"CROSS SECTIONAL STUDY OF LIPID PEROXIDATION, ANTIOXIDANT STATUS AND SERUM CALCIUM IN BENIGN PROSTATE HYPERTROPHY AND PROSTATE CANCER".

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Endorsement by the HOD, Principal/Head of the Institution

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LIST OF ABBREVIATIONS USED

ΔOD	-	Change in optical density
$^{\bullet}CCl_3$	-	Trichloromethyl radical
^{0}C	-	Degree centigrade
$A^{\bullet}$	-	Armstrong
ARE	-	Antioxidant Response Elements
AUA	-	American Urologist Association
BPH	-	Benign Prostate Hypertrophy
Ca	-	Serum calcium
CAT	-	Catalase
CCl_4	-	Carbon tetrachloride
$CCl_3^{\bullet}$	-	Trichloromethyl radical
CH_2	-	Methylene
COX-2	-	Cyclo-oxygenase-2
DNA	-	Deoxy ribonucleic acid
D/W	-	Distilled water
Da	-	Dalton
DHT	-	Dihydrotestosterone
dL	-	Deciliter
EPS	-	Expressed Prostate Secretions
Fe	-	Iron
Fe^{2+}	-	Ferrous iron
Fe^{3+}	-	Ferric iron
FAD	-	Flavin adenine dinucleotide
g or gm	-	Gram

GSSG	-	Oxidized glutathione
GSH	-	Reduced glutathione
GR	-	Glutathione reductase
H_2O_2	-	Hydrogen peroxide
$HCOO^{\bullet}$	-	Hydroperoxyl radical
HOCl	-	Hypochlorus acid
HCl	-	Hydrochloric acid
Hb	-	Hemoglobin
mmol	-	Milli mol
μmol	-	Micro mol
mg/dL	-	Milligram/deci litre
Min	-	Minute
ml	-	Milli litre
Mn	-	Manganese
MPO	-	Myeloperoxidase
MDA	-	Malondialdehyde
$NO^{\bullet}$	-	Nitric oxide radical
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NADH	-	Reduced nicotinamide adenine dinucleotide
nmol	-	Nano mol
$O_2CCl_3^{\bullet}$	-	Peroxyl radicals
$ONOO^{-}$	-	Peroxynitrite anion
O_2	-	Singlet oxygen
$OH^{\bullet}$	-	Hydroxyl radical
$O_2^{\bullet-}$	-	Superoxide anion radical

PC	-	Prostate Cancer
PIN	-	Prostate Intraepithelial Neoplasia
PSA	-	Prostate Specific Antigen
PUFA	-	Polyunsaturated fatty acid
RBC	-	Red blood cells
$RS^{\bullet}$	-	Thiyl radical
$R^{\bullet}$	-	Lipid radical
$ROO^{\bullet}$	-	Lipid peroxide radical
RDA	-	Recommended daily allowance
ROS	-	Reactive Oxygen Species
rpm	-	Revolutions per minute
RSH	-	Thiol compounds
Se	-	Selenium
SOD	-	Super Oxide Dismutase
SNP	-	Single Nucleotide Polymorphism
TBA	-	Thiobarbituric acid
TBAS	-	Thiobarbituric acid reactive substances
TAG	-	Triacyl glycerol
UV	-	Ultraviolet
Vc	-	Volume of cuvette
W/W	-	Weight by weight
1,25-VD	-	1α, 25-dihydroxyvitamin D

ABSTRACT

Background and Objectives:

The most dramatic organic manifestation of andropause in terms of decrease in quality of life is Benign Prostate Hypertrophy (BPH), in its ultimate consequences, a life threatening condition due to obstruction of urethra and finally uremia. Carcinoma of prostate is the second most common malignant disease in men. It often grows so slowly that, many men with prostate cancer "die with it, rather than from it." Lipid peroxidation is a well established mechanism of cellular injury. This is widely accepted to be involved in the pathogenesis of human disease. Malondialdehyde is an end product derived from peroxidation of polyunsaturated fatty acids (PUFA) and related esters. Thus measurement of MDA levels provides a convenient in-vivo index of lipid peroxidation and represents a non invasive bio-marker of oxidative stress, often clinically employed to investigate radical mediated physiological and pathological conditions.

To protect the body from these devastating free radicals, scavenging system is present in the body in the form of antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione reductase, catalase (CAT) and non enzymatic antioxidants include vitamin E and vitamin C that is alpha-tocopherol and ascorbate respectively. There is good evidence that men whose diet include a lot of calcium or those who take calcium supplements are at increased risk for prostate cancer. But since there is little relationship between dietary calcium and serum calcium, this study does not address the question of how much calcium men should eat. Men whose serum calcium levels fall within the high end of normal range are three times more likely to develop fatal prostate cancer.

Thus this study was done to know the extent of oxidative stress in BPH and Prostrate Cancer by estimating MDA, the role of antioxidants namely Glutathione Reductase, Vitamin C in protecting the body from these oxidative stress and Serum Calcium in prediction of Prostate Cancer.

Materials and methods:

All cases of BPH and prostate cancer in the age group of 65 to 75 years admitted or attending Urology unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. Based on previous hospital records minimum sample size will be 30 BPH and 30 Prostate Cancer.

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 preparing hemolysate and 6 ml in plain tubes, centrifuged, serum separated and kept at 4^0C which was analysed within 24 hours using colorimeter.

Methods of assay

Blood

- Malondialdehyde – Thiobarbituric acid method.

Hemolysate

- Glutathione Reductase – Beutler E method.

Serum

- Vitamin C – Evelyn and Melloy method.
- Serum calcium – Modification of O-Cresolphthalien complexone method.

Results:

The mean age of controls was 70.46±3.58 years (59-78 years), the mean age in Benign Prostate Hypertrophy cases was 72.26±4.70 years (65-81 years) and in Prostate Cancer cases was 72.76±4.98 years (65-81 years).There was no statistically significant difference between mean age of three groups (p= 0.117). The groups were age and sex matched.

The mean level of MDA in controls was 6.17±1 nmol/ml. The value in BPH cases was 11.44±0.82 nmol/ml and in Prostate Cancer cases was 15.83±3.63 nmol/ml. The level was significantly increased (P< 0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

The mean glutathione reductase level in controls was 8.96±1.04 IU/g of Hb. The level in BPH cases was 3.39 ± 0.74 IU/g of Hb and in Prostate Cancer cases was 3.32±0.76 IU/g of Hb. The level was significantly decreased (P< 0.001) in all the cases of BPH and also in cases of Prostate Cancer when compared to controls.

The mean vitamin C level in controls was 0.87 ±0.01 mg/dl. The level in BPH cases was 0.42 ±0.14 mg/dl and in Prostate Cancer cases was 0.50 ±0.15. The level was significantly decreased (p <0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

The mean value of total serum calcium in controls was 9.08 ± 0.37 mg/dl. The level in BPH cases was 9.92±0.97 mg/dl and in Prostate Cancer cases was 9.55±1.35mg/dl. The value of total serum calcium was significantly increased (p= 0.05) in all the BPH cases when compared to controls. But there was no statistical significant difference (p=0.187) in total serum calcium levels in Prostate Cancer cases when compared to controls.

Interpretation & Conclusion:

Our study revealed that there was increased oxidative stress and decreased antioxidant defense in patients of Benign Prostate Hypertrophy and Prostate Cancer. Also it was found that serum calcium levels were in higher end of normal range in patients of Benign Prostate Hypertrophy and Prostate Cancer.

Supplementation of natural antioxidants and drugs lowering serum calcium levels to the individuals who are prone for Benign Prostate Hypertrophy and Prostate Cancer may prevent the progression of these diseases to a certain extent. Since serum calcium levels were increased statistically only in BPH cases but not in Prostate Cancer, follow up is necessary to establish the association in these disease processes.

Key words: Benign Prostate Hypertrophy, Prostate Cancer, Malondialdehyde, Glutathione Reductase, Vitamin C, Serum Calcium.

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INTRODUCTION

The most dramatic organic manifestation of andropause in terms of decrease in quality of life is Benign Prostate Hypertrophy (BPH), in its ultimate consequence, a life threatening condition due to obstruction of urethra and finally uremia. Though the pathology has been well established that it is nodular Hypertrophy, its cause is not known definitely. It is a disease with well defined age incidence. It is essentially a disease of old age. The process of prostatic hypertrophy involves all four major cell types in prostate (Smooth muscle cells, fibroblasts, acinar cells and basal epithelial cells).[1]

Carcinoma of prostate is the second most common malignant disease in men. Its incidence being 25.3 per 100000 men and increasing due to early detection. The mortality rate being 8.1 per 100000. It often grows so slowly that many men with Prostate Cancer "die with it, rather than from it." It is the commonest malignant condition in men after 60 years of age. In a series of autopsies in men over age of 80 years, 75% had Prostate Cancer. In the light of these findings serum malondialdehyde (MDA) was analysed in order to clarify the oxidative stress for Benign Prostate Hypertrophy (BPH) and Prostate Cancer.[2]

Lipid peroxidation is a well established mechanism of cellular injury. This process leads to the production of lipid peroxidases and other by-products which ultimately affect the membrane function and integrity. This is widely accepted to be involved in the pathogenesis of human disease. Malondialdehyde is an end product derived from peroxidation of Polyunsaturated Fatty Acids (PUFA) and related esters. In contrast to free radicals, aldehydes are relatively stable and therefore able to diffuse within or out of cell and to attack targets, distant from the site of original free radical

initiated events. Furthermore MDA does not just reflect lipid peroxidation, but is also a by product of cycloxygenase activity in platelets and persistent platelet activation is a common feature of many clinical syndromes associated with enhanced lipid peroxidation. Thus measurement of MDA level provides a convenient in-vivo index of lipid peroxidation and represents a non invasive bio-marker of oxidative stress, often clinically employed to investigate radical mediated physiological and pathological conditions.[3]

Oxidative stress is an imbalance of antioxidants and pro-oxidants in favour of pro-oxidant. When the pro-oxidant systems outbalance the antioxidant, this results in oxidative damage to lipids, proteins, carbohydrates and nucleic acids, ultimately leading to cell death. Antioxidants are substances that are capable of competing with the other oxidizable substrate and thus significantly delay or inhibit the oxidation of these substances.[4]

A small fraction of oxygen is diverted to form reactive oxygen species (ROS) either accidentally / deliberately. ROS are products of partial reduction of oxygen and are highly reactive. To protect the body from these devastating free radicals, scavenging system is present in the body in the form of antioxidants. Enzymatic antioxidants include superoxide dismutase, glutathione reductase, glutathione peroxidase, catalase, and non enzymatic antioxidants include vitamin E (alpha-tocopherol) and vitamin C (ascorbate). [4]

There is good evidence that men whose diet include a lot of calcium or those who take calcium supplements are at increased risk for Prostate Cancer. But since there is little relationship between dietary calcium and calcium in the blood, this study does not address the question of how much calcium men should eat. Often serum calcium is measured during routine blood tests. Pre-diagnostic levels of serum

calcium strongly predict risk of fatal Prostate Cancer which has important implications for clinical decision making and also for Prostate Cancer prevention. Men whose serum calcium levels fall within the high end of normal range are three times more likely to develop fatal Prostate Cancer.[5]

Thus this study was done to know the extent of oxidative stress in BPH and Prostrate Cancer by estimating MDA, the role of antioxidants namely Glutathione Reductase, Vitamin C in protecting the body from these oxidative stress and Serum Calcium in prediction of Prostate Cancer.

OBJECTIVES OF THE STUDY

1. To assess lipid peroxidation by estimating the levels of MDA and antioxidant defense, by measuring enzymatic antioxidant like Glutathione Reductase and non-enzymatic antioxidant like Vitamin C in BPH and Prostate Cancer.

2. To estimate the Serum Calcium levels in BPH and Prostate Cancer patients.

3. To compare the values of the above parameters with that of controls.

REVIEW OF LITERATURE

In ancient Greek the masculine term "prostatēs" meant "president" and was exclusively used in a non-medical sense. It was not until the Renaissance that anatomists discovered the organ naming it a "glandulous body." The term ‘prostate’ was first used by Herophilus of Alexandria in 335 B. C. to refer to the organ situated in front of the bladder.[6]

In 1600 the French physician du Laurens introduced the metaphoric denomination "prostatae." However it was only in the 1800s that this anatomical error was corrected while the grammatical one lived on.The history of the term "prostate" is a prime example of the difficulties with which the development of a precise urologic terminology had to struggle.[7]

The word cancer came from the father of medicine, Hippocrates, a Greek physician. Hippocrates used the Greek words, “carcinos” to describe tumors, thus calling cancer "karkinos." The Greek term was actually used to describe a crab, which Hippocrates thought a tumor resembled. Although Hippocrates may have named "Cancer," he was certainly not the first to discover the disease. The history of cancer actually begins much earlier. The prostate (from Greek - *prostates*, literally "one who stands before", "protector", "guardian") is a compound tubule-alveolar-exocrine gland of the male reproductive system in most mammals. In male it is the largest accessory reproductive gland which surrounds the first part of urethra, known as prostatic urethra.[8]

Gross anatomy

The mean weight of the “normal” prostate in adult men is about 11 grams (range 7–16 grams).[9] It surrounds the urethra just below the urinary bladder and can be felt during per rectal examination. It is the only exocrine organ located in the midline in humans and similar animals. The ducts are lined with transitional epithelium. Within the prostate, the urethra coming from the bladder is called the prostatic urethra and merges with the two ejaculatory ducts. The prostate is sheathed in the muscles of the pelvic floor, which contract during the ejaculatory process. The prostate can be divided in two ways: by zone, or by lobe.[10]

Zonal anatomy of prostate

The "zone" classification is more often used in pathology. The idea of "zones" was first proposed by McNeal in 1968. McNeal found that the relatively homogeneous cut surface of an adult prostate in no way resembled "lobes" and thus led to the description of "zones."[11]

Table No-1: Anatomical lobes of prostate

Anterior lobe	Roughly corresponds to part of transitional zone
Posterior lobe	Roughly corresponds to peripheral zone
Lateral lobes	Spans all zones
Median lobe	Roughly corresponds to part of central zone

Table No-2: Anatomical zones of prostate

NAME	FRACTION OF GLAND	DESCRIPTION
Peripheral zone(PZ)	65%	The sub-capsular portion of the posterior aspect of the prostate gland that surrounds the distal urethra. It is from this portion of the gland that ~70-80% of **Prostate Cancer** originate.
Central zone (CZ)	25%	This zone surrounds the ejaculatory ducts. The central zone accounts for roughly 2.5% of Prostate Cancers although these cancers tend to be more aggressive and more likely to invade the seminal vesicles.
Transition zone (TZ)	5%	~10-20% of Prostate Cancers originate in this zone. The transition zone surrounds the proximal urethra and is the region of the prostate gland that grows throughout life and is responsible for the disease of **Benign Prostate Hypertrophy.**
Anterior fibro-muscular zone (or stroma)	5%	This zone is usually devoid of glandular components, and composed only, as its name suggests, of muscle and fibrous tissue.

