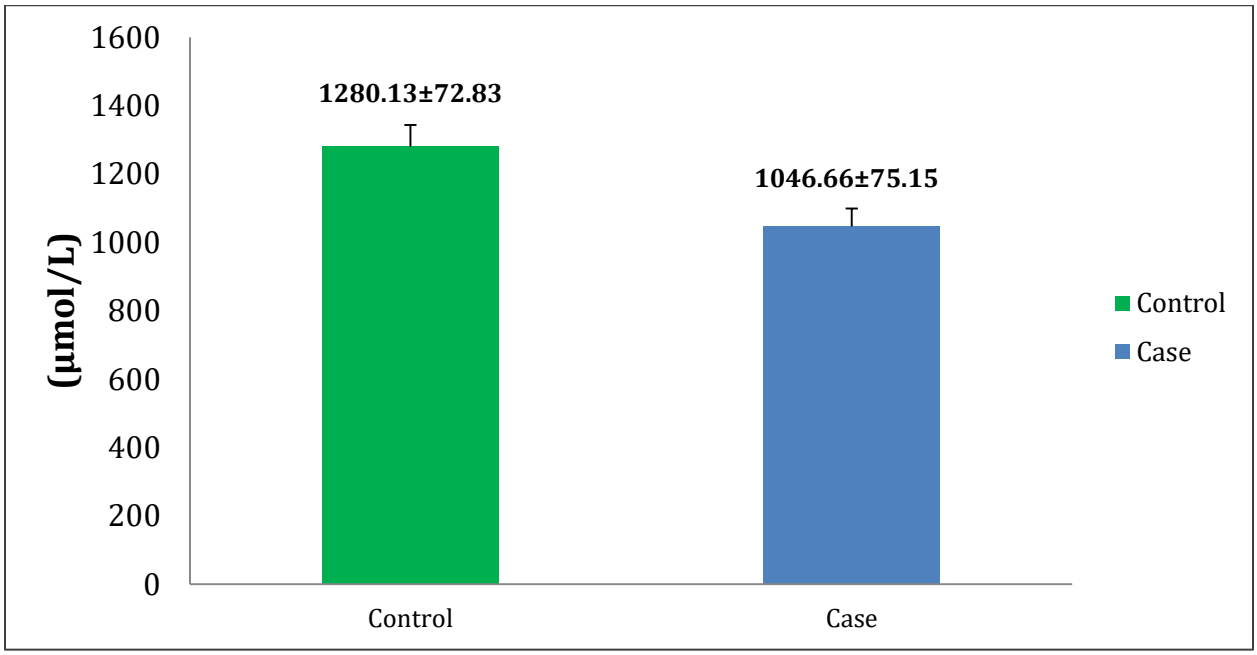


Fig 4. Mean of FRAP compared between Cases and Control.

Karl Pearson's Coefficient of Correlation among the Parameters

Correlations				
Parameters	**Age (years)**	**Weight (kg)**	**MDA (µmol/L)**	**FRAP (µmol/L)**
Age (years)	1	0.115	0.149	0.055
Weight (kg)	-	1	0.171	0.067
MDA (µmol/L)	-	-	1	**-0.468****
FRAP (µmol/L)	-	-	-	1
****. Correlation is significant at the 0.01 level (2-tailed).**				
FRAP: (Ferric Reducing Ability of Plasma) MDA: (Malondialdehyde)				

Table 3. P-value between the various parameters.

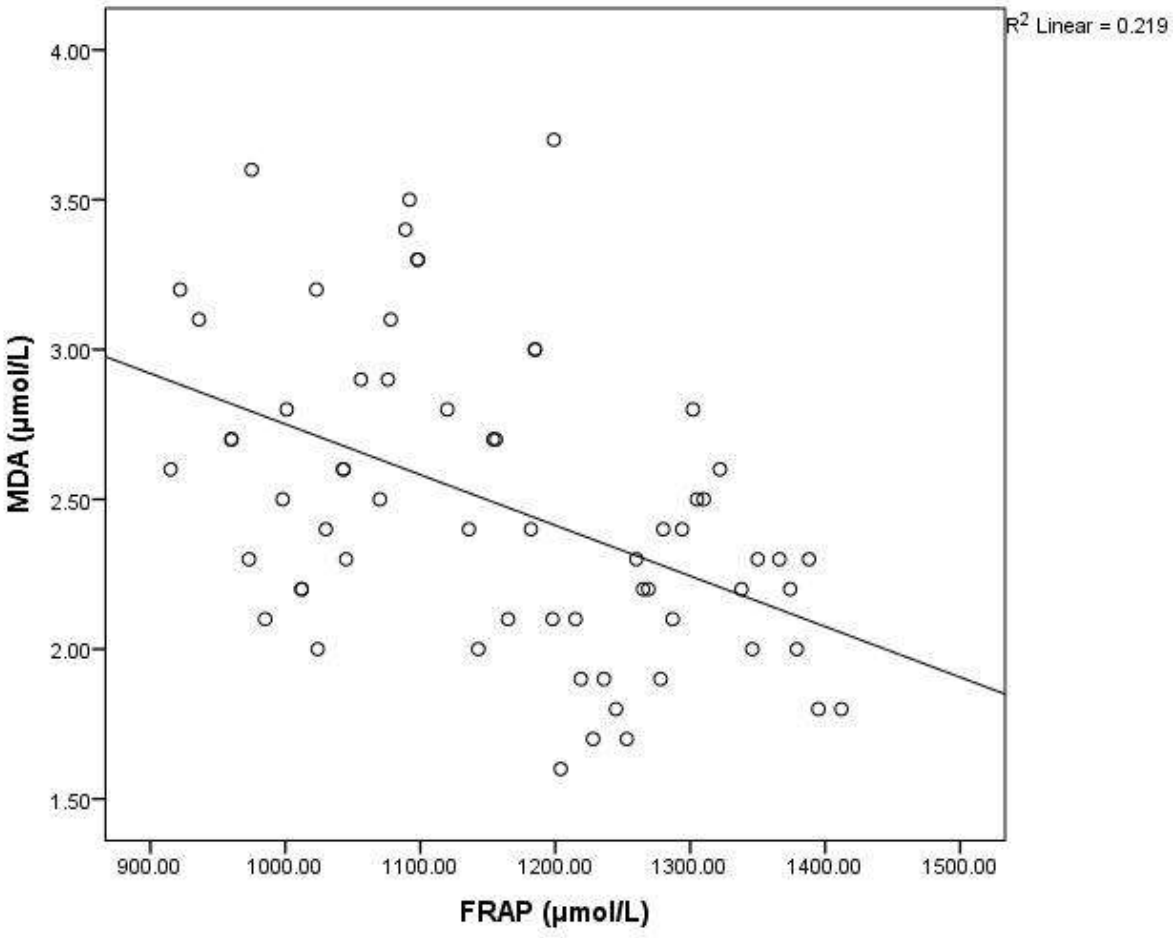


Fig 5. Scatter Diagram of MDA compared to FRAP in Cases

DISCUSSIONS

From this study, the MDA concentration values showed a mean of **2.80±0.45** and **2.15±0.30** in case and control respectively. MDA acts as a marker of oxidative stress, produced as a result of lipid peroxidation. Both free radicals and oxidants are heavily involved in signaling pathways. However any imbalance in its production and elimination is lethal to the bio molecular integrity. Oxidative stress has been discussed as an important part of the development of many diseases [**Mas-Bargues, C., et al 2021]**. Lipid peroxidation occurs in response to the oxidation of lipids (PUFA) by these reactive oxygen species. Lipid peroxidation occurs in 3 phases; initiation, elongation and termination. Pamplona, R.. et al noted that a single initiation cycle can give rise to 200-400 propagation cycles that give rise to aldehydes and dialdehydes that are considered more harmful [**Pamplona, R., et al 2019].** The two most commonly determined aldehydes are 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). 4-HNE has been proven to have the highest biological activity whereas MDA is the most highly produced during lipid peroxidation, and is commonly used as a marker of oxidative stress. [**Barrera, G. et al 2018]**.

