METABOLIC SYNDROME AND OXIDATIVE STRESS IN GHANAIANS PRESENTING WITH PROSTATE CANCER

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST and the Komfo Anokye Teaching Hospital. This work has not been submitted for any other degree.

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ABSTRACT

The latest estimates of global cancer incidence show that prostate cancer has become the second most common cancer among men in the world. A number of reports have linked both oxidative stress and certain features of the metabolic syndrome (MetS) to prostate cancer. Although oxidative stress and MetS have been found to be more prevalent among the Ghanaian population no data exist on its prevalence in Ghanaian prostate cancer patients. This study seeks to investigate metabolic syndrome and oxidative stress in Ghanaians presenting with prostate cancer. This cross-sectional study was conducted at the out-patient department of the department of surgery, Komfo Anokye Teaching Hospital, Kumasi, between the period of November, 2010 and April, 2012. In all, one hundred and twenty four (124) adult males (87 case subjects and 37control subjects) aged at least forty two years were enrolled. Prevalence of MetS was diagnosed using The World Health Organization (WHO), International Diabetes Federation (IDF) and the National Cholesterol Education Programme, Adult Treatment Panel III (NCEP ATP III) criteria for defining MetS was employed. The overall percentage prevalence of MetS in the PCa population was 9.2%, 18.4% and 12.6% using NCEP-ATP III, IDF and WHO criteria respectively. Using all the three criteria, the MetS prevalence was highest among the highly aggressive PCa group compared to the other PCa groups and control subjects. Malondialdehyde, an oxidative stress marker, and uric acid were significantly raised whereas the measured antioxidant (vitamin C) was significantly reduced among the PCa patients compared to the controls. The indication is that oxidative stress with reduced antioxidant levels is common in PCa patients. Oxidative stress and MetS may have a significant role in prostate cancer. Based on the findings, it may seem reasonable to propose that therapeutic regimens aimed at beefing up the antioxidant defences could offer some degree of protection for PCa patients against oxidative stress.

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LIST OF ABBREVIATION

AFMS – Anterior FibromuscularStroma

ALP – Alkaline Phosphatase

ASAP – Atypical Small Acinar Proliferation

ATP –Adenosine Triphosphate

ATP III – Adult Treatment Panel III

BMI – Body Mass Index

BP – Blood Pressure

BPH – Benign Prostatic Hyperplasia

CAT – Catalase

Cm – Centimetre

cTNM – Clinical (stage) Tumour Node Metastasis

CHO – Cholesterol

CHRPE – Committee on Human Research Publications and Ethics

CI – Confidence Interval

Cor – Correlation

COX – Cyclooxygenase

CR – Coronary Risk

CRE – Creatinine

Cu – Copper

$CUSO_4$ – Copper (II) Sulphate (IV)