Fig no- 1: Anatomical zones of prostate

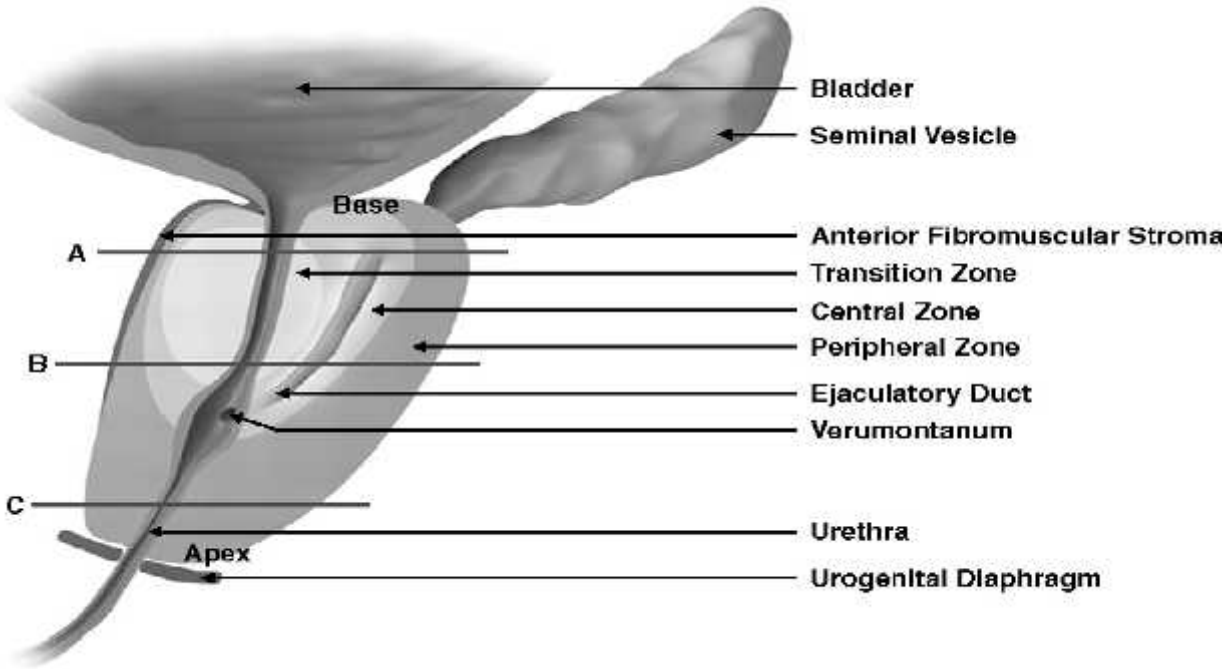
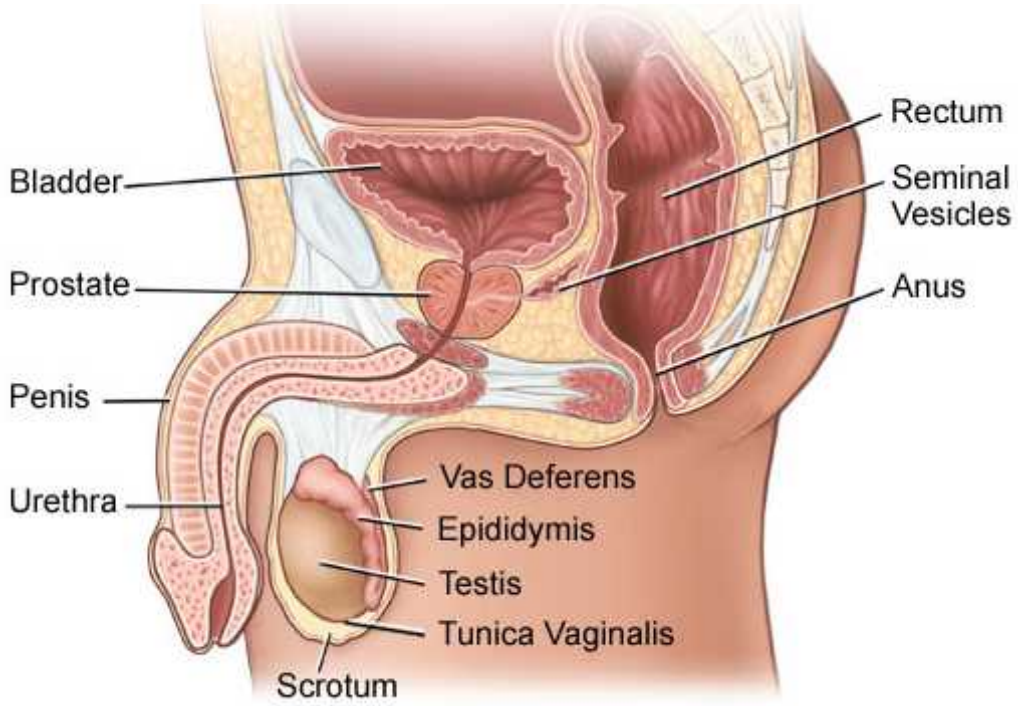


Fig no-2: Anatomical location of prostate



Secretions:

Prostatic secretions vary among species. They are generally composed of simple sugars and are often slightly alkaline. In human prostatic secretions, the protein content is less than 1% and includes proteolytic enzymes, prostatic acid phosphatase and prostate-specific antigen. The prostatic fluid is expelled in the first ejaculate fractions, together with most of the spermatozoa. In comparison with the few spermatozoa expelled together in seminal vesicular fluid, those expelled in prostatic fluid have better motility, longer survival and better protection of the genetic material (DNA). The prostate also contains some smooth muscles that help expel semen during ejaculation.The prostatic fluid is rich in inorganic cations, notably sodium, potassium and calcium but not enough inorganic anions are found to balance the cations so as to account for the observed pH of 6.45. The lactate, citrate and other organic ions make up for the anion deficiency.[12]

The functional activities of the prostate involve secretion, transport and reabsorption of a variety of materials into and out of the glandular lumen and that these activities are directly related to the basic structural organization of the gland. These functional activities are constantly occurring in the prostate even under basal (ie, non-ejaculating) conditions. Due to these functional activities, the prostatic fluid in the glandular lumen is a complex mixture of a variety of components derived, not only from the synthetic activity of the glandular epithelial cells of the gland itself, but also from the blood.[13]

Development

The prostatic part of the urethra develops from the pelvic (middle) part of the urogenital sinus (endodermal origin). Endodermal outgrowths arise from the prostatic part of the urethra and grow into the surrounding mesenchyme. The

glandular epithelium of the prostate differentiates from these endodermal cells and the associated mesenchyme differentiates into dense stroma and the smooth muscle of the prostate. The current paradigm suggests that the regional differences seen in the prostate of both animal models and humans are a consequence of specific epithelial-mesenchyme interactions along the cranial-caudal axis of the urogenital sinus.[14]

The prostate gland represent the modified wall of the proximal portion of the male urethra and arises by the 9th week of embryonic life in the development of the reproductive system. Condensation of mesenchyme, urethra and wolffian ducts give rise to the adult prostate gland, a composite organ made up of several glandular and non-glandular components tightly fused within a common capsule.[15]

FREE RADICALS

Definition:

A free radical is any species capable of independent existence that contains one or more unpaired electrons. An unpaired electron is one that is alone in an orbital.[16] The unpaired electron gives certain characteristic properties to the free radical, such as para magnetism. The chemical reactivity of free radicals is usually high. They may be positively charged, negatively charged or electrically neutral.[17] A free radical is conventionally represented by a superscript dot ($R^{\bullet}$).

Types

A compound becomes a free radical by gaining an additional electron, as in the case of reduction of molecular oxygen to superoxide anion radical ($O_2^{\bullet-}$).

- Hydroperoxyl radical ($HOO^{\bullet}$),

- Hydroxyl radical ($OH^{\bullet}$),
- Lipid peroxyl radical ($ROO^{\bullet}$).

The sequential univalent reduction steps of oxygen may be represented as, [18]

$$O_2 \xrightarrow{(+)\ e^-} O_2^{\bullet -} \xrightarrow{(+)\ e^{-},\ 2H} H_2O_2 \xrightarrow{(+)\ e^{-},\ H^+} OH^{\bullet} \xrightarrow{(+)\ e^{-},\ H^+} H_2O\ \text{-}H_2O$$

Generation & Sources of Free Radicals:

Free radicals may be formed by,

- Covalent bond cleavage.
- Loss or addition of a single electron from a normal molecule.

Oxidants related to human disease are derived from three sources.

- Exaggerated normal intracellular biologic processes.
- Release from inflammatory cells.
- Detoxification reactions - includes oxidants or induce oxidant generation within cells.[19]

Fig no-3: Sources & effects of free Radicals

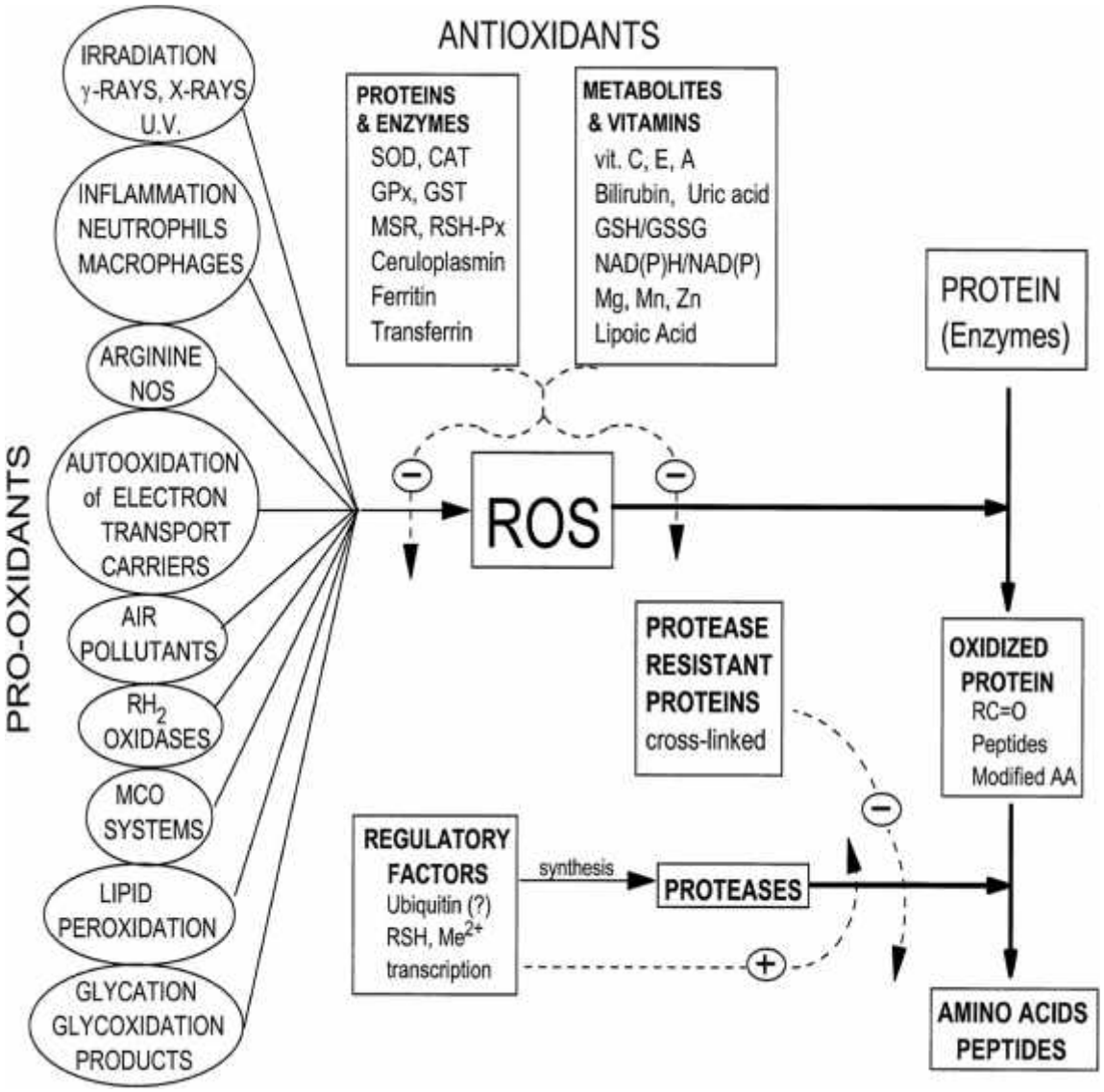
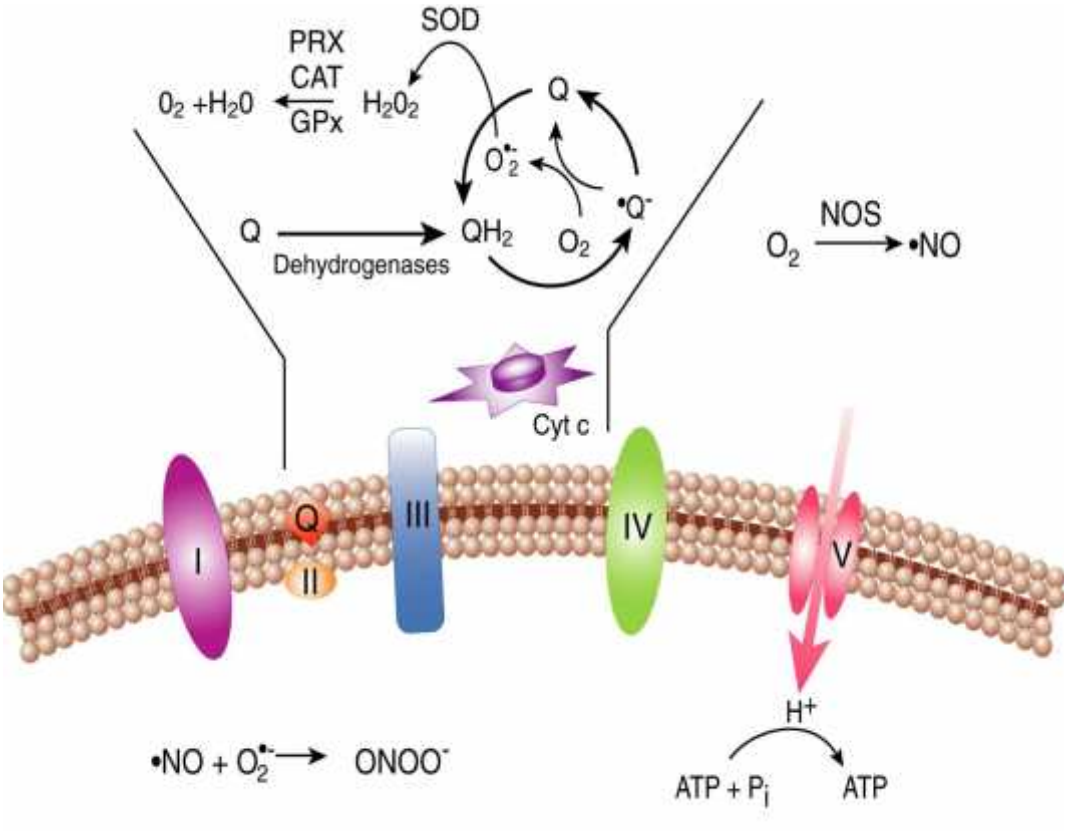


Fig No 4.: Mitochondrial Pathways of ROS Generation



i) Endogenous Sources:

a) Oxidation-reduction reactions generate free radicals constantly within the body. These can be mediated by the action of enzymes or non-enzymatically, often through the redox chemistry of transition metal ions[20].

b) Intracellular free radicals are generated from the auto oxidation and consequent inactivation of small molecules such as reduced flavins and thiols, and from the activity of certain oxidases, cycloxygenases, lipoxygenases, dehydrogenases and peroxidases.[21]

c) A variety of enzyme systems catalyze the univalent reduction of molecular oxygen to superoxide anion radical. Such univalent reduction of molecular

oxygen also occurs in-vivo in non-enzymatic electron-transfer oxidation-reduction reactions. (E.g. hydroquinone + O_2 ⟶ semiquinone + $O_2^{\bullet -}$ + H^+)

d) The generation of ROS is due to the rapid assembly and activation of a multicomponent oxidase (NADPH oxidase, also called phagocyte oxidase) which oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) and in the process, reduces oxygen to superoxide anion. In neutrophils, this rapid oxidative reaction is triggered by activating signals which is accompanied by phagocytosis, and is called the *respiratory burst* [22] (Fig No.5).

e) Transition metals such as iron and copper donate or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction ($H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH + OH^-$). Because most of the intracellular free iron is in the ferric (Fe^{3+}) state, it must be reduced to the ferrous (Fe^{2+}) form to participate in the Fenton reaction. This reduction can be enhanced by sources of iron and thus may cooperate in oxidative cell damage[23, 24].

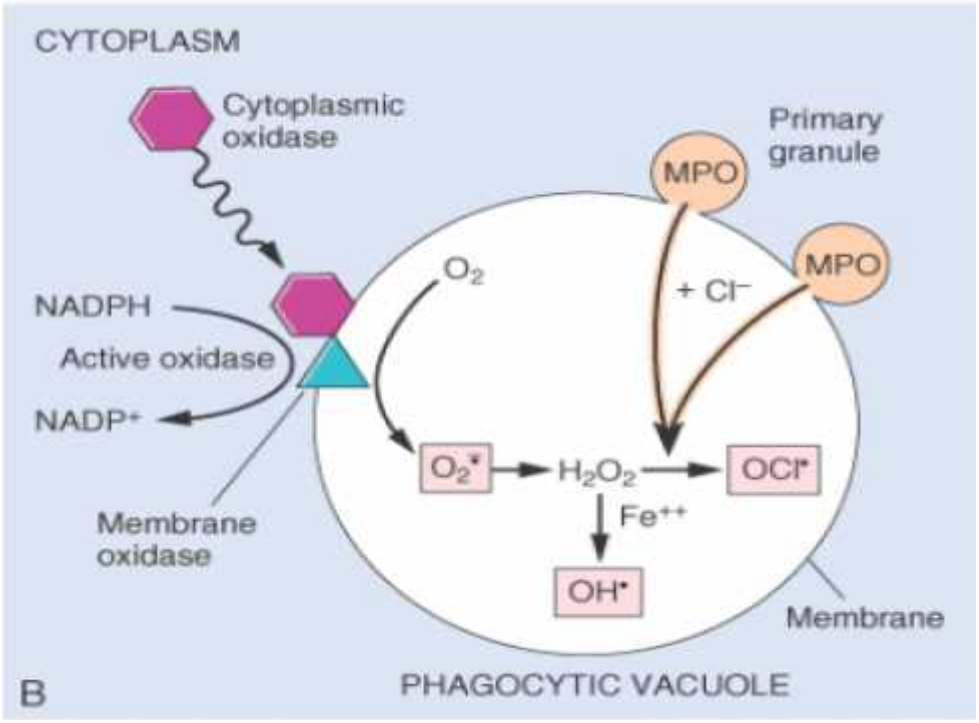
$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$ (Fenton reaction)

$O2^{\bullet -} + H_2O_2 \xrightarrow{\text{Fe salt catalyst}} O_2 + OH^{\bullet} + OH^-$.