This study also shows a significant negative correlation between MDA and FRAP (**r = -0.468, p<0.01)**. This data indicates there being an inverse relationship; an increased MDA concentration usually signals a reduction in the antioxidant machinery. FRAP indicates the body's mechanism to react towards the increased OS that the body undergoes. The data shows a significantly lower concentration of FRAP in cases when compared to control groups signaling the fact that in diseased state, the body's ability to fight a counter measure to the increased OS is compromised (**Muhammad Y et al 2021).**

There is a marked increase in production of reactive oxygen species that results in processes such as lipid peroxidation; to which MDA serves as a good marker. In recent years, it

has been proven that the OS plays a role in the aging process **[Liguori et al 2018].** BPH is considered an age-related illness, with increasing cases being isolated to aging males. It can therefore be mentioned that in aging males, there is a high chance that OS localized within the prostate gland leads to development of prostatic diseases such as BPH.

The data obtained shows no correlation between FRAP levels and age. Simply put, there is no statistical evidence to show that the decline in antioxidant capacity of individuals is brought about by aging. Rather, total antioxidant capacity is a result of increased oxidative stress. To say that the reduction of antioxidant capacity is as a result of aging would be misleading, unless data proves otherwise. Antioxidant systems are overwhelmed when there exists an imbalance between rate of production of free radicals and rate of elimination. Thus, a reduction in antioxidant capacity signals an increased molecular output of free radicals, and not aging [**Adwas AA et al 2019]**.

SUMMARY AND CONCLUSION

SUMMARY

Oxidative stress and antioxidant status in BPH were meant to be assessed in this research work through the concentration of Malondialdehyde (MDA) and Ferric Reducing Ability of Plasma (FRAP) respectively.

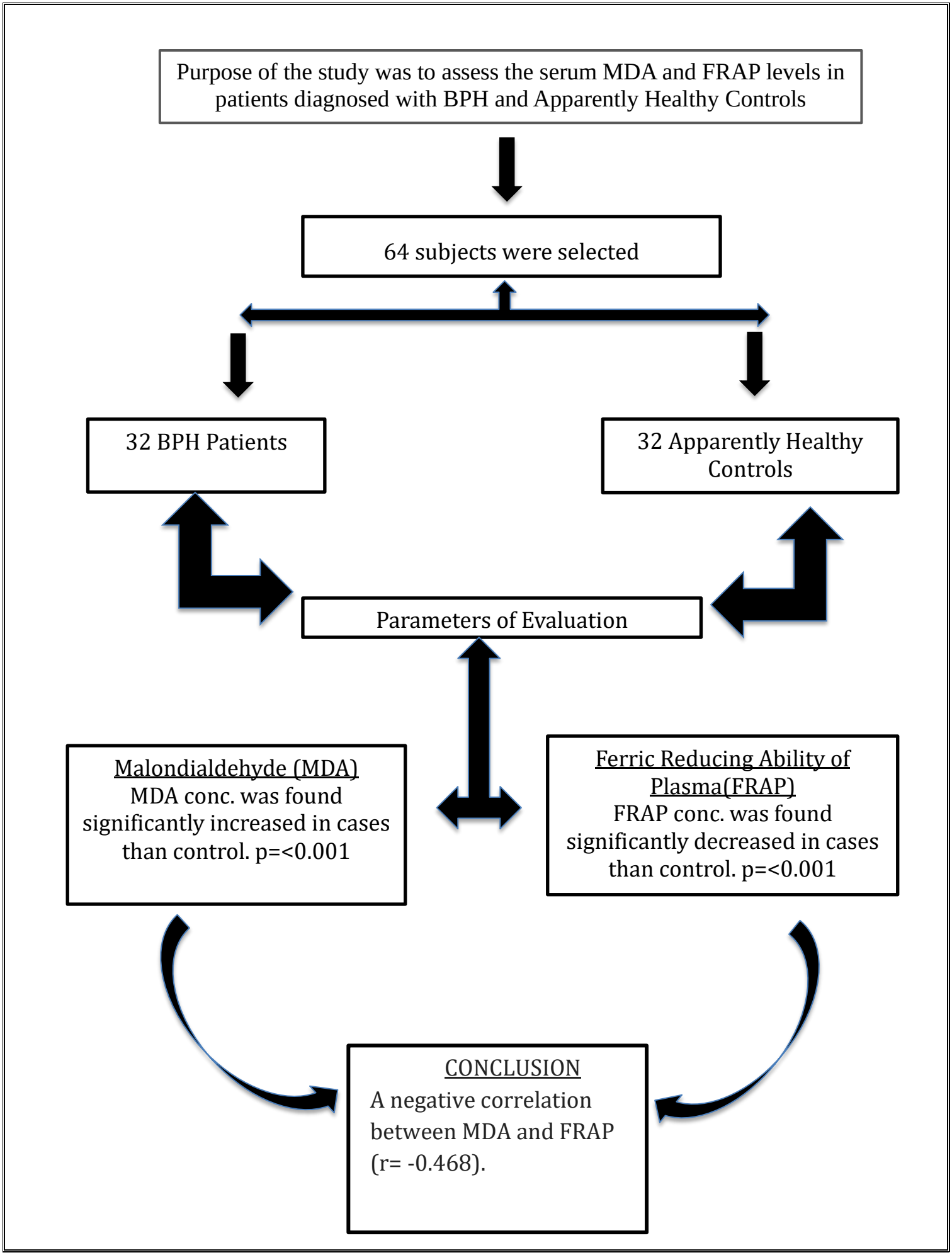
To sum up the research, we observed that:

1. Levels of MDA were significantly increased in cases than control.
2. Ferric Reducing Ability of Plasma was decreased in cases than control.
3. Comparison within the same age means, (50.16±6.29) in cases and 50.56±3.67 in controls, the levels of MDA were higher in cases than in controls, as well as reduced FRAP concentrations in cases than in controls.

CONCLUSION

In the study, it was observed that;

- There was significant difference in MDA concentration between the cases and control group.
- We found out that there was also statistical significance in the FRAP activity in cases compared to control.
- A negative correlation was observed between MDA and FRAP as observed in the sampled patients.
- Further research is needed on the roles MDA and FRAP play in Benign Prostatic Hyperplasia to determine any association.


Purpose of the study was to assess the serum MDA and FRAP levels in patients diagnosed with BPH and Apparently Healthy Controls
64 subjects were selected
32 BPH Patients
32 Apparently Healthy Controls
Parameters of Evaluation
Malondialdehyde (MDA)
MDA conc. was found significantly increased in cases than control. p=<0.001
Ferric Reducing Ability of Plasma(FRAP)
FRAP conc. was found significantly decreased in cases than control. p=<0.001
CONCLUSION
A negative correlation between MDA and FRAP (r= -0.468).