DRE – Direct Rectal Examination

dL – Decilitre

DNA – Deoxyribonucleic Acid

DBP – Diastolic Blood Pressure

ELISA – Enzyme Linked Immunosorbent Assay

FBG – Fasting Blood Glucose

FBS – Fasting Blood Sugar

Fe – Iron

GSH – Glutathione

HC – Hip Circumference

HDL-C – High density lipo-protein cholesterol

HHT – Hydroxyheptadecatrienoate

HPC – Hereditary Prostate Cancer

HOCl – Hypochloric Acid

H_2O_2 – Hydrogen Peroxide

IDF – International Diabetes Federation

IGF-1 – Insulinlike growth factor–1

KATH – KomfoAnokye Teaching Hospital

KNUST – Kwame Nkrumah University of Science and Technology

LDL – Low density lipo-protein

LDL-C – Low density lipo-protein cholesterol

MDA – malondialdehyde

MetS – Metabolic Syndrome

MRI – Magnetic Resonance Imaging

NAC –N – acetylcysteine

NADPH – Nicotinamide Adenine Dinucleotide Phosphate

NO – Nitric Oxide

NCEP – National Cholesterol Education Program

OFR – Oxygen Free Radical

PCa – Prostate Cancer

PIH – Pregnancy-Induced Hypertension

PIN – Prostatic Intraepithelial Neoplasia

PSA – Prostate Specific Antigen

pTNM – Pathologic (stage) Tumour Node Metastasis

PUFA – Poly-unsaturated Fatty Acid

RBS – Random Blood Sugar

RNASEL – Ribonuclease L

RNS – Reactive Nitrogen Species

ROS – Reactive Oxygen Species

rpm –Revolutions per minute

SBP – Systolic Blood Pressure

SEER – Surveillance, Epidemiology, and End Results

SEM – Standard Error of Mean

SOD – Superoxide dismutase

SVI – Seminal Vesicle Involvement

TCA – Tricarboxylic Acid

TBA – Thiobarbituric Acid

TC – Total Cholesterol

TG – Triglyceride

TNM – Tumour Node Metastasis

TUR – Transurethral Resection

TURP – Transurethral Resection of the Prostate

TRUS – Transrectal Ultrasonography

TZ – Transition Zone

UA – Uric Acid

VLDL – Very low density lipoprotein

VIT C – Vitamin C

WC – Waist Circumference

WHO – World Health Organization

WHR – Waist to Hip Circumference Ratio

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Prostate cancer (PCa) is a type of cancer that develops in the prostate, a gland in the male reproductive system. Though age, obesity and family history are among the primary risk factors, a complete understanding of the causes of this dreadful disease however remains unclear. It is particularly uncommon in men below 50 years, but becomes particularly more common with advancing age, specifically above 50 years. The disease may be initially asymptomatic but most patients may present with symptoms as the disease progresses. Most forms of prostate cancers are known to be slow growing. However, a number of cases of the aggressive type have been identified. The cancer cells may spread from the prostate to other parts of the body, especially the bones and lymph node. PCa is classified as an adenocarcinoma or glandular cancer that begins when normal semen-secreting prostate gland cells mutate into cancer cells (Hsing et al., 2006).

Prostate cancer is gradually taking a centre stage around the globe with a dreadful epidemiology. The latest estimates of global cancer incidence for instance, has it that prostate cancer has become the second most common cancer among men in the world, accounting for almost 11.7% of new cancer cases overall, which constitutes 19% of cancers in developed countries and 5.3% in developing countries (Parkin et al., 2001). In Europe, an estimated 2.6 million new cases of cancer are diagnosed each year. Out of this, prostate cancer alone constitutes about 11% (Bray et al., 2002). A study conducted by Black et al. (1997) also revealed that prostate cancer also accounts for about 9% of all cancer deaths among men within the European Union.

Despite its precarious nature, a number of biological processes and health abnormalities which were previously thought to be somewhat innocuous or pose indirect and long term harmful effects to the human body but are presently scaring, are now being claimed to be associated with this dreadful killer. Notable among these include lipid peroxidation, oxidative stress, metabolic syndrome, etc. Lipid peroxidation is a reaction whereby molecular oxygen is incorporated into poly-unsaturated fatty acids (PUFA) to yield lipid peroxides. Ozan et al. (2002) reported that that lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Oxidative stress is characterized by disequilibrium between oxidant and antioxidant forces in favour of oxidation. The lipid peroxidation process, initiated by the reaction of free radicals with polyunsaturated fatty acids (Hubel et al., 1989) is used as a marker of oxidant force.

Cell membranes are generally made up of lipid bilayers and thiol containing proteins. The unsaturated lipid component and thiol containing proteins of the cell membranes are susceptible to free radical attack. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals, or oppose their actions (Sies, 1991). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Krishna Mohan and Venkataramana, 2007). Defense mechanisms of the body however, play an important role in the formation of antioxidants, putting up remarkable attempt to minimize the damage, as an adaptation to stressful situations.

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and is used as an indicator of oxidative stress in cells and tissues. It can also be generated

during prostaglandin biosynthesis in cells. MDA reacts with amino groups on proteins and other biomolecules to form a variety of adducts, including adducts with DNA bases that are mutagenic and carcinogenic (Marnette, 1994). Increased levels of lipid peroxidation products, by measurement of MDA, have been associated with benign prostatic hypertrophy (BPH) (Rosaria et al., 2003). MDA can be found in most biological samples including serum, plasma, tissues and urine, as a result of lipid peroxidation. According to Rumley et al. (2004), the distribution of plasma lipid peroxides in men and women, determined by MDA, was found to be well approximated by a normal distribution. The median level increased by about 10% between 30 and 70 years of age in both sexes, which may be relevant to the increasing prevalence of atherosclerosis with age.