(Haber Weiss reaction)

The chelated Fe^{3+} can be reduced to Fe^{2+} by thiols, ascorbate and most of the other reductants. Fe^{2+} can then auto-oxidize, producing $O_2^{\bullet -}$

Fig. No. 5. Generation of Oxygen Free Radicals in Respiratory Burst



ii) Exogenous Sources:

a) Carbon tetrachloride, drugs, air pollutants, anaesthetics, organic solvents, hypertoxic environments and pesticides will increase the production of free radicals[21].

b) Ionising radiation damages tissues by producing hemolytic fission of background water.

$$H_2O \longrightarrow (\text{gamma, UV radiation}) \longrightarrow H^{\bullet} + OH^{\bullet}$$

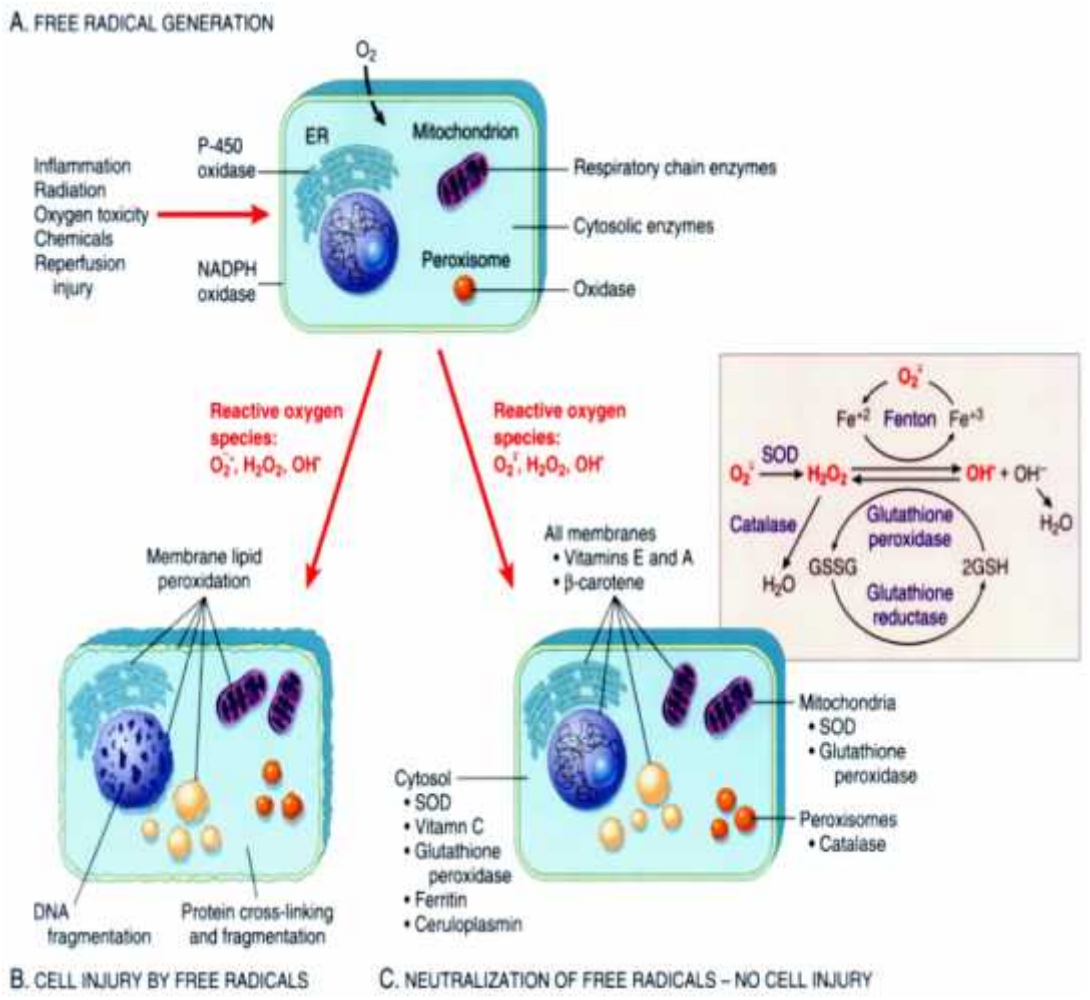
c) Light of appropriate wavelengths can cause excitation of oxygen in presence of photosensitizers to produce singlet oxygen.

Sites of Free Radical Generation:

Main sites of free radical generation are

- Mitochondria,
- Lysosomes,
- Peroxisomes,
- Nuclei,
- Endoplasmic Reticulum,
- Plasma membranes and cytosol [21].

Fig no-6: Free Radical Generation



REACTIVE OXYGEN SPECIES (ROS)

The oxygen derived free radicals and related non-radical species are collectively known as reaction oxygen species (ROS).

Superoxide Radical ($O_2^{\bullet -}$):

- This ROS is formed, when oxygen takes up one electron and leaks in the mitochondrial electron transport but its formation is easily increased when exogenous components (redox cycling compounds) are present. Its first production site is the internal mitochondrial membrane (NADH ubiquinone reductase and ubiquinone cytochrome-c-reductase).[25]
- This species is reduced and forms hydrogen peroxide (H_2O_2). The production of superoxide radicals at the membrane level (NADPH oxidase) is initiated in specialized cells with phagocytic functions (macrophages) and contributes to their bactericidal action (oxidative burst) [26].
- The flavin cytosolic enzyme xanthine oxidase found in quite all tissues and in milk fat globules generates superoxide radicals from hypoxanthine and oxygen and is supposed to be at the origin of vascular pathologies.

Hydrogen Peroxide (H_2O_2):

Hydrogen peroxide is mainly produced by enzymatic reactions. These enzymes are located in microsomes, peroxisomes and mitochondria. Even in normal conditions, the hydrogen peroxide production is relatively important and leads to a constant cellular concentration between 10^{-9} and 10^{-7} M. The natural combination of superoxide dismutase and catalase contributes to remove H_2O_2 and thus has a true cellular antioxidant activity. H_2O_2 is also able to diffuse easily through membranes.[27]

Hydroxy Radical ($OH^{\bullet}$):

In the presence of Fe^{2+}, H_2O_2 produces the very active species $OH^{\bullet}$ by the Fenton reaction.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

The iron-catalyzed decomposition of oxygen peroxide is considered the most prevalent reaction in biological systems and the source of various deleterious lipid peroxidation products.[22]

Nitric Oxide ($NO^{\bullet}$):

Nitric Oxide is produced in vascular endothelium. This species is not too reactive (poorly oxidizing function), it reacts readily with $O_2^{\bullet -}$ and gives the extremely reactive peroxynitrite ($ONOO^{-}$). This ROS is naturally formed in activated macrophages[28] and endothelial cells.[29]

Single Oxygen (O_2):

This chemical form of oxygen is not a true radical but is reported to be an important ROS in reactions related to ultraviolet exposition (UVA 320-400 nm). Several natural sensitizers are known to catalyze oxidative reactions such as tetrapyrroles (bilirubin), flavins, chlorophyll, hemoproteins and reduced pyridine nucleotides (NADH). It has been suggested that singlet oxygen may be formed during the degradation of lipid peroxides and thus may cause the production of other peroxide molecules. This singlet oxygen formation may account for the chemiluminescence observed during lipid peroxidation.[17]

Thiyl Radicals ($RS^{\bullet}$):

Thiol compounds (RSH) are frequently oxidized in the presence of iron or copper ions.

$$RSH + Cu^{2+} \longrightarrow RS^{\bullet} + Cu^{+} + H^{+}$$

These thiyl radicals have strong reactivity in combining with oxygen.[31, 32]

$$RS^{\bullet} + O_2 \longrightarrow RSO^{\bullet}_2$$

Furthermore, they are able to oxidize NADH into NAD, ascorbic acid and to generate various free radicals ($^{\bullet}OH$ and $O2^{\bullet-}$). These thiyl radicals may also be formed by hemolytic fission of disulfide bonds in proteins.

Carbon Centered Radicals:

The formation of this reactive free radical is observed in cells treated with carbon tetrachloride (CCl_4). The action of the cytochrome P_{450} systems generates the trichloromethy1 radical ($^{\bullet}CCl_3$), which is able to react with oxygen to give several peroxy1 radicals (i.e. $^{\bullet}O_2\,CCl_3$).[32]

DAMAGE PRODUCED BY FREE RADICALS

Free radicals are extremely reactive. Their mean effective radius of action is only $30A^0$. Their half-life is only a few milliseconds. When a free radical reacts with a normal compound, other free radicals are generated. This chain reaction leads to thousands of events. The peroxidation of polyunsaturated fatty acids (PUFA) severely damages the cell membrane leading to loss of membrane functions like absorption, secretion etc. Almost all biological macromolecules are damaged by the free radicals e.g.

a) Peroxidation of PUFA in plasma membranes,

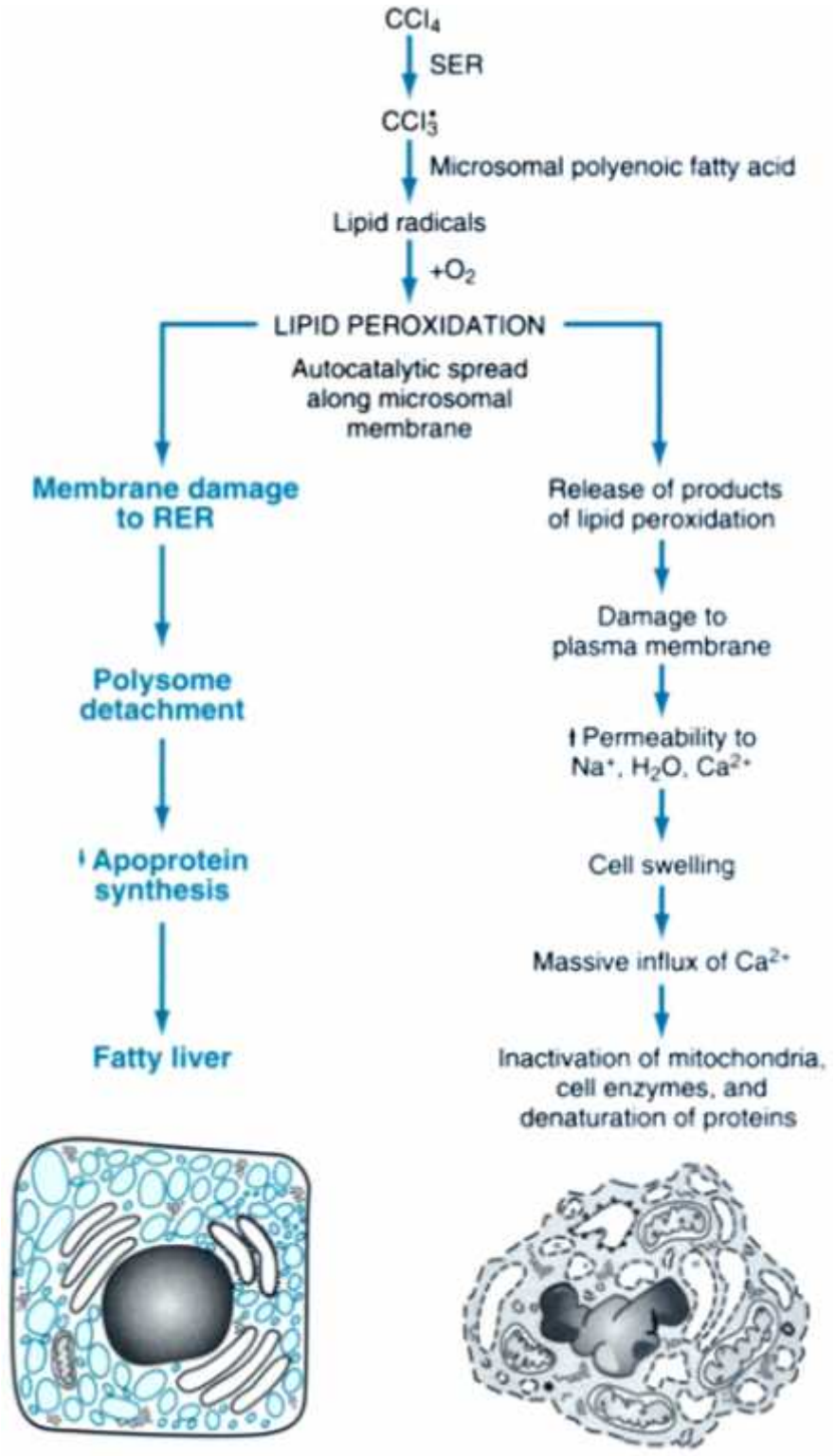
b) Oxidative inactivation of sulfhydry1 containing enzymes,

c) Polysaccharide depolymerization and DNA breaks.

d) DNA damage may directly cause inhibition of protein and enzyme synthesis, indirectly it also cause cell death or mutation and carcinogenesis.

e) Lipid peroxidation and consequent degradation product such as MDA seen in biological fluid. Their effect in the serum is often employed to assess the oxidant stress[33].

Fig No-7: Effects of Reactive Oxygen Species



Proteins:

Proteins containing tryptophan, tyrosine, phenylalanine histidine, methionine and cysteine can undergo free radical mediated amino acid modification. Free radical promotes sulfhydryl mediated cross-linking of such labile amino acids as well as cause fragmentation of polypeptide chains. Oxidative modifications enhance degradation of critical enzymes by cytosolic neutral proteases.[34] Enzymes undergo cross-linking with resulting increase in molecular weight and such enzymes cross-link with their neighbours in a random destructive reaction. The normal precise arrangement of protein and enzymes in sub-cellular membranes and organelles is badly disrupted and their biological properties are lost or impaired.[35]

Carbohydrates:

Hyaluronic acid undergoes polymer fragmentation following exposure to free radical system, which leads to destabilization of connective tissue and loss of synovial fluid viscosity. Therefore, it is not surprising that glucose and other related monosaccharides undergo oxidation when conditions are appropriate.[34]

Nucleic Acid:

DNA is readily attacked by oxidizing radicals if they are formed in its vicinity and has been clearly demonstrated by radiation biologists. It must therefore be considered as a vulnerable and important target. Cell mutation and death from ionizing radiation is primarily due to free radical reactions with DNA. Mutations and cell death resulting from free radicals generated during normal metabolism have also been ascribed to reactions with DNA[36].

Lipids:

All of the major classes of biomolecule may be attacked by free radicals but lipids are probably the most susceptible.[37] Cell membranes are rich sources of polyunsaturated fatty acids. Biomembrane and organelles are the major sites of lipid peroxidation damage. Major constituents of biological membranes are lipids and proteins. Lipid peroxidation can damage membrane proteins as well as lipids.[38] The membrane fluidity is due to the presence of polyunsaturated fatty acid side chain in many membrane lipids which lower the melting point of the interior membrane. Lipid peroxidation decreases membrane fluidity. Conditions which favour lipid peroxidation are:

i) A high degree of unsaturation in the lipid substrate.

ii) A rich supply of oxygen and

iii) The presence of transitional metal catalysts.[39]

LIPID PEROXIDATION

Lipid peroxidation is defined as "oxidative deterioration of polyunsaturated lipids". Lipid peroxidation is particularly damaging because it proceeds as a self perpetuating chain reaction.[40]

Fig. No-8: The lipid Peroxidation process

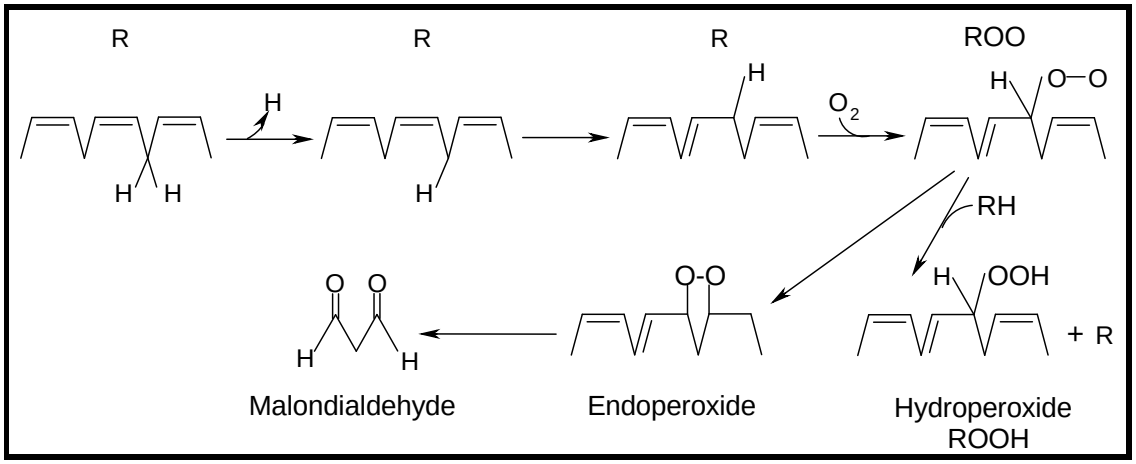
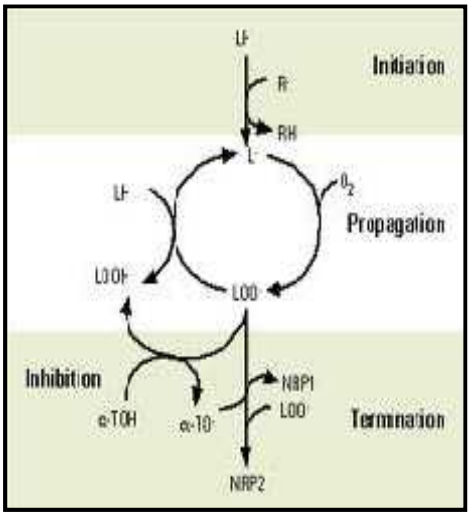