REFERENCES

- Adwas AA, Elsayed A, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. J. Appl. Biotechnol. Bioeng. 2019 Feb 21;6(1):43-7.
- Ahmad M, Suhail N, Mansoor T, Banu N, Ahmad S. Evaluation of oxidative stress and DNA damage in benign prostatic hyperplasia patients and comparison with controls. Indian Journal of Clinical Biochemistry. 2012 Oct;27(4):385-8.
- Aoun F, Marcelis Q, Roumeguère T. Minimally invasive devices for treating lower urinary tract symptoms in benign prostate hyperplasia: technology update. Research and reports in urology. 2015 Aug 19:125-36.
- Bell MA, Campbell JD, Joice G, Sopko NA, Burnett AL. Shifting the paradigm of testosterone replacement therapy in prostate cancer.The World Journal of Men's Health. 2018 May 1;36(2):103-9.
- Besiroglu H, Ozbek E. Re: Preliminary assessment of neck circumference in benign prostatic hyperplasia in patients with metabolic syndrome. International braz j urol. 2017 Sep;43:1000-1.
- Bid HK, Konwar R, Singh V. Benign prostatic hyperplasia: Is it a growing public health concern for India?. Indian journal of medical sciences. 2008 Sep 1;62(9):375-6.
- Chughtai B, Forde JC, Thomas DD, Laor L, Hossack T, Woo HH, Te AE, Kaplan SA. Benign prostatic hyperplasia. Nature reviews Disease primers. 2016 May 5;2(1):1-5.
- Cohen KB, Demner-Fushman D. Biomedical natural language processing. John Benjamins Publishing Company; 2014 Feb 15.
- Corrêa LL, Vieira Neto L, Lima GA, Gabrich R, Miranda LC, Gadelha MR. Insulin-like growth factor (IgF)-I, IgF binding protein-3, and prostate cancer: correlation with gleason score. International braz j urol. 2015 Jan;41:110-5.

- De Nunzio C, Presicce F, Tubaro A. Inflammatory mediators in the development and progression of benign prostatic hyperplasia. Nature reviews urology. 2016 Oct;13(10):613-26.
- Domoslawska A, Zduńczyk S, Kankofer M, Bielecka A. Oxidative stress biomarkers in dogs with benign prostatic hyperplasia. Irish Veterinary Journal. 2022 Dec;75(1):1-6.
- Gandaglia G, Zaffuto E, Fossati N, Cucchiara V, Mirone V, Montorsi F, Briganti A. The role of prostatic inflammation in the development and progression of benign and malignant diseases. Current opinion in urology. 2017 Mar 1;27(2):99-106.
- Golden TR, Hinerfeld DA, Melov S. Oxidative stress and aging: beyond correlation. Aging cell. 2002 Dec;1(2):117-23.
- Griffin SP, Bhagooli R. Measuring antioxidant potential in corals using the FRAP assay. Journal of Experimental Marine Biology and Ecology. 2004 May 12;302(2):201-11.
- Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. Journal of Biological Chemistry. 2008 Aug 8;283(32):21837-41.
- Halliwell B, Gutteridge JM. Free radicals in biology and medicine.Oxford university press, USA; 2015.
- Hamid AR, Umbas R, Mochtar CA. Recent role of inflammation in prostate diseases: chemoprevention development opportunity. Acta Med Indones. 2011 Jan 1;43(1):59-65.
- Kawee-Ai A, Srisuwun A, Tantiwa N, Nontaman W, Boonchuay P, Kuntiya A, Chaiyaso T, Seesuriyachan P. Eco-friendly processing in enzymatic xylooligosaccharides production from corncob: Influence of pretreatment with sonocatalytic–synergistic Fenton reaction and its antioxidant potentials. UltrasonicsSonochemistry. 2016 Jul 1;31:184-92.

- Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. Cancer letters. 2009 Sep 18;282(2):125-36.
- Kohen R, Nyska A. Invited review: oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic pathology. 2002 Oct;30(6):620-50.
- Lerner LB, McVary KT, Barry MJ, Bixler BR, Dahm P, Das AK, Gandhi MC, Kaplan SA, Kohler TS, Martin L, Parsons JK. Management of lower urinary tract symptoms attributed to benign prostatic hyperplasia: AUA guideline part I—initial work-up and medical management. The Journal of urology. 2021 Oct;206(4):806-17.
- Liu CC, Huang SP, Li WM, Wang CJ, Chou YH, Li CC, Huang CH, Wu WJ. Relationship between serum testosterone and measures of benign prostatic hyperplasia in aging men. Urology. 2007 Oct 1;70(4):677-80.
- Livingstone DE, Barat P, Di Rollo EM, Rees GA, Weldin BA, Rog-Zielinska EA, MacFarlane DP, Walker BR, Andrew R. 5α-Reductase type 1 deficiency or inhibition predisposes to insulin resistance, hepatic steatosis, and liver fibrosis in rodents. Diabetes. 2015 Feb 1;64(2):447-58.
- Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clinical interventions in aging. 2018 Apr 26:757-72.
- Mas-Bargues C, Escriva C, Dromant M, Borrás C, Vina J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. Archives of Biochemistry and Biophysics. 2021 Sep 30;709:108941.
- Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. Free Radical Biology and Medicine. 2000 Jun 15;28(12):1745-50.

- Merendino RA, Salvo F, Saija A, Di Pasquale G, Tomaino A, Minciullo PL, Fraccica G, Gangemi S. Malondialdehyde in benign prostate hypertrophy: a useful marker?. Mediators of inflammation. 2003 Apr 1;12:127-8.
- Minciullo PL, Inferrera A, Navarra M, Calapai G, Magno C, Gangemi S. Oxidative stress in benign prostatic hyperplasia: a systematic review. Urologia Internationalis. 2015 Dec 5;94(3):249-54.
- Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty Ahmad A, Kabir MB, Umar Bindawa K, Ahmed AU. Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa, Northwestern Nigeria. SAGE open medicine. 2021 Jan;9:2050312121991246.
- Nazarko L. A guide to diagnosing and treating benign prostatic hypertrophy.British Journal of Community Nursing. 2023 Jul 2;28(7):344-50.
- Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: past, present and future. Differentiation. 2011 Nov 1;82(4-5):184-99.
- Pamplona R, Borras C, Jové M, Pradas I, Ferrer I, Viña J. Redox lipidomics to better understand brain aging and function. Free Radical Biology and Medicine. 2019 Nov 20;144:310-21.
- Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: the interplay. BioMed research international. 2014 Oct;2014.
- Reynard JM. Does anticholinergic medication have a role for men with lower urinary tract symptoms/benign prostatic hyperplasia either alone or in combination with other agents?. Current opinion in urology. 2004 Jan 1;14(1):13-6.

- Srivastava DS, Mittal RD. Free radical injury and antioxidant status in patients with benign prostate hyperplasia and prostate cancer. Indian Journal of Clinical Biochemistry. 2005 Jul;20:162-5.
- Thorpe A, Neal D. Benign prostatic hyperplasia. The Lancet. 2003 Apr 19;361(9366):1359-67.
- Tong Y, Guo YJ, Zhang Q, Bi HX, Kai K, Zhou RY. Combined treatment with dihydrotestosterone and lipopolysaccharide modulates prostate homeostasis by upregulating TNF-α from M1 macrophages and promotes proliferation of prostate stromal cells. Asian Journal of Andrology. 2022 Sep 1;24(5):513-20.
- Vaselkiv JB, Ceraolo C, Wilson KM, Pernar CH, Rencsok EM, Stopsack KH, Grob ST, Plym A, Giovannucci EL, Olumi AF, Kibel AS. 5-alpha reductase inhibitors and prostate cancer mortality among men with regular access to screening and health care. Cancer Epidemiology, Biomarkers & Prevention. 2022 Jul 1;31(7):1460-5.
- Vieira SA, Zhang G, Decker EA. Biological implications of lipid oxidation products. Journal of the American Oil Chemists' Society. 2017 Mar;94:339-51.
- Vignozzi L, Rastrelli G, Corona G, Gacci M, Forti G, Maggi M. Benign prostatic hyperplasia: a new metabolic disease?. Journal of endocrinological investigation. 2014 Apr;37:313-22.
- Wong CP, Bray TM, Ho E. Induction of proinflammatory response in prostate cancer epithelial cells by activated macrophages. Cancer letters. 2009 Apr 8;276(1):38-46.