Oxygen radicals react with polyunsaturated fatty acid residues in phospholipids resulting in the production of a plethora of products, many of them reactive toward protein and DNA. One of the most abundant carbonyl products of lipid peroxidation is malondialdehyde (MDA) which reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine, and deoxycytidine. The deoxyguanosine adduct (M(1)G) has been detected in liver, white blood cells, colon, pancreas, and breast from healthy human beings at abnormally high levels. Random and site-specific mutagenesis experiments indicate that MDA-DNA adducts are mutagenic in bacteria and in mammalian cells. M(1)G for instance is highly mutagenic when incorporated into viral genomes then replicated in E. coli. It is repaired by the nucleotide excision repair pathway. Lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to cancer and other genetic diseases linked to lifestyle and dietary factors (Rosaria et al., 2003). The measurement of MDA, which is the most abundant lipid peroxidation product, is a convenient in vivo index of lipid

peroxidation and represents a non-invasive biomarker of oxidative stress. Therefore, the measurement of MDA concentrations in our study subjects will thus provide an index of lipid peroxidation and the extent of oxidative stress to which they are subjected to.

1.1.1 PROSTATE CANCER AND METABOLIC SYNDROME: GENERAL OVERVIEW

In 1988, Reaven considered the following abnormalities in his description of the metabolic syndrome or syndrome X: resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinaemia, increased VLDL triglycerides, decreased HDL cholesterol and hypertension. Other metabolic abnormalities that have been considered as part of the syndrome include abnormal weight or weight distribution, microalbuminuria, inflammation, hyperuricaemia and abnormalities of fibrinolysis and of coagulation (Meigs, 2000).

A number of recent reports have suggested that prostate cancer may be associated with features of the metabolic syndrome. This can be inferred from the following studies. Using data from a clinical study of men with prostate cancer in Sweden, Hammarsten and Hogstedt (2004) observed that certain features of the metabolic syndrome, including both hypertension and obesity, were more common in men who had stage T3 cancer compared with men who had stage T2 cancers. Again, according to Beebe-Dimmer et al. (2007), features of the metabolic syndrome, specifically abdominal obesity and hypertension, are associated with prostate cancer in African-American men. However, though a number of these metabolic syndrome features

have been observed in the prostate cancer patients, an explanation linking them is quite unclear. Varying degrees of insulin resistance are present in overweight and obesity and closely related to type 2 diabetes (DeFronzo and Ferrannini, 1991). Insulin resistance is associated with increased morbidity and mortality when accompanied by other cardiovascular risk factors such as abnormal glucose tolerance, hypertension, hyperlipidaemia, or obesity components of the insulin resistance syndrome or the metabolic. The prevalence of the metabolic syndrome increases with increasing age and Body Mass Index (BMI). Meanwhile, obesity has been reported to be associated with greater production of inflammatory mediators. However, inflammation has been suggested to be involved in prostate cancer development (Nelson et al., 2003).

Metabolic syndrome (MetS) has been linked with prostate cancer though its exact role in PCa development and progression remains unclear. However, the link between specific features of the metabolic syndrome and PCa focuses primarily on altered serum concentration of insulin-like growth factors (IGFs) and associated binding proteins, sex steroid hormones and sex steroid binding globulin (Hammarsten and Högstedt, 2005). It has been reported that insulin resistance and hyperinsulinaemia suppress the production of IGF binding protein 1 and 2 in the liver while increasing the production of IGF-1. It has also been reported that IGF-1 contributes to the initiation of PCa through a number of mechanisms such as increased proliferation of both normal and neoplastic cells as well as inhibition of apoptosis (Hammarsten and Högstedt, 2005). Meanwhile, it has also been reported that increased insulin production suppresses the production of sex steroid binding globulin thereby resulting in a higher concentration of bioavailable testosterone and estradiol which have been proposed that it may also contribute to the development of PCa.

Another metabolic pathway possibly involved consists of increased cholesterol levels. Cholesterol is an essential component of plasmatic membrane of the cell, but when its levels become higher than a critical concentration, it can inhibit cell apoptosis through structures known as lipid rafts, which alter the mechanisms of signal transduction which is reported to be involved in the development of PCa (Zhuang et al., 2005). However, Hsing et al. (2003) observed a correlation between PCa and insulin resistance among Chinese men which was independent of IGF, sex hormone binding globulin, and sex steroid concentrations. This suggests that there could be other mechanisms of prostate carcinogenesis such as oxidative stress.