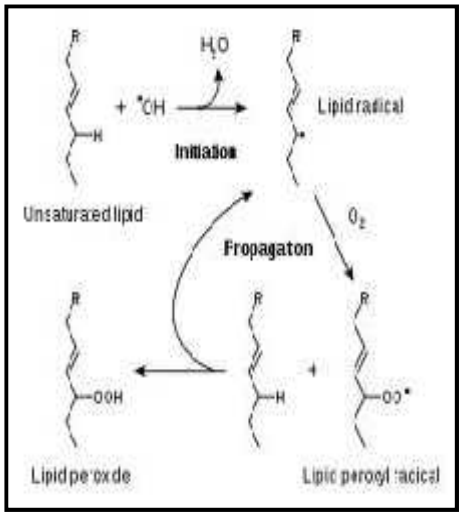
Figure 8 : Illustrate lipid peroxidation. The reaction is initiated by an existing free radical ($X^{\bullet}$) by light or by metal ions. Malondialdehyde is only formed by fatty acids with three or more double bonds, and is used as a measure of lipid peroxidation together with ethane from the terminal two carbon of ω3 fatty acids and pentane from the terminal five carbon of ω6 fatty acids.[41]

Peroxidation of polyunsaturated fatty acids usually involves three operationally defined processes.[38]

Fig no.-9 (a & b): Phases of Lipid Peroxidation Process



(a)



(b)

Initiation Phase:

During this phase, the primary event is the abstraction of hydrogen atom from bisallylic site of PUFA. Initiation of a peroxidation sequence in membrane or PUFA[29] is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom from a methylene (CH_2) group. This leaves behind an unpaired electron on the carbon, -CH-. The carbon radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which then easily reacts with an oxygen molecule to give peroxy radical, R-OO$^{\bullet}$. The presence of the redox active metals such as iron or copper can facilitate the initiation process.[38]

$$ROOH + Metal\ (n)^{+} \longrightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$$

Propagation Phase:

During this phase lipid peroxidation relies on the interaction of molecular oxygen with carbon-centered free radicals to form lipid hydroperoxides.[34] The peroxy

radical abstract a hydrogen atom from another lipid molecule and once the process begins it tends to continue. The peroxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxides R-OOH. A probable alternative fate of peroxy radicals is to form cyclic peroxides. With the help of metal catalysts, the decomposition of hydroperoxides results in the formation of alkoxyl or peroxyl radicals. These radicals are capable of further reactions and thus the propagation of lipid peroxidation continues.[34, 38]

$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$ etc

Termination Phase:

The propagation reactions of lipid peroxidation will not proceed very far before they meet a protein molecule, which can then be attacked and damaged, in addition aldehyde can attack amino groups on the protein molecule to form both intramolecular cross links and also cross links between different protein molecules. eg. malondialdehyde.

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow RO\text{-}OR + O_2$

$ROO^{\bullet} + R \longrightarrow ROOR$

$R^{\bullet} + R^{\bullet} \longrightarrow RR$

TOXIC EFFECTS OF LIPID PEROXIDATION

The uncontrolled peroxidation of bio-membranes can lead to profound effects on membrane structure and function and may be sufficient to cause cell death. The toxic products generated during lipid peroxidation may be involved in damage of specific protein and transport systems critical to cell function.[42] Malondialdehyde

produced by lipid peroxidation can cause cross linking and polymerization of membrane components. This can alter the intrinsic membrane properties such as ion transport and enzyme activity. Because malondialdehyde is diffusible, it will also react with nitrogenous bases of DNA[43] and lipid hydroperoxides can directly inhibit enzymes.[42]

ANTIOXIDANT DEFENSE SYSTEMS

Detoxification of reactive oxygen species is one of the prerequisite of aerobic life. Many defense systems have evolved by providing an important antioxidant defense system for prevention, interception and repair, consisting of non-enzymatic and enzymatic scavengers and quenchers (Fig. No. 10).[44]

Prevention:

The important function of these antioxidant defenses is to prevent the generation of free radicals. Antioxidants defense remove peroxides that are reacting with transition metal ions to produce reactive free radicals. These peroxides include both hydrogen peroxide and also the lipid hydroperoxides, which are produced during lipid peroxidation. Catalase and glutathione peroxidases are the enzymes, whose role is to safely decompose peroxides.[38] Transferrin and iron binding protein lactoferrin also function as preventive antioxidant by binding iron and stopping it from participating in radical reactions. Uric acid, albumin, haptoglobin and hemopexin have also been shown to inhibit various free radical reactions.[45,46] Cerulloplasmin is an important extracellular antioxidant [42] which oxidizes Fe^{2+} to Fe^{3+} that swiftly binds to transferrin and any iron mobilized from serum ferritin.[48]

Interception:

The free radical scavenging enzyme is superoxide dismutase whose substrate is a free radical; other scavengers include lipid soluble vitamin E, vitamin A, ubiquinol and the aqueous phase compounds such as free radical scavengers like ascorbic acid glutathione, uric acid etc.[23]

Repair:

Another category of natural antioxidant defense is repair process, which removes damaged biomolecule before their presence, which alter cell metabolism or viability. Specific enzymes repair oxidatively damaged nucleic acids. Oxidized proteins are removed by proteolytic systems and oxidized membrane lipids acted upon by lipases, peroxidases and acyl transferases. Antioxidants oppose the toxic effect of lipid peroxides and oxygen radicals, and they limit the amount of lipid peroxides that are formed.[49]

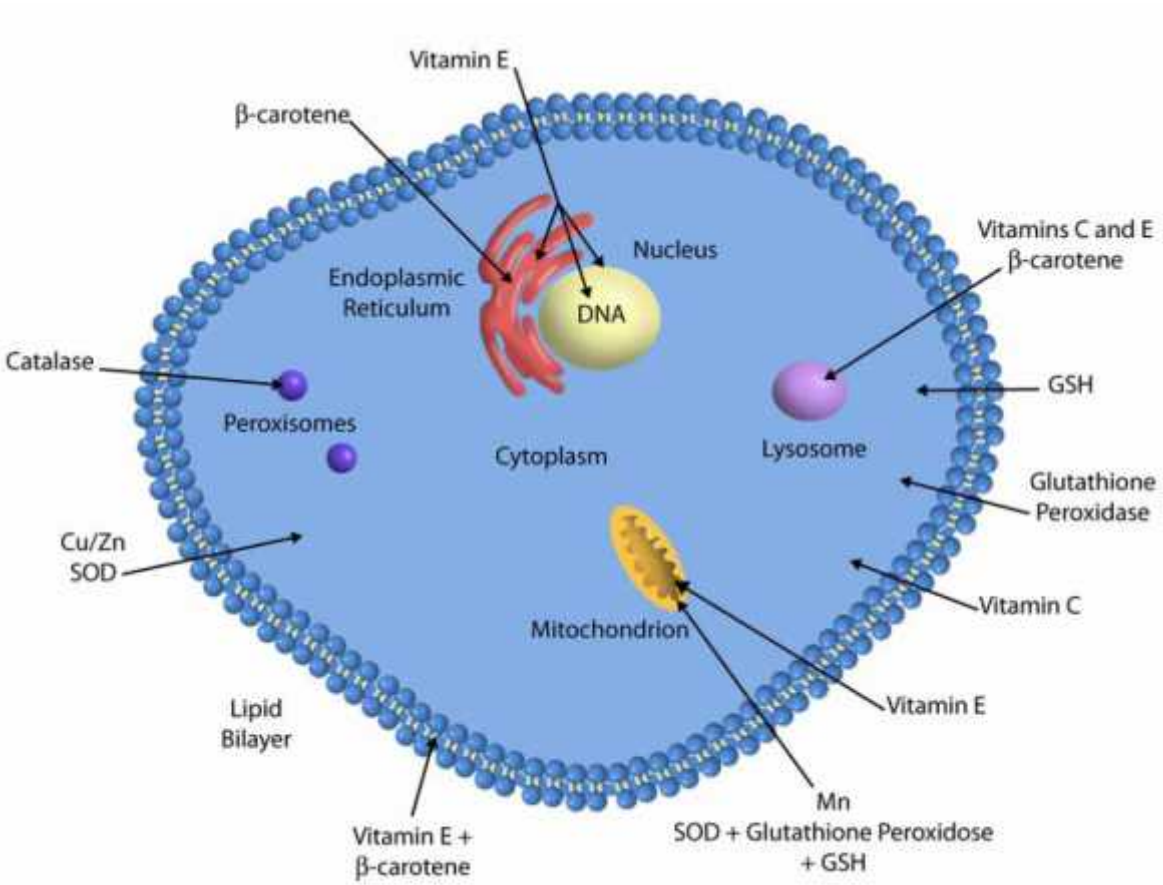
Antioxidants can be classified depending on the mechanism of action

- Preventive : superoxide dismutase, catalase and glutathione peroxidase
- Chain breaking : alpha tocopherol, ubiquinone, beta-carotene, bilirubin and water-soluble substances such as ascorbate, GSH and urate.

The defense of living eukaryotic cells against the damage caused by the activated oxygen is a complex process which involves a interrelated protective agencies for activated oxygen targets like DNA, proteins and polyunsaturated fatty acids. Peroxidation of polyunsaturated fatty acids will result in a disruption of membrane. So, the antioxidants lie functionally at the heart of this protective mechanism.

Intracellular defense system is largely dependent on the antioxidant enzymes such as GSH-Reductase where riboflavin functions as an antioxidant cofactor (FAD).

Fig No-10: Antioxidant protection within the cell



ENZYMATIC ANTIOXIDANT

GLUTATHIONE REDUCTASE

Glutathione reductase is present in the liver, kidney, pancreas, heart, thyroid and erythrocytes. It has a molecular weight of 44000. It catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of reduced coenzyme II ($NADPH_2$)

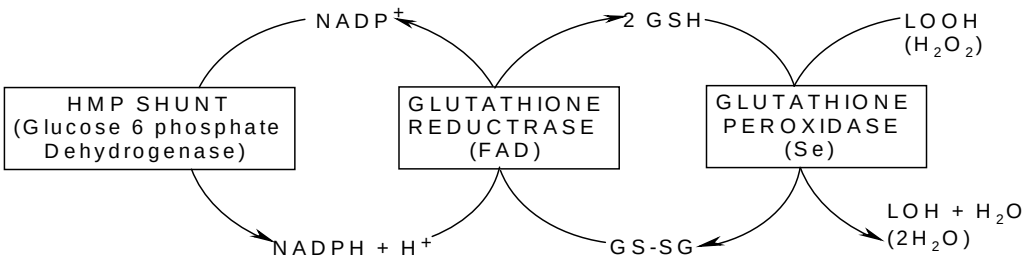
Glutathione reductase is NADPH dependent and in erythrocytes the main source of NADPH2 is from Hexose monophosphate shunt activity.[49] The ratios of GSH/GSSG in normal cells are kept high so there must be a mechanism for reducing GSSG back to GSH, which is achieved by glutathione reductase.

Glutathione reductase contains the FAD at its active site. NADPH reduces the FAD, which then passes its electrons on to a disulfide bridge (S-S) between two cysteine residues in the protein. The two-sulphydryl groups so formed then interact with GSSG and reduce it to 2GSH, reforming the protein disulfides.[38] Glutathione reductase is present in two forms.

1. An active form associated with FAD
2. An inactive form not bound to FAD.

Reduction of GSSG to GSH mediated by glutathione reductase whose substrate is NADPH and Glucose -6-Phosphate dehydrogenase as a source of NADPH guarantees the reduction of GSSG and maintenance of constant pool of GSH.

Fig no-11: Glutathione system and its components



VITAMIN-C (ASCORBIC ACID)

Vitamin-C or Ascorbic Acid is a water-soluble non-enzymatic antioxidant. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with superoxide and hydroxyl radical and various lipid hydroperoxides. In addition it can restore the antioxidant properties of oxidized vitamin-E, suggesting that a major function of vitamin-C is to recycle the vitamin-E radicals. Ascorbic acid is widely distributed in mammalian tissues, but it is present in relatively high amounts in the adrenal and pituitary glands, lesser amounts are found in the liver, spleen, pancreas and brain. Ascorbic acid serves as both an antioxidant and a pro-oxidant. As an antioxidant, vitamin-C exerts a sparing effect on the antioxidant actions of vitamin-E and selenium. On the other hand, excess amount (≅ mM) may act as a prooxidant in the presence of transition metals Fe^{3+} or Cu^{2+}. Many studies have shown that ascorbate's pro-oxidant action, which induces lipid peroxidation, resides in its ability to reduce Fe^{3+} to the Fe^{2+} state. Fe^{2+} is known to be a potent free radical inducer.[34]

Ascorbic acid appears to trap virtually all peroxyl radicals in the aqueous phase before they can diffuse into the plasma lipids. The ability of ascorbate to show antioxidant properties is related to its fast reaction with many reactive oxygen species (peroxyl radicals) and to the fact that the resulting semidehydroascorbate radical is

poorly reactive. Enzymatic systems exist invivo to reduce semidehydroascorbate back to ascorbate at the expense of NADH (NADH-semidehydroascorbate reductase enzyme).[51]

BENIGN PROSTATE HYPERTROPHY.

Incidence and prevalence.

BPH is the most common urological problem of ageing men, manifested as severe obstruction in urinary flow with discomfort and pain. BPH is a complex disease from the etiological and pathogenesis point of view.[47] A recent AUA guideline suggests an increase in the incidence of BPH worldwide and predicts that by the age of 60 years, more than 50% of men will have microscopic evidence of the disease and by the age of 85 years, as many as 90% of men will be affected.[53]

RISK FACTORS

Senescence

Studies have demonstrated that the initial development of BPH begins as early as 25-30 years of age. With advancing age the prevalence of histologically identifiable BPH increases progressively such that by sixth decade the prevalence is more than 50% and by 85 years the prevalence will be 90%. Thus these findings clearly demonstrate that BPH occurs very early in life and that nearly all men will develop microscopic BPH if they live long enough.[54]

Role of MDA.

Programmed death of cells by apoptosis is a protective mechanism of the organism against accumulation and spread of defective cells. A decreased susceptibility of senescent cells in vitro and in vivo to apoptosis by oxidative stress has been observed. Highly reactive aldehydes (e.g. 4-hydroxynonenal, MDA), products of the lipid peroxidation, are capable of modifying both DNA and proteins,

resulting in mutagenic, genotoxic and cytotoxic events. Therefore, high levels of MDA, such as of other reactive aldehydes, may explain DNA base modifications demonstrated not only in Prostate Cancer, but also in BPH epithelium.[55]

Role of androgens

It is also known that prostatic levels of dihydrotestosterone (DHT) as well as the androgen receptor (AR) remain high with aging despite the fact that peripheral levels of testosterone are decreasing. Moreover, androgen withdrawal leads to partial involution of established BPH. Although androgens do not cause BPH, the development of BPH requires the presence of testicular androgens during prostate development, puberty and aging. Patients castrated prior to puberty or who are affected by a variety of genetic diseases that impair androgen action or production do not develop BPH.[56]

Role of Growth Factors

Growth factors are small peptide molecules that stimulate, or in some cases inhibit, cell division and differentiation processes. Cells that respond to growth factors have on their surface receptors specific for that growth factor that in turn are linked to a variety of transmembrane and intracellular signaling mechanisms. Interactions between growth factors and steroid hormones may alter the balance of cell proliferation versus cell death to produce BPH and Prostate Cancer.[57]

The Potential Role of Inflammatory Pathways and Cytokines

In the last 5 years, specific inflammatory mediator pathways have been studied in detail to elucidate the potential role of these pathways in BPH pathogenesis. A large number of cytokines and their receptors are seen in BPH tissue. Specifically, significant levels of IL-2, IL-4, IL-7, IL-17, interferon γ (IFN-γ), and their relevant receptors are found in BPH tissue. IL-2, IL-7, and IFN-γ stimulate the proliferation of

prostatic stromal cells in vitro. Prostatic epithelial cell senescence results in increased expression of IL-8, which can promote proliferation of nonsenescent epithelial and stromal cells .Macrophage inhibitory cytokine 1 is expressed in normal prostate tissue but significantly downregulated in BPH. Chronic inflammation in BPH is also associated with focal upregulation of cyclooxygenase 2 (COX-2) in the glandular epithelium To date, however, no firm cause-and-effect relationships have been established between prostatic inflammation and related cytokine pathways and stromal-epithelial hypertrophy.[58]

Serum Vitamin D and Calcium

1α, 25-dihydroxyvitamin D3 (1,25-VD) has been known to reduce the incidence of colon, breast and Prostate Cancers in human as well as in chemically induced animal cancer models. Much research has focused on vitamin D receptor (VDR)-mediated antiproliferative effects of 1,25-VD, the active metabolite of vitamin D. In general, VDR binds to vitamin D response elements (VDREs), and regulates target genes' expression to inhibit cancer cells proliferation. However, little information is available on the antioxidant property of 1,25-VD. Gene expression profiling revealed that 1,25-VD and its analogs induced several genes controlling redox balance, including G6PD, glutathione peroxidase (GPx).[59]

Since vitamin D has important role in maintaining serum calcium, so the levels of serum calcium was estimated to know its indirect role in the disease process of BPH.