ANNEXURES

INFORMED CONSENT FORM (FOR CASE)

- I, ARNOLD RATEMO MULUHYA, MSc. Medical Biochemistry 3rd Year student IIMSR Lucknow.
- For this study, I will take your **4 ml** blood sample for the determination of malondialdehyde and ferric reducing ability.
- The blood is only subjected for determination of serum MDA and ferric reducing ability.
- There will be no charges /fees/any consideration given or taken for the study.
- Your identity will be confidential and information and the result of your blood test will not be revealed to any other except you if you desire.
- This study has nothing to do with your treatment nor is it going to hamper the same if you refuse to participate.
- I am not associated with your treating doctor panel.
- The study has nothing to do with your current treatment but may improve the knowledge and understanding of the disease process and that knowledge may or not be helpful in future.
- After knowing all the above details, would you like to participate in our study?

Yes…… /No…….

CONSENT FORM

I……..… Aged.....

S/O.....UID.....……. Here, I state that I have been duly informed about the study titled "**A STUDY ON MALONDIALDEHYDE (MDA) AND FERRIC REDUCING ABILITY OF PLASMA (FRAP) IN BENIGN PROSTATE HYPERPLASIA PATIENTS AND CONTROL SUBJECTS**", its prospects and consequences. I hereby give informed and written consent for the collection of my blood sample for the above said study only.

Date:

Signature/thumb impression of the patient: Name of research scholar

Signature/thumb impression of the witness Signature of research scholar:

INFORMED CONSENT FORM (FOR CONTROL)

- I, ARNOLD RATEMO MULUHYA, MSc. Medical Biochemistry 3rd Year student IIMSR Lucknow.
- You are an apparently healthy individual.
- For this study, I will take **4 ml** of your blood sample for the determination of malondialdehyde and ferric reducing ability.
- The blood is only subjected for determination of serum MDA and ferric reducing ability.
- There will be no charges /fees/any consideration given or taken for the study.
- Your identity will be confidential and information and the result of your blood test will not be revealed to any other except you if you desire.
- The study may improve the knowledge and understanding of disease processes and that knowledge may or may not be helpful in future.
- After knowing all the above details, would you like to participate in our study?

Yes…… /No…….

CONSENT FORM

I……..… Aged.....

S/O.....UID.....……. Here, I state that I have been duly informed about the study titled "**A STUDY ON MALONDIALDEHYDE (MDA) AND FERRIC REDUCING ABILITY OF PLASMA (FRAP) IN BENIGN PROSTATE HYPERPLASIA PATIENTS AND CONTROL SUBJECTS**", its prospects and consequences. I hereby give informed and written consent for the collection of my blood sample for the above said study only.

Date:

Signature/thumb impression of the patient: Name of research scholar

Signature/thumb impression of the witness Signature of research scholar:

सूचित सहमति प्रपत्र (मामले के लिए)

- मैं, अर्नोल्ड रेटमो मुलुह्या, एमएससी मेडिकल बायोकेमिस्ट्री तृतीय वर्ष का छात्र आईआईएमएसआर लखनऊ।
- इस अध्ययन के लिए, मैं मैलोंडायल्डिहाइड और फेरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा के आकलन के लिए आपके **4** मिलीलीटर रक्त का नमूना लूंगा।
- रक्त केवल सीरम एमडीए और फेरिक कम करने की क्षमता के आकलन के अधीन है।
- अध्ययन के लिए कोई शुल्क/शुल्क/कोई विचार नहीं किया जाएगा।
- आपकी पहचान गोपनीय और जानकारीपूर्ण होगी और यदि आप चाहें तो आपके रक्त परीक्षण का परिणाम आपके अलावा किसी अन्य को प्रकट नहीं किया जाएगा।
- इस अध्ययन का आपके उपचार से कोई लेना-देना नहीं है और न ही यदि आप भाग लेने से इंकार करते हैं तो इससे इसमें बाधा नहीं आएगी।
- मैं आपके उपचार करने वाले डॉक्टर पैनल से संबद्ध नहीं हूं।
- अध्ययन का आपके वर्तमान उपचार से कोई लेना-देना नहीं है, लेकिन यह रोग प्रक्रिया के ज्ञान और समझ में सुधार कर सकता है और यह ज्ञान भविष्य में सहायक हो सकता है या नहीं।
- उपरोक्त सभी विवरणों को जानने के बाद, क्या आप हमारे अध्ययन में भाग लेना चाहेंगे?

हां..... नहीं.....

सहमति पत्र

मैं ..... आयु.....सुपुत्र.....यूआईडी.. ..... यहां, मैं बताता हूं कि मुझे "**डटर्मीनेशन ऑफ़ मालोंडियाल्डहाइड ऐंड फैरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा इन बिनाइन प्रोस्टेट हाइपरप्लेशिया पेशेंट्स ऐंड कंट्रोल सब्जेक्ट्स**" शीर्षक वाले अध्ययन, इसकी संभावनाओं और परिणामों के बारे में विधिवत जानकारी दी गई है। मैं केवल उपरोक्त अध्ययन के लिए अपने रक्त के नमूने के संग्रह के लिए सूचित और लिखित सहमति देता हूं।

दिनांक:

रोगी के हस्ताक्षर/अंगूठे का निशानः शोधार्थी का नाम

साक्षी के हस्ताक्षर/अंगूठे का निशान शोधार्थी के हस्ताक्षरः

सूचित सहमति प्रपत्र (नियंत्रण के लिए)

- मैं, अर्नोल्ड रेटमो मुलुह्या रिसर्च स्कॉलर मेडिकल बायोकैमिस्ट्री IIMSR लखनऊ।
- आप स्पष्ट रूप से स्वस्थ व्यक्ति हैं।
- इस अध्ययन के लिए, मैं मैलोंडायल्डिहाइड और फेरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा के आकलन के लिए आपके रक्त के नमूने का **4** मिलीलीटर लूंगा।
- रक्त केवल सीरम एमडीए और फेरिक कम करने की क्षमता के आकलन के अधीन है।
- अध्ययन के लिए कोई शुल्क/शुल्क/कोई विचार नहीं किया जाएगा।
- आपकी पहचान गोपनीय और जानकारीपूर्ण होगी और यदि आप चाहें तो आपके रक्त परीक्षण का परिणाम आपके अलावा किसी अन्य को प्रकट नहीं किया जाएगा।
- अध्ययन रोग प्रक्रियाओं के ज्ञान और समझ में सुधार कर सकता है और यह ज्ञान भविष्य में सहायक हो भी सकता है और नहीं भी।
- उपरोक्त सभी विवरणों को जानने के बाद, क्या आप भाग लेना चाहेंगे हमारे अध्ययन में?

हां ….. नहीं……

सहमति पत्र

मैं …… आयु…….सुपुत्र……यूआईडी.. ..... यहां, मैं बताता हूं कि मुझे "**डटर्मीनेशन ऑफ़ मालोंडियाल्डहाइड ऐंड फैरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा इन बिनाइन प्रोस्टेट हाइपरप्लेशिया पेशेंट्स ऐंड कंट्रोल सब्जेक्ट्स**" शीर्षक वाले अध्ययन, इसकी संभावनाओं और परिणामों के बारे में विधिवत जानकारी दी गई है। मैं केवल उपरोक्त अध्ययन के लिए अपने रक्त के नमूने के संग्रह के लिए सूचित और लिखित सहमति देता हूं।

दिनांक:

रोगी के हस्ताक्षर/अंगूठे का निशानः शोधार्थी का नाम

साक्षी के हस्ताक्षर/अंगूठे का निशान शोधार्थी के हस्ताक्षरः

ANNEXURE II (A)

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, LUCKNOW

1. INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA	YES	NO
1. Subject diagnosed with Benign Prostate Hyperplasia	☐	☐
2. Age group 45 years and above	☐	☐
3. Signed consent form.	☐	☐

EXCLUSION CRITERIA

1. History of Renal diseases	☐	☐
2. History of Liver disease.	☐	☐
3. History of Cardiac failure.	☐	☐

Subject is eligible for the study, if all **INCLUSION** criteria are **YES** and all **EXCLUSION** criteria are **NO**

INVESTIGATOR'S STATEMENT

I have verified the data entered in the case report form and have determined that it is complete, accurate and compatible with the source documents.