1.2 STUDY HYPOTHESIS

Studies around the world have linked metabolic syndrome and oxidative stress to PCa. It is suggested that there is a counter association between PCa and oxidative stress on one hand as well as that between PCa and metabolic syndrome (Hammarsten and Högstedt, 2005; Rosaria et al., 2003). The study therefore hypothesized that PCa leads to oxidative stress and that the derived oxidative stress may also lead to the development of metabolic syndrome which worsens the complications of PCa.

1.3 JUSTIFICATION

Numerous studies on oxidative stress and MetS have been done in Ghana and across the African continent involving varying age groups. However, none of them involved PCa. Currently, PCa is the second most common cancer and the sixth leading cause of cancer deaths among men in the world. Global incidence of PCa is on the ascendancy. Annually, about 899,100 new cases of PCa are reported worldwide, 39,600 for Africa and 13,300 for West Africa. It is estimated that every year, about 258,100 PCa deaths

are reported worldwide for which 28,000 and 10,700 occur in Africa and West Africa respectively (Ferlay et al., 2010).

The global PCa burden is also expected to reach 1.7 million new cases and 499,000 new deaths by the year 2030 (Ferlay et al., 2010). In Ghana, about 921 new cases are reported every year with as many as 758 PCa deaths being reported annually (WHO, 2002). This dreadful disease also results in sexual, urinary and bowel dysfunction as well as bone pain. A substantial number of sufferers also experience significant reduction in their quality of life due to physical pain, mental anguish as well as economic hardship.

It is therefore necessary to investigate metabolic syndrome and oxidative stress among Ghanaians with prostate cancer for which if proven suggests that prevention or control of these conditions eventually may lead to a significant reduction in the prostate cancer incidence and deaths in Ghana and the world at large.

1.4 GENERAL OBJECTIVE

To investigate metabolic syndrome and oxidative stress among Ghanaians presenting with prostate cancer.

1.4.1 SPECIFIC OBJECTIVES

1. To determine the prevalence of metabolic syndrome among Ghanaians presenting with prostate cancer.
2. To determine the effect of oxidative stress among Ghanaians presenting with prostate cancer.

CHAPTER 2

LITERATURE REVIEW

2.1 THE PROSTATE GLAND

2.1.1 EMBRYOLOGY AND DEVELOPMENT OF THE PROSTATE GLAND

In all mammals, androgen formed in the developing testes is responsible for the aspects of male development in which the Wolffian ducts, urogenital sinus and urogenital tubercle are transformed into the epididymis/vas deferens, prostate and penis. It is also well known that androgens and mesenchymal-epithelial interactions are required for the formation and growth of the prostate. Development of glandular organs such as the prostate involves the process of branching morphogenesis (Lowsley, 1912).

During the third month of gestation, the prostate gland develops from epithelial invaginations from the posterior urogenital sinus under the influence of the underlying mesenchyme (Lowsley, 1912). The developing prostate lobes begin as an epithelial bud that invades the surrounding mesenchyme, projecting dividing epithelial cords or tubes away from the site of initiation. Growth of the prostatic ductal network during the prepubertal period is considered nonuniform, with ductal growth being highest in the distal region, at the ductal tips, and much lower in the proximal region closest to the urethra (Lee, 1996). The normal formation of the prostate gland requires the presence of 5α-dihydrotestosterone, which is synthesized from foetal testosterone by the action of 5α-reductase. This enzyme is localized in the urogenital sinus and external genitalia of humans (Wilson et al., 1981). Consequently, deficiencies of 5α-reductase will cause a rudimentary or undetectable prostate in addition to severe

abnormalities of the external genitalia, although the epididymides, vasa deferentia, and seminal vesicles remain normal (Imperato-McGinley et al., 1975). During the prepubertal period, the constitution of the human prostate remains more or less identical but begins to undergo morphologic changes into the adult phenotype with the beginning of puberty. The gland enlarges continuously in size to reach the adult weight of approximately 20 g by 25–30 years of age (Lowsley, 1912).