Oxidant and Anti-oxidants in BPH

Malondialdehyde is also a second product of lipid peroxidase and is used as an indicator of free radical tissue damage. Plasma levels of Vitamin E and Vitamin C

were lower than the healthy controls. The decrease in the levels suggested an increased defence mechanism playing against pro-oxidants. These two vitaminic antioxidants act in synergism in the membrane and cytosol of the cell. Vitamin E scavenges lipid peroxy free radicals and interrupts chain reaction of lipid peroxidation becoming oxidised itself in the process. Ascorbate present in the aqueous compartments for example cytosol, plasma and other body fluids acts as a water soluble chain breaking antioxidants, converts the locoperoxyl radical back into alpha-tocopherol that is vitamin E, thereby replenishing antioxidant activity of alpha-tocopherol. In addition it has been suggested that antioxidants have protective role against BPH as well as progressive Prostate Cancer.[60]

PROSTATE CANCER

Incidence and Mortality Trends

Prostate Cancer has been the most common visceral malignant neoplasm in U.S. men since 1984, now accounting for one third of all such cancers. The estimated lifetime risk of disease is 17.6% for whites and 20.6% for African Americans, with a lifetime risk of death of 2.8% and 4.7%, respectively The incidence of Prostate Cancer peaked in 1992, approximately 5 years after introduction of prostate-specific antigen (PSA) as a screening test; it fell precipitously until 1995 and has been rising slowly since, at a slope similar to that observed before the PSA era .The fall in incidence between 1992 and 1995 has been attributed to the "cull effect" of identifying previously unknown cancers in the population by the use of PSA, followed by a return to baseline, when fewer cases were detected in previously screened individuals. For 2005, the American Cancer Society estimates 232,090 new cases of Prostate Cancer in the United States. Before reliable data were available from African countries, rates of Prostate Cancer in Africa were thought to be much

the same as those in Asia. However in Uganda and Nigeria, Prostate Cancer is common and it is the most common cancer in men in Nigeria.[61]

Prostate Cancer is the second most common male malignant neoplasm worldwide. Its incidence varies widely between countries and ethnic populations, and disease rates differ by more than 100-fold between populations. The average age adjusted incidence rates for Prostate Cancer in India are ranged from 6.98/100,000 in Delhi. At Mumbai the age adjusted incidence rate was 5.2/100,000. At Bangalore the incidence was 5.6/100,000, and in Chennai 4.1/100,000.[62]

Age at Diagnosis

Prostate Cancer is rarely diagnosed in men younger than 50 years, accounting for less than 0.1% of all patients. Peak incidence occurs between the ages of 70 and 74 years, with 85% diagnosed after the age of 65 years .At 85 years of age, the cumulative risk of clinically diagnosed Prostate Cancer ranges from 0.5% to 20% worldwide, despite autopsy evidence of microscopic lesions in approximately 30% of men in the fourth decade, 50% of men in the sixth decade, and more than 75% of men older than 85 years. PSA-based screening has induced an important age migration effect; the incidence of Prostate Cancer in men 50 to 59 years has increased by 50% between 1989 and 1992 with important implications for deciding on the need for, type of, and complications after therapy.[63, 64]

RISK FACTORS

Genetic factors

Extensive efforts have been made to identify genetic susceptibility loci for Prostate Cancer, both through analyses of hereditary factors associated with familial risk of early-onset disease, and more recently through genome-wide association studies. In particular, Prostate Cancer susceptibility loci associated with HPC have

been mapped a gene of uncertain function. Additionally genome-wide association studies have identified numerous Single Nucleotide Polymorphisms (SNPs) that are associated with cancer risk .[65,66] In particular, a major locus identified in these genome-wide association studies is identified by multiple sequence polymorphisms at 8q24, proximal to MYC. Disappointingly, however, many of the other loci identified in genome-wide association studies have not been easily replicated in other population-based studies, including analyses of groups with high-risk for Prostate Cancer, such as African-Americans.[67]

Inflammation.

Inflammatory cells, particularly macrophages that are attracted to the site of inflammation can be found in the Expressed Prostatic Secretions (EPS) characterizing Chronic Prostatitis. Non-specific immune defense, mediated by inflammatory cells, activated as a result of chronic prostatitis has been labelled as the primary cause for a rapid increase in the amount of hydroxyl radicals, superoxides and peroxides in prostate tissue.[68] The continual exposure of prostate tissue to the source of inflammation can lead to a dramatic increase in ROS, causing changes in protein structure and function, somatic genetic alterations and post translational DNA modifications.[69] These changes can lead to further tissue damage resulting in enhanced epithelial cell proliferation to compensate for the tissue damage and can therefore induce prostatic neoplasia.[70]

Telomere Shortening

Another event that has been implicated in Prostate Cancer initiation is the shortening of telomeres, which is generally associated with DNA damage and may lead to chromosomal instability.[71] Telomere length has been correlated with disease

outcome, while prostate carcinomas as well as many highgrade PINs display increased telomerase activity, which is not observed in benign prostate tissue.[72]

Senescence

Cellular senescence corresponds to a form of cell cycle arrest in which cells remain fully viable, but are non-proliferative despite exposure to mitogenic signals. Much recent work has identified cellular senescence as a potent mechanism of tumor suppression that prevents manifestation of the malignant phenotype after oncogenic insults. In particular, activated oncogenes are believed to induce senescence through a variety of molecular mechanisms, including replicative stress or formation of ROS, or as a response to DNA damage. Thus, oncogene-induced senescence may play a central role in preventing the progression of preneoplastic lesions to the fully malignant state.[73]

Serum Calcium

Several epidemiologic studies have investigated the role of dietary calcium in Prostate Cancer, with mixed results.[74, 75, 76] Calcium levels in serum are tightly controlled over a wide range of dietary calcium and generally are not correlated with dietary calcium levels. But some studies have shown an increased risk for advanced or for fatal Prostate Cancer among men whose diets are unusually high in calcium.[77]

Both normal and cancerous prostate cells posses the calcium-sensing receptor, a G-protein coupled receptor that is activated by extracellular calcium. Prostate cells also express calcium-dependent channels that regulate cell proliferation via the control of calcium entry into the cells.[78] Levels of calcium in serum were significantly correlated with serum levels of free PSA. It is unlikely that serum PSA acts to increase serum calcium, whose serum levels are under strict homeostatic control.[79]

Specifically, if serum levels of PSA increased serum levels of calcium then men with advanced Prostate Cancer would develop hypercalcemia. However, the prevalence of hypercalcemia in Prostate Cancer is very low.[80] Rather it was hypothesized that calcium levels in serum promote growth in normal prostate cells, akin to the known effects of calcium on cancerous prostate cells. A role for calcium in Prostate Cancer is supported by several observations. For example, several epidemiologic studies have shown an increased risk for advanced or for fatal Prostate Cancer among men whose diets are unusually high in calcium.[77]

Similarly, two prospective studies in men without clinical Prostate Cancer have shown that high levels of calcium in serum were associated with a significantly increased risk of fatal Prostate Cancer.[81, 82]

Each individual is believed to have his or her own set point for serum calcium that is under genetic control.[83] The concentration of ionized serum calcium in a given individual normally does not deviate by >2% from its set point. Conversely, there is considerable variation in calcium levels between individuals, with normal levels of total serum calcium ranging from 8.5 to 10.5 mg/dL. Measurement of calcium in serum preceded death from Prostate Cancer by an average of 5.3 years (SD 2.5). Because the latency between blood draw and death was relatively short, the presence of occult Prostate Cancer could have influenced the levels of calcium in serum ('reverse causality'). Compared with men in the lowest tertile of ionized serum calcium, the relative hazard for fatal Prostate Cancer for men in the middle tertile was 2.58 (95% CI, 0.66-10.02) and for men in the highest tertile was 4.65 (95% CI, 1.40-15.49). Thus reverse causality is an unlikely explanation for the positive association between ionized serum calcium and Prostate Cancer mortality. [84]

Thus it was hypothesized that calcium intakes that exceed the recommended allowance may increase the risk of fatal Prostate Cancer because these intakes may increase the levels of calcium in serum. While some large cohort studies (with >500 cases) have reported no significant positive associations between dairy products and/or calcium intake and risk of Prostate Cancer.[77]

A recent clinical trial of calcium supplementation ($1200\,mg\,day^{-1}$) in the prevention of colorectal adenomas found no significant increase in Prostate Cancer (70 cases) risk with supplementation in secondary analyses, and even a suggestion of a protective effect.[85]

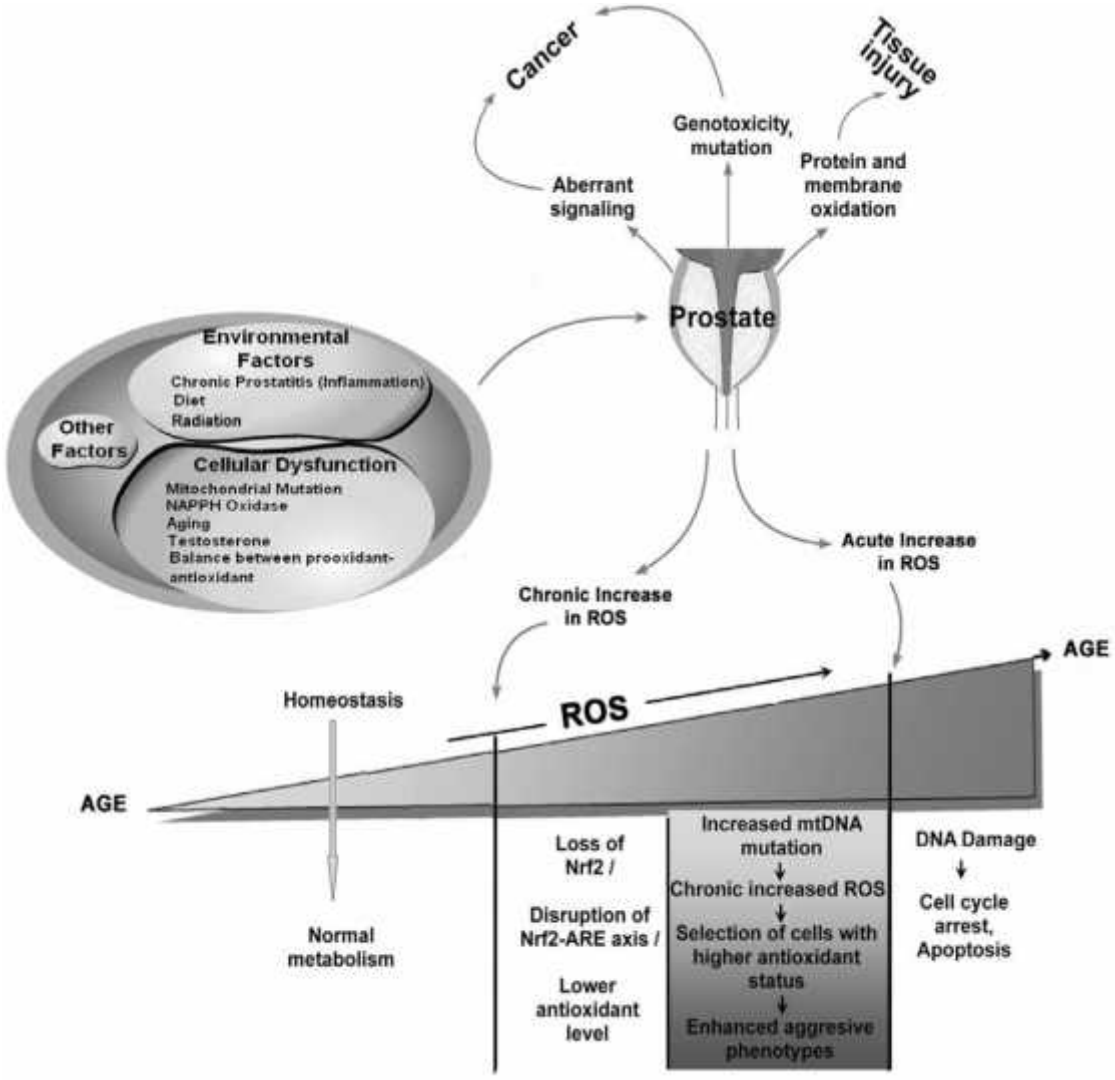
A 2005 meta-analysis of prospective cohort studies reported increased RR of 1.11 (95% confidence interval, 1.00–1.22) and 1.39 (1.09–1.77) for all Prostate Cancer among men with the highest intakes of dairy products and calcium, respectively, and stronger associations for advanced Prostate Cancers (RR=1.33 and 1.46, respectively). The team analyzed data on 2814 men who had participated in the National Health and Nutrition Examination Survey (NHANES-1) and found 85 cases of Prostate Cancer and 25 deaths from Prostate Cancer. The team also looked at serum calcium levels in blood samples taken at an average of 9.9 years before Prostate Cancer was diagnosed and found that men with higher levels had a significantly higher risk for fatal Prostate Cancer. These men had calcium levels in the range of 9.9 to 10.5 mg/dL, which the researchers described as the "high end of the normal range."[85]

OXIDATIVE STRESS AND DNA DAMAGE

Oxidative stress results from the imbalance of reactive oxygen species (ROS) and detoxifying enzymes that control cellular levels of ROS, which leads to cumulative damage to lipids, proteins, and DNA. Several lines of evidence have

suggested that one of the major aging-associated influences on prostate carcinogenesis is oxidative stress and its cumulative impact on DNA damage.[87] .

Fig No.12: Effects of Reactive Oxygen Species on Prostate



ROLE OF ROS IN PROSTATE CANCER

Prostate Cancer cells that proliferate in the absence of androgens typically have an aggressive phenotype, though multiple factors and signalling pathways have been implicated in the development of aggressive Prostate Cancer.[88] Prostate Cancer is mainly a disease of aging, with most cases occurring in men over the age of 55. Therefore, progressive inherent or acquired changes in cellular metabolism occurring over the years may play a very important role in the development of this disease. Many factors like diet, environmental carcinogens, and other inflammatory diseases have been linked to an increased risk of Prostate Cancer. Hydroxyl radicals, peroxides and superoxides are ROS that are generated during everyday metabolic processes in a normal cell. ROS, generated either endogenously (mitochondria, metabolic process, inflammation etc.) or from external sources[89], play a vital role in regulating several biologic phenomena. While increased ROS generation has traditionally been associated with tissue injury or DNA damage which are general manifestations of pathological conditions associated with infection, aging, mitochondrial DNA mutations and cellular proliferation; new and exciting information points to an essential role for increased ROS generation in several cellular processes associated with neoplastic transformation and aberrant growth and proliferation.[90,91]

Processes associated with proliferation, apoptosis, and senescence may be a result of the activation of signalling pathways in response to intracellular changes in ROS levels. Thus, excessive production of ROS or inadequacy in a normal cell's antioxidant defense system (or both) can cause the cell to experience oxidative stress and the increased ROS may play a broader role in cellular processes associated

with initiation and development of many cancers including Prostate Cancer. Over the last decade association between Prostate Cancer risk and oxidative stress has been recognized, and epidemiological, experimental and clinical studies have unequivocally proven a role for oxidative stress in the development and progression of this disease. Differences in Prostate Cancer incidence among various races, environment, diet, life style, genetic constitution and hormone of an individual/community are some of the contributing risk factors for occurrence of Prostate Cancer.[92, 93] Though recent studies have indicated that oxidative stress is higher in the epithelium of Prostate Cancer patients than men without the disease, the association of ROS mediated oxidative stress and Prostate Cancer risk remains to be elucidated. Theories abound regarding their role in initiation of Prostate Cancer, and include but are not limited to, failure of antioxidant defense mechanism (due to persistent oxidative stress that leads to inherited and acquired defects in the defense system), mtDNA mutations, chronic inflammation, defective DNA repair mechanism and apoptosis etc., finally leading to the development of Prostate Cancer. Thus, many of the factors that are associated with Prostate Cancer like aging, imbalance of androgens, antioxidant system, dietary fat and pre malignant conditions like high grade Prostate Intraepithelial Neoplasia. may be linked to oxidative stress. In recent years several anti-oxidant trails have been conducted against Prostate Cancer, but the usefulness of such therapies needs extensive research before put into practice.[94]

Oxidants and Antioxidants in Prostate Cancer

Prostate Cancer is commonly associated with a shift in the antioxidant-pro-oxidant balance towards increased oxidative stress. Previous studies highlighted the altered pro-oxidant and antioxidant status in prostatic tissue of man, rat and also in cell lines, where the imbalance between these antagonist played a major role in the

initiation of prostate carcinogenesis.[95] However, there is very little idea about the cause of this imbalance. Androgens are considered to be the most powerful candidates that regulate ROS balance in the prostate, though the mechanistic relation between androgen status and redox homeostasis in the prostate is not proven.[96]

Tam et al.[97] in this context indicated that replacement of androgens reduced the oxidative stress level by down-regulating NADPH Oxidase (Nox) expression, thereby bringing the antioxidant level to normalcy. Besides androgen, the transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) mediates the expression of key protective enzymes through the antioxidant-response element (ARE) in Prostate Cancer.[98, 99] Recent studies suggested that Nrf2 and several of its target genes are significantly down regulated in human Prostate Cancer and as a result, cells were continually exposed to increased oxidative stress and may have resulted in their progression to metastatic disease.[100]

Another major component involved in the maintenance of redox balance in the cell is the Glutathione oxidation-reduction system. Somatic mutations, causing inactivation of the Glutathione S Transferase gene (GSTP1) have been identified in almost all the Prostate Cancer cases examined by Nelson and colleagues.[87]

Therefore, the sensitive balance between the oxidant and antioxidant components of the cells and their regulatory mechanisms seem to play a major role in developing a malignant state in prostate tissue.