Investigator's Name and Signature Date

_____ _____ _____

Unique Identification No:

IDENTIFIERS- CASES

Registration No: …….

Contact No: ……..

Name: …….

Father's Name: ……

Address: ……

DEMOGRAPHICS- CASES

Age: …….

Place of Residence: Urban ☐ Rural ☐

Social / Economical Status: a) Upper b) Upper Middle c) Lower Middle d) Upper Lower e) Lower

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CASES

Height (mts) ……..……...

Weight (kgs) …….

Body Mass Index (kg/ m^2) …….

Unique Identification No:

IDENTIFIERS- CONTROL

Registration No: ……

Contact No: …….

Name: ……

Father's Name: ……

Address: ……

DEMOGRAPHICS- CONTROL

Age: ……..

Place of Residence: Urban ☐ Rural ☐

Social / Economical Status: a) Upper b) Upper Middle c) Lower Middle d) Upper Lower e) Lower

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CONTROL

Height (mts) : …….

Weight (kgs) : ……

Body Mass Index (kg/m^2): ……..

INSTITUTIONAL ETHICS COMMITTEE (IEC)

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CERTIFICATE

This is to certify that research work entitled **"A Study of Malondialdehyde and Ferric Reducing Ability of Plasma in Benign Prostatic Hyperplasia Patients and Control Subjects"** submitted by **Arnold Ratemo Muluhya, Dr.Roshan Alam** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **30th December 2022**.

Dr.Q.S.Ahmed
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Page count:	20
Word count:	3,389
Character count:	18,323
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Introduction

Benign prostatic hyperplasia (BPH) is a prevalent condition commonly diagnosed in aging males, leading to lower urinary tract symptoms (LUTS). The prevalence of BPH is increasing, and there are various risk factors, both modifiable and non-modifiable, that can contribute to its development and progression. The symptoms associated with BPH and LUTS can be classified as obstructive (causing difficulties in initiating urination, weak urine stream, straining, or prolonged voiding) or irritative (resulting in increased frequency and urgency of urination, nocturia, urge incontinence, and reduced volume of voiding). Additionally, post-micturition symptoms like post void dribble or incomplete emptying can also affect patients. **(Chughtai et al. 2016)**

In 2021, the American Urological Association (AUA) published an article that discusses BPH as a very prevalent among aging males, with onset in the early 40s, increasing to 80% by the age of 80 **(Lerner LB et al., 2021)**. Analyses of multiple studies show that every year in the fourth decade, there are pathologically identifiable prostate enlargements in autopsy **(Lumpkin et al. 2019)**. In India, although not well performed, epidemiological research data from 2008 suggest that the incidence rate of prostate enlargement is about 82.45% to 93.3% **(Bhat et al 2008)**. A well-designed research encouraged by urologists is needed to have a realistic and updated survey in India.

Histological diagnosis of patients with BPH reveals abnormal proliferation of epithelial cells. Among all the research reasons and increased contractility issues, all indicate the prostatic transition zone **(Roehrborn et al. 2017)**.The previous hand also in the anatomic relationship that

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Introduction

Benign prostate hyperplasia (BPH) is a prevalent condition commonly diagnosed in aging males, leading to lower urinary tract symptoms (LUTS). The prevalence of BPH is increasing, and there are various risk factors, both modifiable and non-modifiable, that can contribute to its development and progression. The symptoms associated with BPH and LUTS can be classified as obstructive (causing difficulties in initiating urination, weak urine stream, straining, or prolonged voiding) or irritative (resulting in increased frequency and urgency of urination, nocturia, urge incontinence, and reduced volume of voiding). Additionally, post-micturition symptoms like post void dribble or incomplete emptying can also affect patients. **[Chughtai et al. 2016]**

In 2010, the American Urological Association (AUA) published an article that discloses BPH as most prevalent among aging males, with onset in the early 40s, increasing to 80% by the age of 80 **[Lerner LB et al. 2021]**. Analyses of multiple studies show that every year in the fourth decade, there are pathologically identifiable prostatic enlargements at autopsy **[Langan et al. 2019]**. In India, although not well performed, epidemiological research data from 2008 suggest that the incidence rate of prostate enlargement is about 92.97% to 93.3% **[Bid et al 2008]**. A well packaged research encouraged by urologists is needed to have a realistic and updated survey in India.

Histological diagnosis of patients with BPH reveals abnormal proliferation of epithelial cells, toning of the smooth muscle and increased connective tissue, all within the prostate transition zone **[Besiroglu et al 2017].**This proves fatal due to the anatomic relationship that

exists between the prostate, the bladder neck and urethra. An enlargement of the prostate from the cellular level leads to a disturbance in the normal functioning of these adjacent organs. Thus, BPH occurs due to prostate enlargement and that may lead to the more severe Benign Prostate Obstruction (BPO). The BPO affects urinary functions; this has become the target for therapeutic intervention as no clear treatment for the disease exists to date.

What stands out, however, is the role the testes plays in progression of BPH. Patients diagnosed with primary hypogonadism, while undergoing testosterone replacement, were found to develop normal prostatic growth as well as progression in prostate enlargement **[Bell MA et al 2018].**Furthermore, it was observed that patients with prostatic diseases (prostate cancer or BPH) who had bilateral orchiectomy performed, there was a significant decline in the disease progression as well as an eventual reversal in the prostate volume **[Nicholson et al 2011]**. This proves the androgen dependence of the prostate gland, without which prostate atrophy is likely. A constant supply and maintenance of circulating androgens is necessary for daily functioning of prostate cells and its overall role in the urogenital system.

The testis is the primary organ for androgen, and estrogen to a lesser extent, production in males. Testosterone serves as the chief androgen released from the testes. It is then transported to the prostate via circulation and once in the prostate, the androgen receptors (AR) located on prostatic cells are activated and lead to cellular growth. AR-dependent transcription in the cell nucleus produces more protein growth factors such as insulin-like growth factors, keratinocyte growth factors, epidermal growth factors, as well as fibroblast growth factors.**[Vignozzi et al. 2014]**. This leads to hyperplasia within the prostate gland.

It is worth mentioning though, that aging occurs, there is a decrease in circulating testosterone hormone levels. This casts a shadow over the idea that testosterone is the sole mitogen responsible for BPH progression as challenged by Liu et al. **[Liu et al 2007]**.

Before scientific advancements, surgery was considered the only viable option for patients. This, however, presents many complications in terms of morbidity and mortality during and after surgery. Recently, the understanding of the action of AR-dependent transcription has led to discovery of ***5 α-reductase Inhibitors*** that has been hailed as a pharmacological breakthrough in alleviating the symptoms resulting from BPH **[Vaselkiv et al 2022]**. The adrenergic inhibitors have proven selective in combating the disease and with varying results among patients **[Livingstone et al 2015]**.