MATERIALS AND METHODS

SOURCE OF THE DATA

The present study comprises of 30 clinically diagnosed cases of BPH and Prostate Cancer respectively. The control group consists of equal number of age and sex matched individuals.

STUDY DESIGN:

Cross sectional study.

STUDY PERIOD

Period of one year from January 2011 to December 2011.

SAMPLE SIZE

On an average 35 patients of Prostate Cancer were admitted in the KLE hospital every year in the last consecutive 3 years. For statistically significant results, minimum sample size taken was 30. Hence the sample size for this study was 30 cases of BPH and Prostate Cancer with equal age matched controls.

STUDY POPULATION.

30 clinically diagnosed cases of BPH and Prostate Cancer respectively, admitted or attending urology unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria

1. Clinically and diagnostically confirmed cases of BPH and Prostate Cancer.
2. Following parameters will be considered for inclusion in the present study.
    - 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 and more than 10ng/mL in Prostate Cancer.

Exclusion Criteria

1. Hepatocellular damage
2. Renal failure
3. Diabetes mellitus
4. Chronic smokers
5. Chronic alcoholics
6. Other systemic disorders

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.

2. Head of Department, Urology unit, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study. (Annexure I)

SCHEDULING:

This study was carried out for a period of one year. It was undertaken during January 2011 to December 2011.

PATIENT INFORMATION

A structured proforma was used to collect socio demographic and clinical information about the study participants. (Annexure II)

COLLECTION OF SAMPLE

Patient preparation:

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 preparing hemolysate and 6 ml in plain tubes, centrifuged, serum separated and kept at 4^0C which was analysed within 24 hours using colorimeter.

Methods of assay

Blood

- Malondialdehyde – Thiobarbituric acid method.[101]

Hemolysate

- Glutathione Reductase – Beutler E method.[102]

Serum

- Vitamin C – Evelyn and Melloy method.[103]
- Serum calcium – Modification of O-Cresolphthalien complexone method.[104]

ESTIMATION OF MALONDIALDEHYDE (MDA) IN WHOLE BLOOD:[101]

Principle:

The reaction depends on the formation of pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having an absorption of maximum at 532 nm.

Thiobarbituric acid reagent:

- 75 mg thiobarbituric acid
- 15 ml trichloroacetic acid
- 2.08 ml – 0.2 N HCI

All were mixed and volume made up to 100 ml with distilled water.

Procedure:

	Blank	**Test**
Whole blood	-	0.75 ml
Distilled water	0.75 ml	-
Thiobarbituric acid reagent	3 ml	3 ml

- Keep in boiling water bath for 15 minutes

- Cool, centrifuge for 10 minutes at 10,000 r.p.m.
- Read absorbance of supernatants of blank and test immediately at 535 nm

Calculation:

Malondialdehyde (nano moles / ml)

$$= \frac{\text{Absorbance of test} \times \text{total volume}}{\text{Nanomolar extension coefficient} \times \text{Sample volume} \times 100}$$

$$= \frac{\text{Absorbance of test} \times 3.75}{1.56 \times 10^5 \times 0.75 \times 100}$$

$$= \frac{\text{Absorbance of test} \times 3205}{100}$$

PREPARATION OF (RBC) HEMOLYSATE

Isolation of Red Blood Cells: [105]

Most of the enzyme activities in red cells are lower than those in white blood cells and platelets, and hence it was of extreme importance to remove virtually all platelets and WBCs. In order to isolate RBCs the whole blood was filtered through a column of α-cellulose and microcrystalline cellulose mixture.

α – Cellulose and microcrystalline cellulose in 1:1 (W/W) was mixed with isotonic (9.0 gm/L) sodium chloride solution. 5ml plastic disposable syringe without barrel

was taken. It was placed in vertical position with the outlet pointing downwards. A small piece of filter paper was placed at the bottom of the syringe. The well – mixed cellulose slurry was poured to the 2-ml mark. The bed was washed with 5-ml isotonic sodium chloride and 1 ml of whole blood was allowed to flow through the column. To ensure efficient removal of WBCs and platelets, the volume of cellulose mixture used was at least twice as that of the blood sample.

The effluent was collected into a centrifuge tube. The saline suspended red cells were washed twice in at least 10 volumes of ice-cold isotonic sodium chloride. After washing, the packed cells were resuspended in isotonic sodium chloride to give an approximately 50% suspension (1:1) dilution. This suspension was subjected to hemolysis.

LYSING OF THE RBCS: [106]

Reagents:

Stabilizing solution: 2.7 mM EDTA (pH 7.0) and 0.7mM β- mercaptoethanol: This solution was prepared by dissolving 100 mg of disodium salt of EDTA in D/W and 5µl of β- mercaptoethanol (Merck) were added to it. Final volume was made to 100 ml with D/W.

Procedure:

In order to prepare the hemolysate, 1 volume of the RBC suspension was mixed with 9 volumes of the stabilizing solution. The hemolysate was frozen rapidly at – 20^0C to – 25^0C in a freezer. Then it was thawed in a water bath at 20^0C to 25^0C. This hemolysate was then ready for the assay. The hemoglobin estimation was performed on hemolysate using Drabkin's reagent, in order to express the enzyme

activities per gram of hemoglobin of the hemolysate. The red cell suspension and its hemolysate were prepared on the day of the assay.

The hemolysate was used for the Glutathione reductase (GR) enzyme activity

DETERMINATION OF HEMOGLOBIN[107]

Principle:

Drabkins reagent contains potassium cyanide and potassium ferricyanide Hemoglobin reacts with ferricyanide to form methemoglobin, which is converted to stable cyanmethemoglobin (HiCN) by the cyanide. The intensity of the colour is proportional to hemoglobin concentration and is compared with a known cyanomethemoglobin standard at 540 nm (green filter).

Reagents:

1. Drabkin's reagent
2. Cyanmethemoglobin standard, (cyanmeth-Hb standard) 15g.

Procedure:

A set of tubes was prepared as follows:

	Bank	**Standard**	**Test**
Drabkin's reagent	5.0 ml	---	5.0 ml
Cyanmethemoglobin standard	----	5.0 ml	----
Unknown	----	----	0.02 ml

The contents in the test were mixed thoroughly and optical density of test and standard (15g %) were measured at 540 nm against blank (Drabkin's reagent)

Calculation:

$$\text{Hemoglobin gm\%} = \frac{\text{OD test}}{\text{OD std}} \times 15$$

PERFORMANCE OF THE ENZYME ASSAYS FROM 1:20 HEMOLYSATE

GLUTATHIONE REDUCTASE[102]

Principle:

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

$$NADPH\ (NADH) + H^+ + GSSG \xrightarrow{GR} NADP^+\ (NAD^+) + 2GSH$$

The activity of this enzyme is measured by following the oxidation of NADPH (NADH) spectrophotometrically at 340nm. Glutathione reductase is a flavin enzyme and it has been found that it is not fully activated by FAD in normal hemolysates. Complete activation of apoenzyme, requires the preincubation of the enzyme with FAD. This must be done before GSSG or NADPH is added to the reaction system, since these seem to interfere with activation of the enzyme by FAD.

Reagents:

1) Tris-HCI; 1M, EDTA: 5mM, pH 8.0: It was prepared as described earlier.

2) Flavin adenine dinucleotide (FAD), 10 µM: 0.8 mg of sodium salt of FAD (SRL, Mwt-829.52) was dissolved in 100 ml of D/W.

3) Glutathione oxidized (GSSG), 0.033M: 20.2 mg of GSSG (SRL, Mwt-612.7) were dissolved in 1ml of D/W.

4) Nicotinamide adenine dinucleotide phosphate reduced (NADPH) 2nmM: It was prepared as described earlier.

Procedure:

The following reagents were added to the cuvettes volume of 1ml

	Blank	**Test**
Tris HCI; 1M EDTA, 5mM, pH 8.0	50 µl	50 µl
1:20 hemolysate	10 µl	10 µl
D/W	790 µl	690 µl
FAD 10 µM	100 µl	100 µl
Tubes were incubated at 37^0C for 10 minutes		
GSSG, 0.033M	--	100 µl
Tubes were incubated at 37^0C for 10 minutes		
NADPH, 2Mm	50 µl	50 µl

The decrease of optical density was measured at 340 nm at 37^0C of the test against the blank.

Calculations:

The number of enzyme units per ml:

$$A = \frac{\Delta OD \times Vc}{\varepsilon \times N \times V_H}$$

Where

ΔOD: Change in optical density per minute

Vc: The volume of the cuvette in ml = 1ml

ε : The millimolar extinction coefficient of the NADPH = 6.22

N: The number of molecules (1) of NADPH converted per molecule of GSSG consumed.

V_H : The volume of hemolysate added to the cuvette in ml = 0.01ml

The enzyme activity in international units / g Hb

$$E = \frac{A \times 100}{Hb}$$

A: The number of enzyme units /ml

Hb: The grams of hemoglobin per 100ml of the Hemolysate.

ESTIMATIONS FROM SERUM[108]

VITAMIN C[103]

Principle:

When ascorbic acid reacts with 2, 6-dichlorophenol indophenols, reduced 2, 6-dichlorophenol indophenol is formed which is colorless. Decrease in color is proportional to the concentration of ascorbic acid present in the solution. Decrease in the optical density is measured at 520nm and concentration is calculated from standards treated similarly.

Reaction:

2, 6-dichlorophenol indophenol (Oxidized form) + Ascorbic acid ⟶

2, 6-dichlorophenol indophenol (Reduced form) + Dehydroascorbic acid

Reagents:

1) 10% sodium tungstate: 10 g of sodium tungstate were dissolved in D/W, and the volume was made up to 100 mL.

2) 2/3 N sulphuric acid: 18.7ml concentrated H_2SO_4 was mixed with distilled water and the volume was made up to 1 liter with D/W.

3) Metaphosphoric acid solution; 5%: 5g of metaphosphoric acid (Robert Johnson) was dissolved in 100ml of D/W without heating. Solution was prepared weekly. Stored in refrigerator.

4) 2, 6 dichlorophenol indophenol solution: 13mg of 2, 6 dichlorophenol indophenol (Loba GR) and 3g of anhydrous sodium acetate trihydrate (Qualigens SQ), were dissolved in 1 liter of D/W. 9ml of this reagent and 1

ml of metaphosphoric acid (reagent 1) were mixed to check the pH which should be around 3.5-3.6 (pH can be adjusted by varying the amount of sodium acetate in the reagent).

5) Ascorbic acid stock standard, 100mg%: 100mg of L-Ascorbic acid was dissolved in 100ml of metaphosphoric acid.

6) Ascorbic acid working standard, 1 mg%: 1ml of the stock standard ascorbic acid was diluted to 100ml with metaphosphoric acid.

Procedure:

In a clean dry test tube, 1 ml of plasma, 3ml of 5% metaphosphoric acid, 0.5ml of sodium tungstate and 0.5ml of 2/3N sulfuric acid were taken. The contents were mixed and filtered after 5 minutes.

Three clean dry test tubes labeled as Blank, Standard and Sample were taken, and following reagents were added as shown as follows.

	Blank	**Standard**	**Sample**
5% Metaphosphoric acid	0.3 ml	-	-
Ascorbic acid standard	-	0.3 ml	-
Plasma	-	-	0.3 ml
2,6-dichlorophenol indophenols	2.7 ml	2.7 ml	2.7 ml

Reading was taken at 520nm against D/W using a double beam Spectrophotometer.

Calculations:

Amount of ascorbic acid in mg% =

OD of Blank – OD of sample x concentration of standard (in mg %)

OD of Blank – OD of Standard

SERUM CALCIUM[104]

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

Alkaline Medium

Calcium + o-Cresolphthalein Complexone ---------->

Calcium – Cresolphthalein Complexone Complex (purple color)

Reagents

Reagent 1:

2-Amino-2-methyl-1-propanol	505mmol/L

Reagent 2:

OCPC	0.06mmol/L
8-hydroxy Quinolone	6.9mmol/L
HCl	45mmol/L

Standard:

Calcium standard:	10 mg/dL

Reagent preparation

Allow the reagent 1 and reagent 2 to attain room temperature. Prepare working reagent by mixing equal volumes of both the reagents.

Assay Procedure:

Three clean dry test tubes labeled as Blank, Standard and Sample were taken, and following reagents were added as shown as follows.

	Blank	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	--	--
Standard	--	10µl	--
Sample	--	--	10µl

Mix well and read the optical density at 578nm against reagent blank using ERBA Chem 5 Semi-auto analyser.

Calculation:

Amount of calcium in mg% =

$$\frac{\text{OD of Blank} - \text{OD of sample}}{\text{OD of Blank} - \text{OD of Standard}} \times \text{concentration of standard (in mg \%)}$$

PHOTOGRAPHS

Photo No-1: ERBA Chem 5 Semi-auto analyser

Photo No-2: Digital spectrophotometer

RESULTS

The present study comprises of 30 clinically diagnosed cases of Benign Prostate Hypertrophy and 30 Prostate Cancer with 30 normal healthy age and sex matched controls. The age group ranges from 65 to 75 years.

STATISTICAL TESTS USED

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 was made using ANOVA. Data was entered in Microsoft excel and analyzed using SPSS (version 17). The mean difference is significant at p value ≤ 0.05.

AGE

The mean age of controls was 70.46±3.58 years (59-78 years), the mean age in Benign Prostate Hypertrophy cases was 72.26±4.70 years (65-81 years) and in Prostate Cancer cases was 72.76±4.98 years (65-81 years).There was no statistically significant difference between mean age of three groups (p= 0.117). The groups were age and sex matched.

HEMOGLOBIN

The mean level of Hb% in controls was 10.49±1.21 gm/dL. The level in BPH cases was 10.47±1.19 gm/dL and in Prostate Cancer cases was 9.71±1.74 gm/dL. There was no statistically significant difference in the three groups (p =0.55).

MALONDIALDEHYDE (MDA)

The mean level of MDA in controls was 6.17±1 nmol/ml. The value in BPH cases was 11.44±0.82 nmol/ml and in Prostate Cancer cases was 15.83±3.63 nmol/ml. The level was significantly increased (P< 0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

GLUTATHIONE REDUCTASE

The mean glutathione reductase level in controls was 8.96±1.04 IU/g of Hb. The level in BPH cases was 3.39 ± 0.74 IU/g of Hb and in Prostate Cancer cases was 3.32±0.76 IU/g of Hb. The level was significantly decreased (P< 0.001) in all the cases of BPH and also in cases of Prostate Cancer when compared to controls.

VITAMIN C

The mean vitamin C level in controls was 0.87 ±0.01 mg/dl. The level in BPH cases was 0.42 ±0.14 mg/dl and in Prostate Cancer cases was 0.50 ±0.15. The level was significantly decreased (p <0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

SERUM CALCIUM

The mean value of total serum calcium in controls was 9.08 ± 0.37 mg/dl. The level in BPH cases was 9.92±0.97 mg/dl and in Prostate Cancer cases was 9.55±1.35mg/dl. The value of total serum calcium was significantly increased (p= 0.05) in all the BPH cases when compared to controls. But there was no statistical significant difference (p=0.187) in total serum calcium levels in Prostate Cancer cases when compared to controls.

Correlation analysis of Lipid Peroxidation product, MDA and Anti-oxidant levels of Glutathione Reductase and Vitamin C levels

There was a strong correlation between plasma MDA with Glutathione Reductase (r = -0.100 & p=0.6) but low correlation with Vitamin C (r=0.41 & p=0.831) in BPH cases. And there was a strong correlation between plasma MDA with Glutathione Reductase (r = -0.031 & p=0.870) and Vitamin C (r= -0.112 & p=0.557) in Prostate Cancer cases.

TABLES

TABLE 3: Age distribution in Controls, BPH and Prostate Cancer.

	CONTROLS	**BPH**	**PROSTATE CANCER**	**p value**
Age (years)	70.46 ±3.58	72.26±4.70	72.76±4.98	0.117

TABLE 4: Mean Hemoglobin concentration in Controls, BPH and Prostate Cancer.

	Controls	**BPH**	**Prostate Cancer**	**p value**
Hemaglobin (gm/dL)	10.49±1.21	10.47±1.19	9.71±1.74	0.55

TABLE 5: Mean PSA levels in Controls, BPH and Prostate Cancer.

	BPH	**Prostate Cancer**	**t**	**df**	**p value**
PSA (ng/mL)	5.33±0.96	18.82±6.23	11.726	58	<0.001

TABLE 6: Concentration of Malondialdehyde (MDA), Glutathione Reductase, Vitamin C and Serum Calcium levels in BPH in comparison with Controls.

Variables	**Controls**	**BPH**	**'t' value**	**df**	**'p' value**
Malondialdehyde	6.17 ± 1	11.44 ± 0.82	-6.467	98	<0.001
Glutathione reductase	8.96 ± 1.04	3.39± 0.74	.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	<0.005

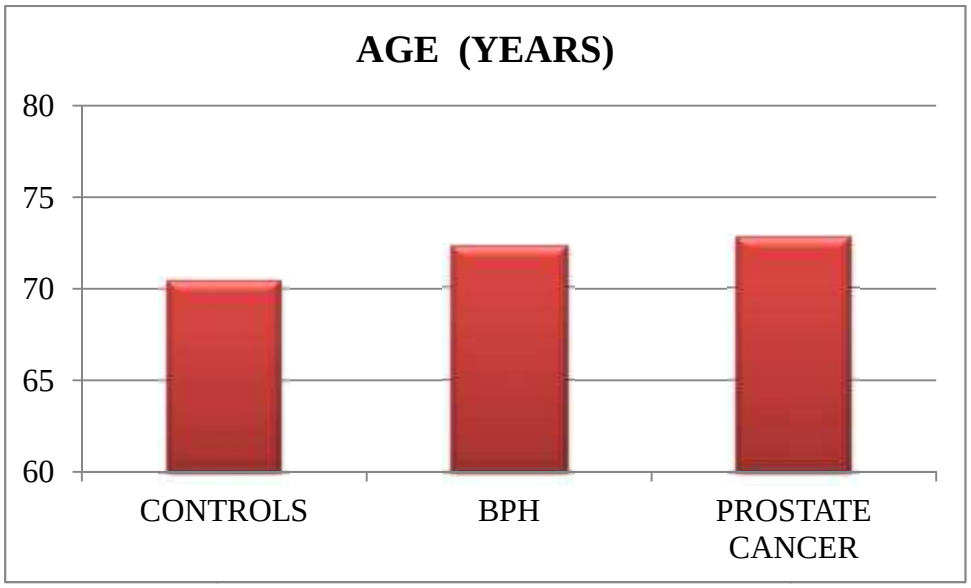
TABLE 7: Concentration of Malondialdehyde (MDA), Glutathione Reductase, Vitamin C and Serum Calcium levels in Prostate Cancer in comparison with Controls.

Variables	**Controls**	**Prostate Ca**	**'t' value**	**df**	**'p' value**
Malondialdehyde	6.17 ± 1	15.83± 3.63	-6.467	98	<0.001
Glutathione reductase	8.96 ± 1.04	3.32 ± 0.76	.350	98	<0.001
Vitamin C	0.87 ± 0.1	0.50 ± 0.15	-2.168	98	<0.001
Serum Calcium	9.08 ± 0.37	9.55 ± 1.35	1.238	98	0.187

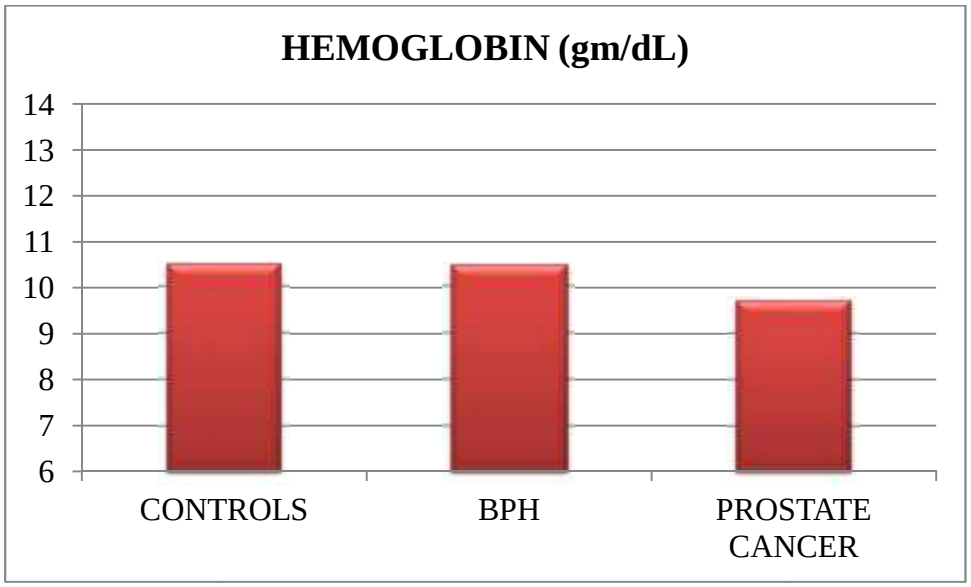
p<0.05 is taken as statistically significant

GRAPHS

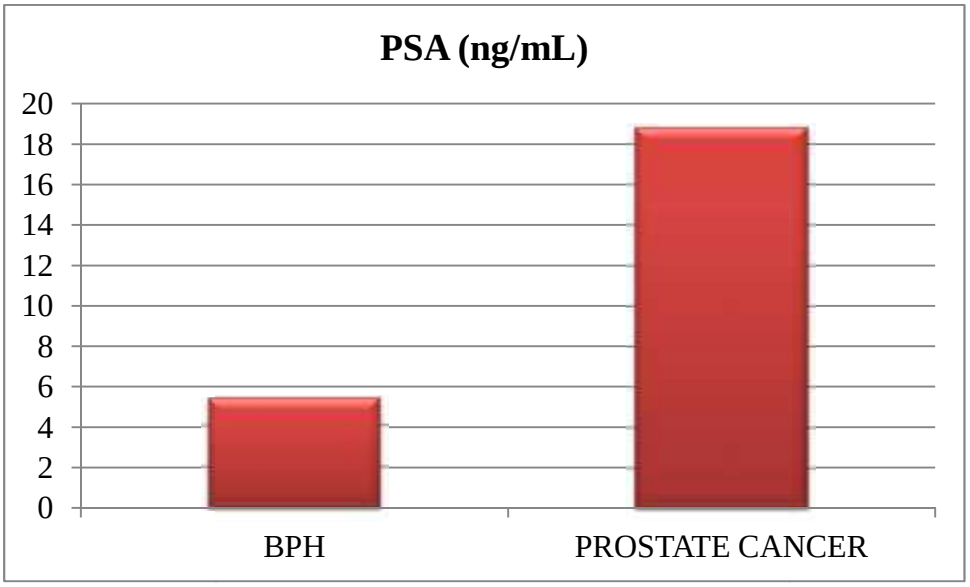
Graph 1: Mean Age distribution in Controls, BPH and Prostate Cancer.



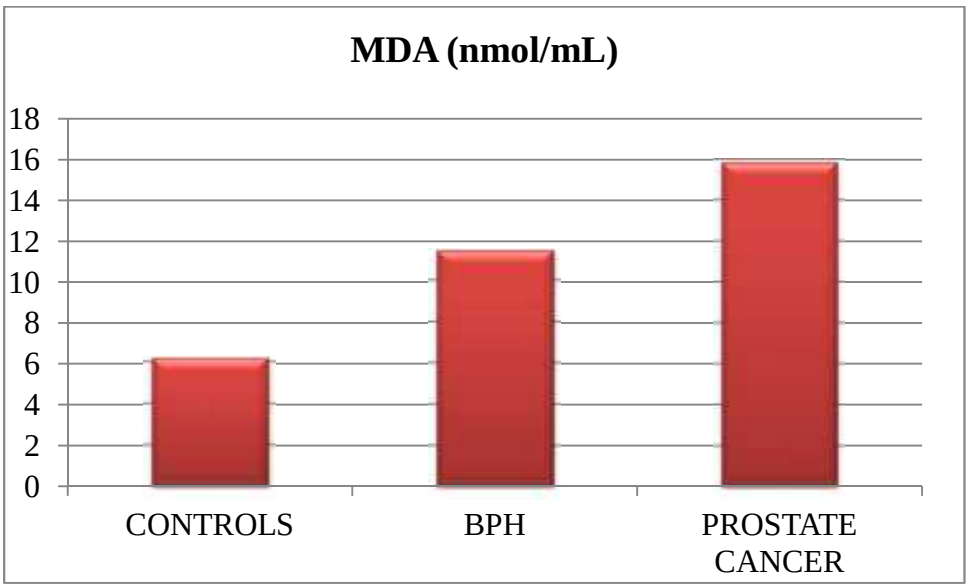
Graph 2: Mean Hemoglobin concentration in Controls, BPH and Prostate Cancer.



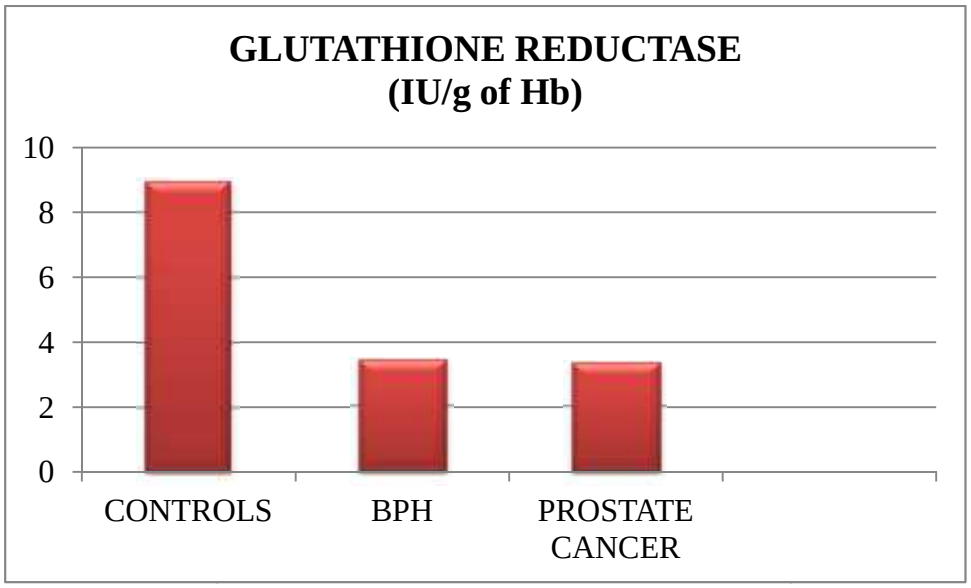
Graph 3: Mean PSA levels in BPH and Prostate Cancer.



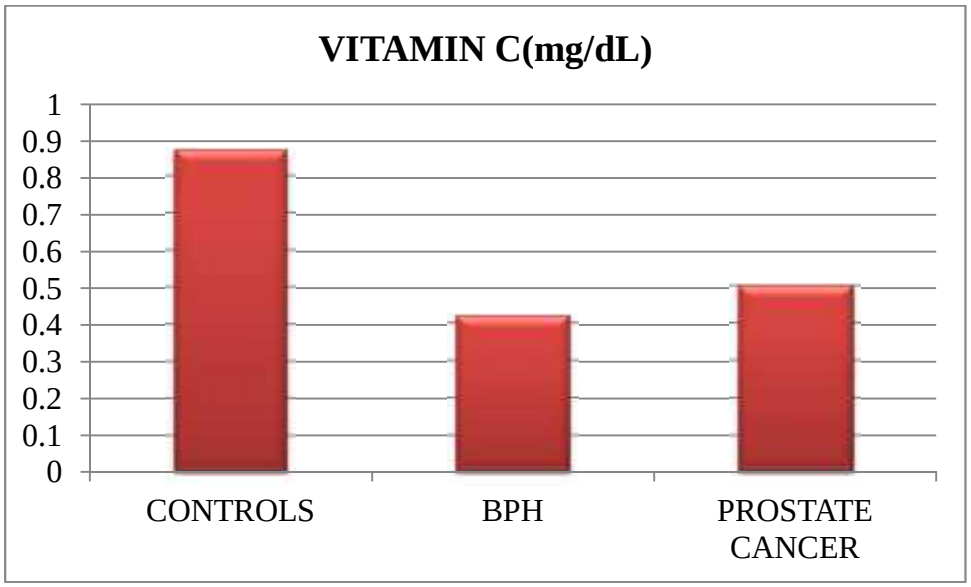
Graph 4: Mean MDA levels in Controls, BPH and Prostate Cancer.



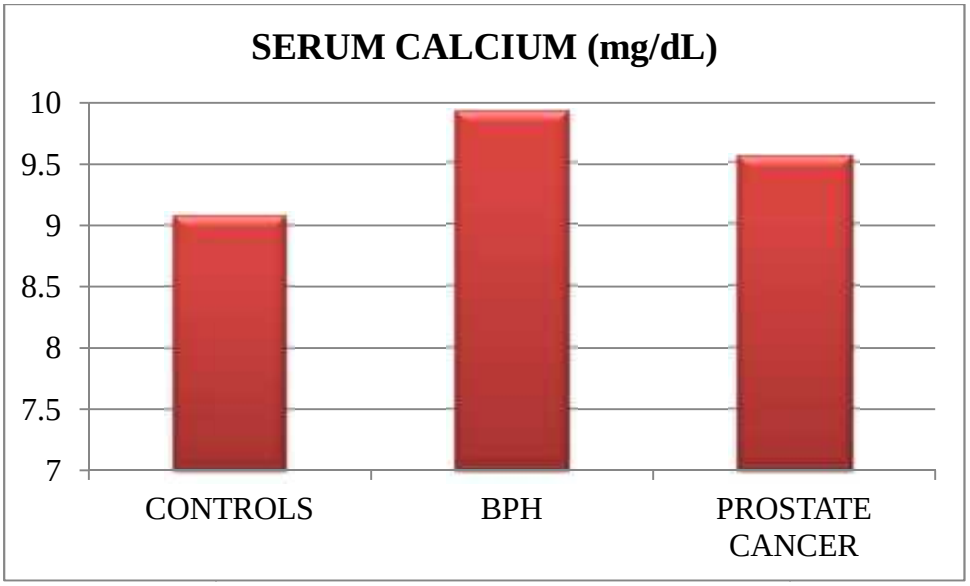
Graph 5: Mean Glutathione Reductase levels in Controls, BPH and Prostate Cancer.



Graph 6: Mean Vitamin C levels in Controls, BPH and Prostate Cancer.



Graph 7: Mean Serum Calcium levels in Controls, BPH and Prostate Cancer.



DISCUSSION

Involvement of oxygen free radicals in the pathophysiology of inflammation and chronic diseases in a number of organs and tissues has been reported in literature[55, 69]. Evidence of ROS generation in patients with Benign Prostate Hypertrophy and Prostate Cancer has been observed by measuring the product of lipid peroxidation malondialdehyde. Antioxidant defense was assessed by measuring, glutathione reductase and vitamin C. Serum calcium levels were estimated in BPH and Prostate Cancer to predict the risk associated with them.

MALONDIALDEHYDE

In the present study mean level of MDA was increased significantly in cases compared to controls. Our findings are in accordance with the study of Aryal M et al [4]. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. According to the study, high concentration of MDA in patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process.

GLUTATHIONE REDUCTASE

The level of glutathione reductase was decreased significantly in BPH ($p<0.001$) and Prostate Cancer ($p<0.001$) compared to controls. The findings of the present study are in accordance with the results of Hayes et al [98]. Glutathione reductase is involved in the reduction of hydrogen peroxide radicals, resulting in a decrease in glutathione reductase levels during that period.

Depressed levels may be associated with an enhanced protective mechanism to oxidative stress in BPH and Prostate Cancer.

VITAMIN C

Vitamin C is the first antioxidant to be depleted upon exposure to both environmental and inflammatory oxidants suggesting that it is the ultimate antioxidant directly scavenging these oxidants (free radicals) or trapping their intermediates. Hence supplementation may be beneficial in patients with BPH and Prostate Cancer.[8]

In the present study the level of vitamin C was significantly decreased ($p < 0.001$) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls. These findings were in accordance with previous reports.[55,]

Vitamin E scavenges lipid peroxy free radicals and interrupts the chain reaction of lipid peroxidation, becoming oxidized itself in the process. Vitamin C present in aqueous compartments functions as a water soluble chain breaking antioxidant, converts the tocopheroxy radical back to active tocopherol, thereby replenishing the antioxidant activity of vitamin E.[39]

In this study low levels of vitamin C were observed in BPH and Prostate Cancer. This might be due to exhaustion in attempt to neutralize heavy load of free radicals in these patients. Therefore supplementation with antioxidant vitamins like vitamin C may be necessary in the management of BPH and Prostate Cancer.

Overall findings of the study suggest that there was significantly increased oxidative stress in patients with BPH and Prostate Cancer assessed by raised MDA and significant decrease in the antioxidant enzymes like erythrocyte Glutathione reductase . Also non enzymatic antioxidants like vitamin C in BPH and Prostate Cancer cases were significantly decreased when compared to healthy controls. Low levels of enzymatic and non-enzymatic anti-oxidants and high levels of lipid peroxidation product like MDA may be due to heavy load of free radicals which cause

the damage leading to oxidative stress. Decreased levels of Vitamin C may be due to exhaustion in an attempt to neutralize the heavy load of free radicals in these patients.

Nutritional supplementation may represent a novel approach in these patients for delaying progress of BPH and Prostate Cancer. Hence the therapeutic benefit of exogenously administered antioxidant like vitamin C further needs to be assessed under carefully controlled clinical setup on a large sample.