Recent studies seem to suggest that oxidative stress may play a role in benign prostate hyperplasia **[Srivastava et al 2005]**. Oxidative stress can be defined as an increased concentration of free radicals in the body that causes oxidative damage to cells, and subsequent decline in the antioxidant systems responsible for combating these free radicals **[Minciullo et al 2015]**. This proves the active role that oxidative stress plays in BPH is not just a phenomenon. Reactive Oxygen Species (ROS) has been reported to promote autocatalytic formation of lipid peroxidation that leads to harmful genotoxic byproducts. These radicals eventually lead to formation of DNA adducts that damage DNA products **[Halliwell et al 2015].**

Understanding the cellular and biochemical changes that accompany BPH may prove useful in proper managing and treating the disease. This research study seeks to find the

correlation between oxidative stress status by measuring malondialdehyde, a marker for lipid peroxidation, and antioxidant profile through the blood serum's ability to perform the Fenton reaction, in patients diagnosed with clinical BPH **[Kawee-Ai et al 2016]**

Review of Literature

Benign Prostate Hyperplasia accounts for approximately 40% of reported urological conditions in men above 60 years **[Aoun et al 2015]**. A study on sexually intact dogs indicated that 80% of them which are 5 years compared to 95% of them that are 9 years showed overall microscopic changes attributed to BPH **[Domosławska et al 2022]**. This proves that the disease is age related and almost unavoidable as age progresses. When compared to other prostatic diseases, there has been some epidemiological similarity that makes it possible to understand the gland in terms of functional anatomy and disease. Their complex pathophysiology includes genetic instability, inflammation, endocrine disruptors, coronary artery disease hormones, and oxidative stress, among other aspects.

In the past, most men did not see the need to seek medical attention; accepting LUTS as part of the aging process. However, with the intense publicity on prostate cancer and the important awareness on men's health, more and more men are coming out to seek more insight and attention in regards to their health **[Taitt HE et al 2018].** The understanding of the molecular pathology and physiology of BPH is due to the advancements in molecular studies and imaging that occur within the prostate gland compared between patients with the disease and healthy individuals. Probable causes are being advanced. It was noted that there was an increased serum-insulin like growth factor 1 among patients with an enlarged prostate compared with those with normal prostate volumes **[Corrêa LL et al. 2015].**

The prostate is a small gland that forms part of the male genital system. Its main role is to provide fluid that goes into semen. This fluid is important for men's fertility. The gland

surrounds the urethra, from the bladder neck downwards. The bladder and urethra form the *lower urinary tract* **[Deters LA 2014]**. Most of the symptoms happen within this region and lead to LUTS. These symptoms are a result of blocked urethra or an overworked bladder.

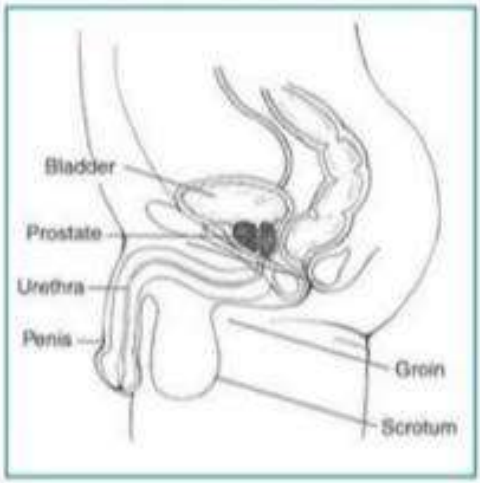

Fig 1. The anatomical position of the Prostate Gland **[Source: Deters LA 2014]**

Lower urinary tract symptoms of BPH include:- **[Deters LA 2014]**

- Increased urinary frequency; up to eight or more times a day
- The inability to delay urination leading to increased urgency
- Delayed micturition caused by trouble starting a urine stream
- An interrupted urine stream
- Dribbling towards the end of urination
- Increased nocturia
- Urinary retention.
- Urinary incontinence

- Painful ejaculation or urination

Diagnosis of LUTS

Diagnosis is based on taking patient history, physical and rectal examination, and urine volume examinations. Confirmation of BPH is normally done by ultrasound check of the volume of the prostate gland in addition to the above mentioned symptoms **[Nazarko et al 2023]**.

Free flow rates, symptoms scores (IPSS), frequency volume charts, and urodynamics are the other aspects of advanced assessments that are used in diagnosis.

Oxidative Stress and BPH

A homeostatic imbalance between the production of reactive oxygen species and their elimination from the body system through antioxidant mechanisms, defines oxidative stress. The body itself has mechanisms that kick in at the expulsion of free radicals into the environment. As discussed earlier, mounting evidence shows that these free radicals are responsible for the formation of DNA adducts that cause formation of protein and protein products outside the norm. These abnormal proteins mediate the formation of hyperplasia. Free radicals such as superoxide and hydrogen peroxide stand in the way of antioxidant systems **[Xiong et al 2020]**

The prostate has been described as an 'immunocompetent organ', referring to the rich immune cells that characterize its tissue **[De Nunzio et al 2016]**. These immune cells act as barriers against easy stimuli, e.g. bacterial infection, sexually transmitted organism or even autoimmune disorders, being easily activated resulting in chronic inflammation **[Gandaglia et**

al. 2017]. Oxidative stress has also been tipped to mediate activation of these inflammation systems leading to release of cytokines and growth factor proteins that trigger abnormal fibro muscular growth, characteristic of BPH.

Wilson, J. D. (1972), in his publication hypothesized the role testosterone plays in BPH. He postulated that while in the prostate, the testosterone hormone is activated to Dihydrotestostrone (DHT) by the ***5 α-reductase*** enzyme found on the stromal cells within the transitional zone **[Tong et al 2022]**. The enzyme exists in two isoforms; *5 α-reductase type 1 & type 2 (5AR1 & 5AR2).* The bio-activate DHT is responsible for activating the AR. One could argue then that BPH is an endocrine disorder based on this.

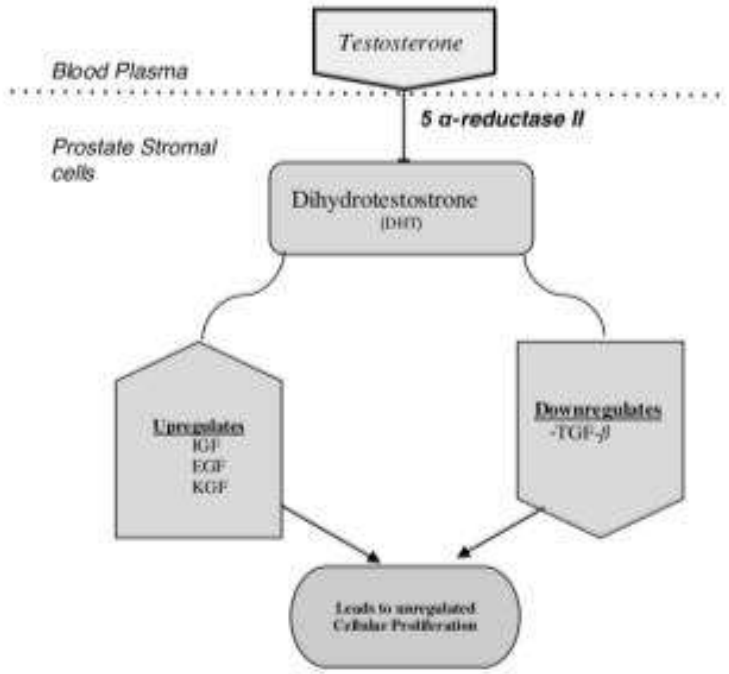

Fig. 2. Pathogenesis of Benign Prostate Hyperplasia [Tong et. al, 2022]

This knowledge has been used therapeutically in the treatment of BPH symptoms and complications. **5 *α-reductase inhibitors*** have been used to inhibit the activation of testosterone in patients with BPH **[Reynard et al 2004]**.

Effects of Oxidative Stress on BPH

Chronic prostatitis has been linked with production of ROS through its effective stromal cellular proliferation leading to oxidative damage and injury. These ROS had been thought to only affect protein products, but studies show that damage is incurred even to the DNA level through mutations and post transcriptional modifications. The mutagenic effect of ROS causes deletion or point mutations that cause a defect from the default operation or a cell. This departure could mean the prostate cells evade cell growth regulation signals leading to hyperplasia and cancerous transformations **[Hamid et al 2011]**.