SERUM CALCIUM

Studies have found a doubling of risk for fatal Prostate Cancer among men in the highest tertile of total serum calcium and a tripling of risk for men in the highest tertile of ionized serum calcium (total and ionised). Numerous studies have investigated the role of dietary calcium in Prostate Cancer, with mixed results.[81,82]

In the present study we found increase in cases of BPH when compared to controls. ($p=0.05$) but we did not find a statistically significant increase in serum calcium levels in cases of Prostate Cancer in comparison with control. ($p=0.187$).

Calcium intakes that exceed the recommended allowance may increase the risk of fatal Prostate Cancer because these intakes may increase the levels of calcium in serum. While some large cohort studies (with >500 cases) have reported significant positive associations between dairy products and/or calcium intake and risk.[85]

The concentration of serum ionized calcium, the biologically active fraction of calcium, is tightly regulated by PTH and normally does not deviate by more that 2% from its set point. This contributes to the stability of total serum calcium levels, of which approximately 50% is ionized. These men had calcium levels in the range of 9.9 to 10.5 mg/dl, which the researchers described as the "high end of the normal range." Most importantly, unlike family history, serum calcium and PTH are factors that can be modified by lifestyle and by pharmacologic means.

CONCLUSION

This study was conducted in the Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 30 Benign Prostate Hypertrophy and 30 Prostate Cancer cases with equal age matched healthy controls.

The findings of the study suggest that there was significantly increased oxidative stress in patients with Benign Prostatic Hypertrophy and Prostate Cancer assessed by raised MDA and significant decrease in the antioxidant enzyme like erythrocyte Glutathione Reductase. Also non enzymatic antioxidant like Vitamin C in the cases of Benign Prostatic Hypertrophy and Prostate Cancer were significantly decreased when compared to healthy controls. Serum calcium levels were raised in cases Benign Prostatic Hypertrophy but not significantly increased in Prostate Cancer. These findings suggest a significant association between oxidative stress and antioxidant defence mechanism. The increased levels in Serum Calcium in BPH may be associated with the disease process. Follow up studies are necessary to prove its association in Prostate Cancer. Thus the disease progression in BPH and Prostate Cancer can be delayed by lifestyle modification, nutritional intervention and also by simple pharmacological drugs which lower serum calcium levels.

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SUMMARY

The objectives of the present study were to assess lipid peroxidation by estimating the levels of MDA and antioxidant defense by measuring enzymatic antioxidants like Glutathione Reductase and non-enzymatic antioxidant like Vitamin C in BPH and Prostate Cancer. To estimate the Serum Calcium levels in BPH and Prostate Cancer patients. To compare the values of the above parameters with that of controls.

The present study was conducted in Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between January 2011 to December 2011 on 30 cases of Benign Prostate Hypertrophy and Prostate Cancer and 30 healthy controls in the age of group of 65 to 75 years. 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 preparing hemolysate, and 6 ml in plain tubes, centrifuged, serum separated and kept at 4^0C which was analysed for Vitamin C and Serum Calcium within 24 hours using colorimeter.

The mean age of controls was 70.46±3.58 years (59-78 years), the mean age in Benign Prostate Hypertrophy cases was 72.26±4.70 years (65-81 years) and in Prostate Cancer cases was 72.76±4.98 years (65-81 years).There was no statistically significant difference between mean age of three groups (p= 0.117). The groups were age and sex matched.

The mean level of MDA in controls was 6.17±1 nmol/ml. The value in BPH cases was 11.44±0.82 nmol/ml and in Prostate Cancer cases was 15.83±3.63 nmol/ml.

The level was significantly increased (P< 0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

The mean glutathione reductase level in controls was 8.96±1.04 IU/g of Hb. The level in BPH cases was 3.39 ± 0.74 IU/g of Hb and in Prostate Cancer cases was 3.32±0.76 IU/g of Hb. The level was significantly decreased (P< 0.001) in all the cases of BPH and also in cases of Prostate Cancer when compared to controls.

The mean vitamin C level in controls was 0.87 ±0.01 mg/dl. The level in BPH cases was 0.42 ±0.14 mg/dl and in Prostate Cancer cases was 0.50 ±0.15. The level was significantly decreased (p <0.001) in all the cases of BPH and in cases of Prostate Cancer in comparison to controls.

The mean value of total serum calcium in controls was 9.08 ± 0.37 mg/dl. The level in BPH cases was 9.92±0.97 mg/dl and in Prostate Cancer cases was 9.55±1.35 mg/dl. The value of total serum calcium was significantly increased (p= 0.05) in all the BPH cases when compared to controls. But there was no statistical significant difference (p=0.187) in total serum calcium levels in Prostate Cancer cases when compared to controls.

Our study revealed that there was increased oxidative stress and decreased antioxidant defense in patients of Benign Prostate Hypertrophy and Prostate Cancer as evidenced by –

- Increased levels of Malondialdehyde.(MDA)
- Decreased levels of enzymatic antioxidant like Glutathione Reductase.
- Decrease in the concentration of non- enzymatic antioxidant like Vitamin C.

Increased in serum calcium levels were observed in cases of BPH but no significant increase in cases of Prostate Cancer when compared to controls. Thus serum calcium levels may have an association with the disease process and also with its progression but follow up is necessary for its association in Prostate Cancer.

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ANNEXURE I

INFORMED CONSENT

Mr./Mrs/Ms.

…… you are invited to participate in "Cross Sectional Study of Lipid Peroxidation, Antioxidant Status and Serum Calcium in Benign Prostate Hypertrophy and Prostate Cancer". Participation in this study is completely voluntary. All the patients with BPH and Prostate Cancer and equal number of healthy volunteers will be enrolled in this study at Jawaharlal Nehru Medical College, Belgaum under the supervision of Dr. _____, Department of Biochemistry, Jawaharlal Nehru Medical College, KLE University, Belgaum. The study will be carried out by Dr._____, Post Graduate Student, Department of Biochemistry, KLE University; Belgaum for his M.D. dissertation ,to be submitted to KLE University, Belgaum.

PURPOSE OF THE STUDY

BPH and Prostate Cancer are common disorders causing increase in morbidity and mortality. Hence it is essential to identify the candidates at risk at an early stage by estimation of MDA, glutathione reductase, vitamin C and Serum Calcium so that they can be diagnosed earlier and helps to reduce morbidity and mortality.

PROCEDURE

For both BPH and Prostate Cancer patients (Cases) and healthy subjects (Controls), 10 ml of venous blood will be collected under aseptic precautionary measures using sterile disposable syringe.

RISKS

Since the blood is drawn under aseptic precautionary measures by trained persons there is no scope for any risks. Further only small volume of blood is collected which will be spontaneously replenished in the body. However there may be minor risks associated with having blood drawn that may include bruising, redness, discomfort or bleeding at the puncture site.

BENEFITS

No direct benefit is guaranteed to you from participating in our study. You can make use of blood levels of studied parameters if desired.

OPTIONS

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

NEW INFORMATION

Does not apply to this research.

PRIVACY AND CONFIDENTIALITY

All information collected about you during the course of the study will be kept confidential to the extent permitted by law. You will be identified in this research record by the code numbers. Information which identifies you personally will not be revealed without your written permission. However your records may be revealed to the sponsor of the study. Information from this study may be published but your identity will be confidential in any publication.

INSTITUTIONAL POLICY

In the event that you are physically injured as a result of participating in this research emergency care will be available. There is no commitment to provide any compensation for research related injury. The Jawaharlal Nehru Medical College will

provide, within the limitations of the laws of the state of Karnataka, facilities and medical attention to subjects who suffered any harm as the result of your participation in this study. In the event you believe that you have suffered any how as a result of your participation in this study you may contact research guide Dr._____Department of Biochemistry, Jawaharlal Nehru Medical College, KLE University, Belgaum.

COST FOR PARTICIPATION

You will not be charged for the test to be carried out on your blood sample.

FINANCIAL INCENTIVE FOR PARTICIPATION

You will not receive any remuneration for participating in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Dr. A.S. Godhi M.S. Principal, Jawaharlal Nehru Medical College, KLE University, Belgaum and Chairman of Jawaharlal Nehru Medical College Institutional Ethics Committee of Human Subjects Research, Telephone No. 0831-2471701.

EMERGENCY PROVISION

If you have questions as a participant in our study, you can contact the study investigator Dr._____, or the research guide Dr _____Phone No. 0831-2473777 (Extension) 4083.

CONSENT TO PARTICIPATE IN A RESEARCH TRIAL

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

_____ _____

Signature of participant Date

_____

Participants Name (Printed):

_____ _____

Name and Signature of witness-1 Date

_____ _____

Name and Signature of witness-2 Date

_____ _____

Signature of researchers or Date

Person obtaining consent

ANNEXURE II

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

"CROSS SECTIONAL STUDY OF LIPID PEROXIDATION, ANTIOXIDANT STATUS AND SERUM CALCIUM IN BENIGN PROSTATE HYPERTROPHY AND PROSTATE CANCER"

Name :Sex:

Age:I.P. No.:

DOA:

Address:

Presenting complaints

Past history

History of similar episode in the past.

Family History

Any complaints in family members.

Personal history

Sleep

Appetite

Bowel and bladder

General Physical Examination

Pallor:	***Vitals***
Pedal oedema :	Pulse rate :
Nail clubbing :	Blood pressure:
Cyanosis :	Respiratory rate:
Icterus :	Temperature :

Lymphadenopathy:

Per abdomen/local examination

Inspection :

Palpation :

Percussion :

Auscultation :

Per-rectal examination :

Examination of external urethra:

Systemic Examination

Respiratory system

Inspection :

Palpation :

Percussion :

Auscultation :

CVS

Pulse :

Blood pressure :

CVS – Central

Inspection :

Palpation :

Percussion :

Auscultation :

Central nervous system

Higher Function Tests:

Cranial Nerves examination:

Motor System:

Sensory System:

Cerebellar Functions:

Skull & Spine:

Investigations:

Blood

- Malondialdehyde

Hemolysate

- Glutathione Reductase

Serum

- Vitamin C
- Serum calcium

Final Diagnosis

ANNEXURE III

MASTER CHART

CONTROLS

Sl.No	Controls	AGE	MDA (nmol/ml)	Glutathione reductase (IU/g of Hb)	Vitamin C (mg/dl)	Calcium (mg/dL)	Hb (gm%)
1	CS01	67	3.85	8.52	0.88	9	11.30
2	CS02	73	5.6	8.34	0.7	9.2	10.23
3	CS03	68	4.6	9.6	1	8	9.32
4	CS04	59	5.89	10.04	0.92	9.2	11.47
5	CS05	71	7.75	9.1	0.84	9.8	10.16
6	CS06	69	6.98	10.52	0.72	9.2	10.38
7	CS07	78	5.6	10.88	0.91	8	10.08
8	CS08	75	6.12	8.62	1.03	9.1	10.69
9	CS09	77	5.38	9.79	0.97	9.3	12.31
10	CS10	66	6.15	8.76	0.73	9	11.93
11	CS11	69	7.46	8.19	0.85	9	12.12
12	CS12	69	7.01	9.79	0.88	9	10.61
13	CS13	74	5.06	10.37	0.88	9	11.11
14	CS14	75	6.92	7.4	0.86	9.1	9.68
15	CS15	67	6.12	8.84	0.83	9.7	9.70
16	CS16	69	5.41	8.19	0.82	9.3	9.80
17	CS17	73	6.98	7.19	0.95	9.2	9.60
18	CS18	71	7.3	7.5	0.85	9.4	8.60
19	CS19	71	6.98	9.78	0.88	9.1	9.70
20	CS20	73	5.6	11.12	1.04	9.3	12.10
21	CS21	69	5.41	8.84	0.96	8.9	9.60
22	CS22	70	6.21	7.9	0.7	9	12.30
23	CS23	70	4.83	8.54	0.85	8.8	9.68
24	CS24	71	5.06	8.19	1.1	9.2	7.30
25	CS25	69	6.15	8.7	0.84	9.5	12.30
26	CS26	71	7.01	9.78	0.73	9.2	9.80
27	CS27	70	7.53	9.6	0.85	9	9.90
28	CS28	70	7.75	8.5	0.82	9	11.43
29	CS29	69	6.79	8.7	0.97	8.9	10.12
30	CS30	71	5.89	7.6	0.82	9	11.60

CASES: BENIGN PROSTATE HYPERTROPHY

Sl.No	Cases	AGE (yrs)	MDA (nmol/mL)	Glutathione reductase (IU/gm Hb)	Vitamin C (mg/dL)	Calcium (mg/dL)	Hb gm/dL	PSA (ng/mL)
1	BPH01	68	11.53	3.67	0.42	10.5	11.30	4.6
2	BPH02	69	10.98	3.4	0.21	11	10.23	5.6
3	BPH03	74	11	3.75	0.31	10.8	9.32	5.2
4	BPH04	80	11.57	4.94	0.21	11.2	11.47	4.8
5	BPH05	74	11.38	3.37	0.36	9.8	10.16	4.2
6	BPHO6	66	11.82	3.67	0.41	10.9	10.38	5.6
7	BPH07	71	12.46	3.56	0.42	11.4	10.08	5.2
8	BPH08	74	11.53	3.75	0.41	10.6	10.69	4.1
9	BPH09	69	10.98	2.77	0.61	9.8	12.31	8.8
10	BPH10	70	11	2.5	0.51	10.4	11.93	4.3
11	BPH11	81	9.9	3.8	0.63	11	12.12	5
12	BPH12	73	10.43	3.7	0.53	9.8	10.61	4.82
13	BPH13	77	11.53	3.1	0.49	7.9	11.11	6.1
14	BPH14	76	11.92	2.3	0.31	8.9	9.68	5
15	BPH15	65	11.18	2.1	0.56	9	9.70	4.2
16	BPH16	69	13.17	1.89	0.42	10.5	9.80	6.2
17	BPH17	68	11.82	4	0.56	11	9.60	6.7
18	BPH18	73	12.2	3.45	0.39	9.8	8.60	4.6
19	BPH19	74	12.46	3.67	0.56	9.9	9.70	5.6
20	BPH20	68	8.9	4.2	0.46	8.8	12.10	4.32
21	BPH21	78	10.9	3.2	0.32	9.7	9.60	5.2
22	BPH22	79	11.5	3.7	0.21	9.1	12.30	5.2
23	BPH23	69	12.24	4.13	0.67	8.5	9.68	4.8
24	BPH24	67	12.2	3.75	0.71	8.7	7.30	6.3
25	BPH25	68	11.53	2.9	0.39	11	12.30	6.1
26	BPH26	65	11.07	3.2	0.31	10.5	9.80	5.9
27	BPH27	71	11.45	2.74	0.34	10.1	9.90	6
28	BPH28	79	11.38	2.18	0.29	8	11.43	5.8
29	BPH29	76	11	3.27	0.21	9.2	10.12	5
30	BPH30	77	12.25	4.99	0.41	10	11.00	4.82

CASES: PROSTATE CANCER

Sl.No	Cases	AGE (yrs)	MDA nmol/mL	Glutathione reductase (IU/gm Hb)	Vitamin C (mg/dL)	Calcium (mg/dL)	Hb gm/dL	PSA (ng/mL)
1	PC01	79	17.8	4.2	0.59	11.2	9.00	10.4
2	PC02	78	14.4	2.8	0.32	12	11.00	12.2
3	PC03	73	16.2	3.7	0.69	8	9.32	32.1
4	PC04	71	19	4.2	0.68	9.3	12.00	28.4
5	PC05	70	12.8	2.4	0.38	9	9.40	26.2
6	PC06	66	11.9	2.8	0.59	10.4	8.00	18.4
7	PC07	69	18.5	3.8	0.32	8	9.40	19.2
8	PC08	67	19.5	4	0.67	12	13.00	16.4
9	PC09	69	20.3	2.9	0.45	8.8	11.00	15.2
10	PC10	68	10.9	2.1	0.78	10	10.00	18.4
11	PC11	70	14.8	3.2	0.39	12	9.60	19.2
12	PC12	71	13.6	3.1	0.48	8.4	6.00	35.4
13	PC13	77	12.7	2.8	0.52	9.8	12.00	28.2
14	PC14	75	26.8	2.5	0.28	9.9	8.00	16.6
15	PC15	79	12.9	2.1	0.54	10.2	11.00	18.2
16	PC16	73	15.5	4.2	0.42	7	10.00	14.4
17	PC17	66	19.5	3.4	0.56	8	9.00	13.2
18	PC18	67	16.5	3.8	0.67	9.2	7.40	18.4
19	PC19	68	19	2.5	0.48	9	8.00	14.3
20	PC20	80	10.5	5	0.55	8.3	9.00	13.8
21	PC21	81	8.2	3.8	0.35	10	10.00	12.3
22	PC22	78	16.7	3.6	0.28	11.2	13.00	18.4
23	PC23	77	14.6	2.9	0.59	10	9.00	19.3
24	PC24	81	17.5	1.9	0.66	9	8.00	13.8
25	PC25	69	17	3.6	0.24	10	11.00	15.4
26	PC26	65	16.5	4.1	0.54	9.4	8.00	16.2
27	PC27	76	14.4	2.9	0.78	7	8.40	19.1
28	PC28	72	17.2	3.1	0.45	9	13.00	18.8
29	PC29	71	12.6	4.2	0.29	9.4	9.00	14.3
30	PC30	77	18.4	4	0.56	11	10.00	28.6