Similarly, ROS has been found to activate the immune response pathway that causes CP **[Wong et al 2009]**. ROS activates NF-κB inducing kinase (NIK) that stimulates the NF-κB; a transcription factor involved in the regulation of cellular immune response. Activation of NIK has been studied and proven to induce signal transduction that leads to formation of CP. Alternatively; NIK may also interfere with the translation process leading to abnormal growth factor proteins.

Indirectly, ROS can contribute to generation of more OS through autocatalytic lipid peroxidation. The result of which is continual generation of genotoxic breakdown byproducts

that are free radicals. These byproducts such as peroxyl radicals and aldehydes have been estimated and analyzed in blood serum **[Vieira et al 2017]**. One of these byproducts, Malondialdehyde, formed the basis of our research. Examining the serum levels of malondialdehyde serves as a non-invasive diagnostic mechanism of estimating oxidative stress levels and determining extent of lipid peroxidation in damaged tissue.

Enhanced lipid peroxidation triggers the immune response protocol and activates platelets. Platelet activation induces cyclooxygenase activity, an enzyme whose byproduct is MDA. Thus, MDA does not just measure lipid peroxidation but also the level of immune response that are triggered due to clinical symptoms arising from lipid peroxidation in tissue **[Merendino et al. 2003]**.

As discussed, ROS caused damage to DNA leading to adducts that are mutant in nature. Cellular component oxidation affects carbohydrate, lipids and protein as well. As is the case with proteins, post translational modification influenced by ROS results in direct oxidation of amino acids residues. The oxidation of methionine and cysteine has been shown to be a frequent phenomenon **[Grimsrud et al 2008]**. Aldehydes contain the most common of these ROS-induced post translational modifications in the form of protein carbonylation.

Antioxidant Systems

In normal systems, Oxidative stress is met by a host of mechanisms that counter the high ingress of free radicals. These systems may be enzymatic with enzymes such as Superoxide

Dismutase, Glutathione Peroxidase and Catalase, as well as vitaminic antioxidants such as **α**-tocopherol and ascorbate. ROS-induced oxidative damage occurs when an imbalance exists between the presence of free radicals and the mechanisms responsible for keeping them at bay. Data from numerous studies show an elevated OS level with a concomitant decrease in antioxidant defense system efficiency **[Khandrika et al 2009]**.

Comparative study between patients with BPH and control groups showed a rise in DNA adduct products compared to activities of SOD in the serum collected **[Javed et al 2017]**. These results signaled a possible association between antioxidant enzymatic activity and DNA base lesions. However, the data is open to debate as some other articles show no significant change in total antioxidant capacity in some studies. Other small-molecular weight molecules, mostly non-enzymatic, scavenge the circulatory system for free radicals. These include vitamins and trace metals **[Rahal et al 2014]**.

Previous studies indicated that oxidative stress and anti-oxidant levels play a major role in etiology of disease. In view of these findings, the present study sought to assess the role played by oxidative stress and antioxidant status in the etiopathogenesis of BPH.

Aim

To find out the association of malondialdehyde and ferric reducing ability of plasma in benign prostate hyperplasia patients and control subjects.

Objectives

1. To determine the concentration of malondialdehyde (MDA) in benign prostate hyperplasia patients and control subjects.
2. To determine the ferric reducing ability of plasma (FRAP) in benign prostate hyperplasia patients and control subjects.
3. To find out the correlation between malondialdehyde and ferric reducing ability of plasma in benign prostate hyperplasia patients, if any.

RESULTS

Mean of age and weight have not shown any significant differences between cases and controls ($p = 0.76$, $p = 0.77$, respectively). Mean of MDA levels was found significantly elevated in cases compared to controls ($p < 0.001$). However, mean of FRAP levels was found significantly low in case compared to controls ($p < 0.001$), shown in **Table No. 1, Figure 3 & 4,** respectively.

Table 1: Baseline characteristics of cases and controls

Parameters	Cases (Mean ± SD) n=32	Controls (Mean ± SD) n=32	p-value	Significance
Age (years)	50.16±6.29	50.56±3.67	0.76	Not Significant
Weight (kg)	73.19±8.01	72.56±8.90	0.77	Not Significant
MDA (µmol/L)	2.80±0.45	2.15±0.30	**<0.001***	**Significant**
FRAP (µmol/L)	1046.66±75.15	1280.13±72.83	**<0.001***	**Significant**

Data represented as Mean ±SD.
**p-value < 0.05, considered as statistically significant.*
FRAP: Ferric reducing ability of plasma, MDA: Malondialdehyde

Table 2. Comparison of mean concentration for MDA (µmol/L)					
Groups	N	Mean	Standard Deviation	p-value	Significance
Cases	32	2.8	± 0.45	0.001	Statistically Significant
Controls	32	2.15	± 0.3		

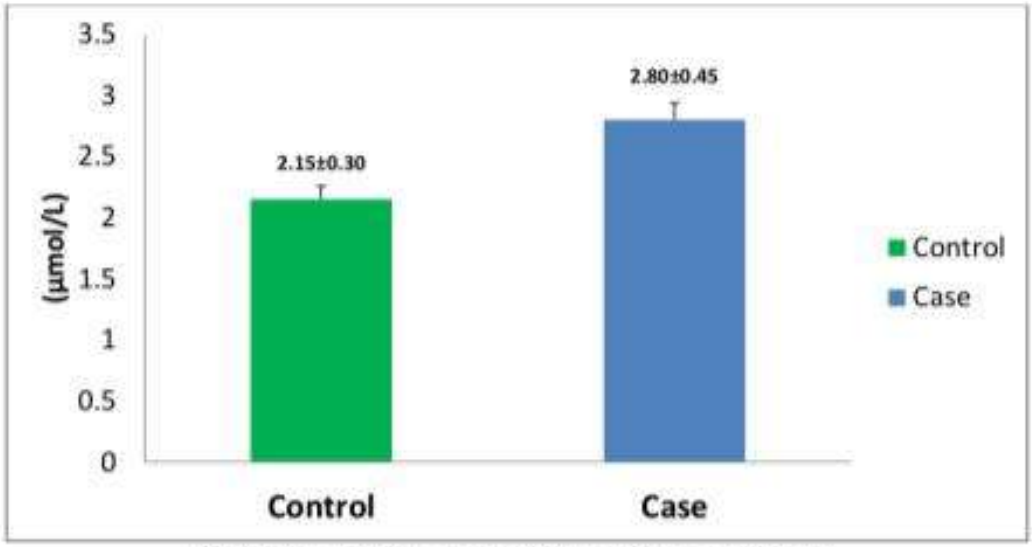

Fig 3. Mean of MDA compared between Cases and Control.

Table 3. Comparison of concentration of FRAP in Cases and Controls (µmol/L)					
Groups	N	Mean	Standard Deviation	p-value	Significance
Cases	32	1046.66	± 75.15	0.001	Statistically Significant
Controls	32	1280.13	± 72.83		

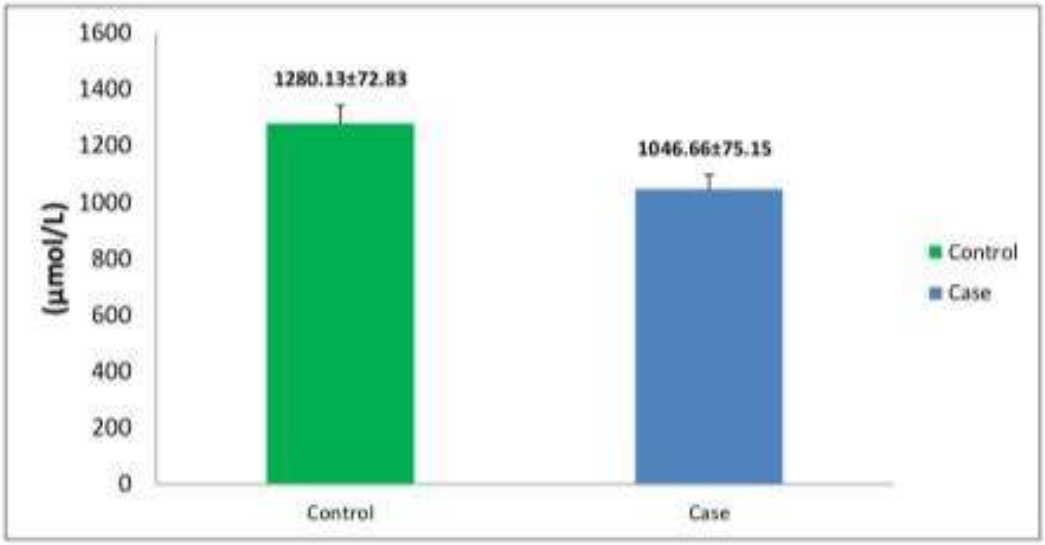

Fig 4. Mean of FRAP compared between Cases and Control.

Karl Pearson's Coefficient of Correlation among the Parameters

Correlations				
Parameters	Age (years)	Weight (kg)	MDA (µmol/L)	FRAP (µmol/L)
Age (years)	1	0.115	0.149	0.055
Weight (kg)	-	1	0.171	0.067
MDA (µmol/L)	-	-	1	-0.468**
FRAP (µmol/L)	-	-	-	1
**. Correlation is significant at the 0.01 level (2-tailed).				
FRAP: (Ferric Reducing Ability of Plasma) MDA: (Malondialdehyde)				

Table 3. P-value between the various parameters.

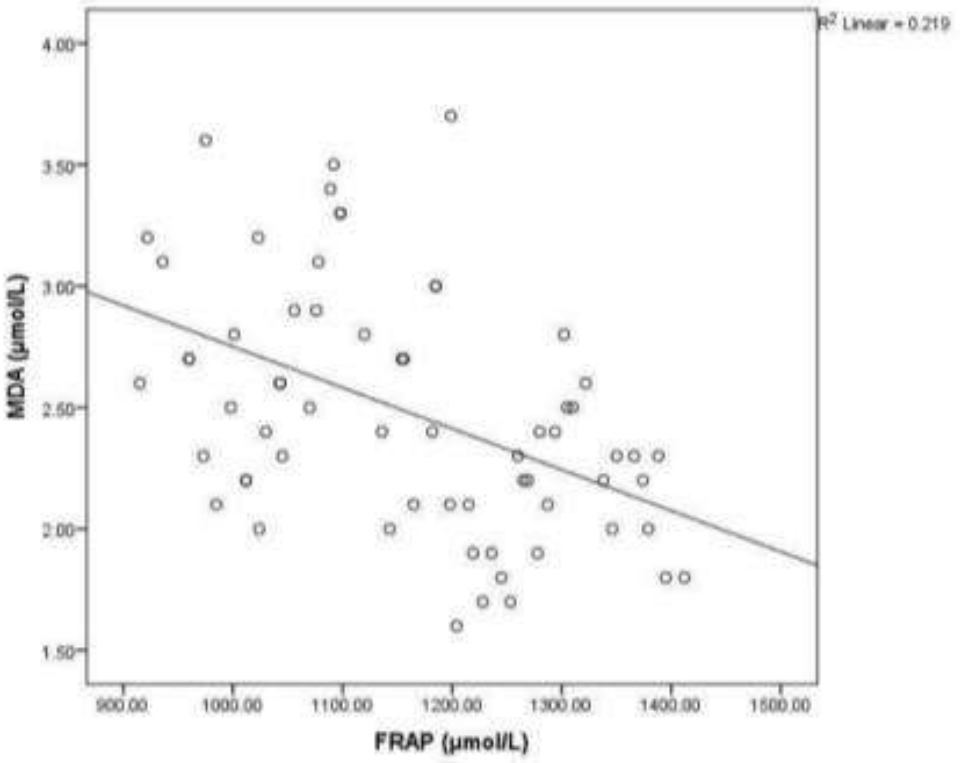

Fig 5. Scatter Diagram of MDA compared to FRAP in Cases

Discussion

From this study, the MDA concentration values showed a mean of **2.80±0.45** and **2.15±0.30** in case and control respectively. MDA acts as a marker of oxidative stress, produced as a result of lipid peroxidation. Both free radicals and oxidants are heavily involved in signaling pathways. However any imbalance in its production and elimination is lethal to the bio molecular integrity. Oxidative stress has been discussed as an important part of the development of many diseases [**Mas-Bargues, C., et al 2021**]. Lipid peroxidation occurs in response to the oxidation of lipids (PUFA) by these reactive oxygen species. Lipid peroxidation occurs in 3 phases; initiation, elongation and termination. Pamplona, R., et al noted that a single initiation cycle can give rise to 200-400 propagation cycles that give rise to aldehydes and dialdehydes that are considered more harmful [**Pamplona, R., et al 2019**]. The two most commonly determined aldehydes are 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). 4-HNE has been proven to have the highest biological activity whereas MDA is the most highly produced during lipid peroxidation, and is commonly used as a marker of oxidative stress. [**Barrera, G. et al 2018**].

This study also shows a significant negative correlation between MDA and FRAP (**r = -0.468, p<0.01**). This data indicates there being an inverse relationship; an increased MDA concentration usually signals a reduction in the antioxidant machinery. FRAP indicates the body's mechanism to react towards the increased OS that the body undergoes. The data shows a significantly lower concentration of FRAP in cases when compared to control groups signaling the fact that in diseased state, the body's ability to fight a counter measure to the increased OS is compromised (**Muhammad Y et al 2021**).

There is a marked increase in production of reactive oxygen species that results in processes such as lipid peroxidation; to which MDA serves as a good marker. In recent years, it has been proven that the OS plays a role in the aging process **[Liguori et al 2018].** BPH is considered an age-related illness, with increasing cases being isolated to aging males. It can therefore be mentioned that in aging males, there is a high chance that OS localized within the prostate gland leads to development of prostatic diseases such as BPH.

The data obtained shows no correlation between FRAP levels and age. Simply put, there is no statistical evidence to show that the decline in antioxidant capacity of individuals is brought about by aging. Rather, total antioxidant capacity is a result of increased oxidative stress. To say that the reduction of antioxidant capacity is as a result of aging would be misleading, unless data proves otherwise. Antioxidant systems are overwhelmed when there exists an imbalance between rate of production of free radicals and rate of elimination. Thus, a reduction in antioxidant capacity signals an increased molecular output of free radicals, and not aging **[Adwas AA et al 2019]**.

SUMMARY

Oxidative stress and antioxidant status in BPH were meant to be assessed in this research work through the concentration of Malondialdehyde (MDA) and Ferric Reducing Ability of Plasma (FRAP) respectively.

To sum up the research, we observed that:

1. Levels of MDA were significantly increased in cases than control.
2. Ferric Reducing Ability of Plasma was decreased in cases than control.
3. Comparison within the same age means, (50.16±6.29) in cases and 50.56±3.67 in controls, the levels of MDA were higher in cases than in controls, as well as reduced FRAP concentrations in cases than in controls.

CONCLUSION

In the study, it was observed that;

- There was significant difference in MDA concentration between the cases and control group.
- We found out that there was also statistical significance in the FRAP activity in cases compared to control.
- A negative correlation was observed between MDA and FRAP as observed in the sampled patients.
- Further research is needed on the roles MDA and FRAP play in Benign Prostatic Hyperplasia to determine any association.

ARNOLD RATEMO

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