2.1.2 NORMAL ANATOMY, PHYSIOLOGY AND HISTOLOGY OF THE PROSTATE

The prostate is a small gland in the male reproductive system that helps produce semen, the thick fluid that carries sperm cells. It is a walnut-sized structure located beneath the bladder of males. It surrounds the upper part of the urethra. The urethra on the other hand is the tube that carries urine from the bladder. The function of the prostate is regulated by testosterone, the male sex hormone produced primarily in the testicles (Wilson et al., 1981).

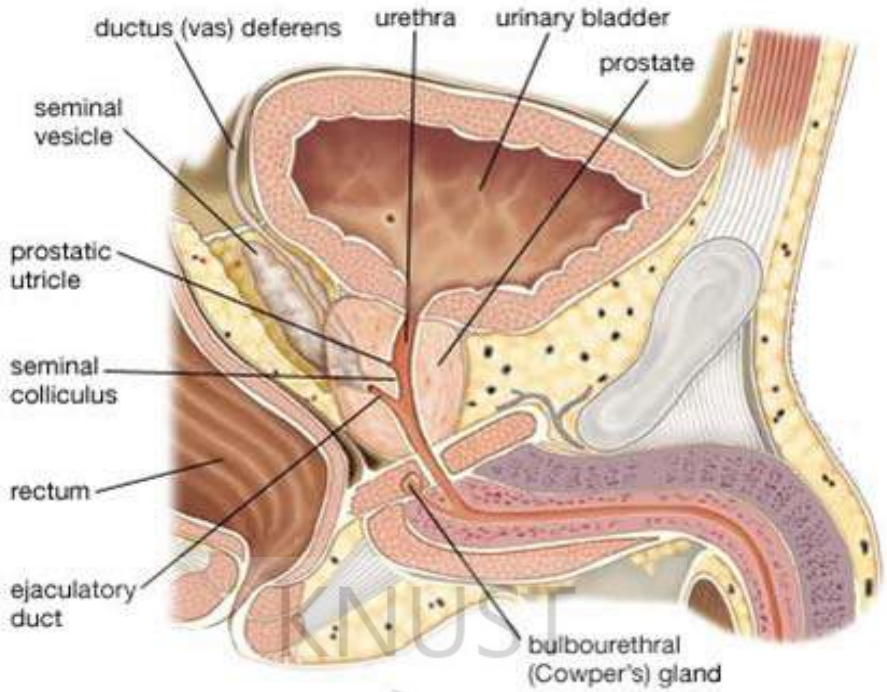

Fig. 2.1 The human prostate location *(Source: Encyclopaedia Britannia, 2007)*

The prostate gland is an exocrine gland found in all mammals. It secretes enzymes, amines, lipids and metal ions, essential for the normal function of the spermatozoa. Accumulation and secretion of exceptionally high levels of citrate is one of the principal functions of the prostate gland of humans and other animals (Costello and Franklin, 1989). The presence of the prostate is universal in mammals; when compared among species the prostate is recognized by variations in its anatomy, biochemistry and pathology. The mature mammalian prostate is a glandular organ which consists of epithelial and stromal cell types that are regulated by hormones. The epithelium consists of a single layer of polarized columnar epithelial cells, together with basal cells and neuroendocrine cells. The epithelial cells provide secretions that empty through ducts into the urethra to form a major component of the seminal plasma of the ejaculate. The surrounding stromal compartment however includes fibroblasts and smooth muscle cells, in addition to neuronal, lymphatic and vascular

components. One main functional difference between murine and human prostate is the presence of prostate specific antigen (PSA) expression in humans. PSA is an androgen-regulated serine protease produced by both prostate epithelial cells and prostate cancer and is the most commonly used serum marker for prostate cancer. It is also widely used to monitor responses to therapy (Karr et al., 1995).

By nature, the prostate gland is inactive during childhood. After puberty, due to the increasing level of circulating androgens, mainly testosterone, it becomes active and then begins to develop. Within the prostate, testosterone is metabolized to dihydrotestosterone (DHT) by 5-alpha reductase, an enzyme which is located mainly on the nuclear membrane. DHT is about two and half times more potent than testosterone. DHT binds to androgen receptor (AR) within the glandular cells. The complex DHT-AR formed activates several cell functions by targeting the DNA sequences in the nuclei resulting in growth and proliferation. A couple of possible functions have been suggested for prostate in the body. First, there is a high production of immunoglobulin in the prostate and the gland thus seems to have a protective function against local infections. Second, is the importance of prostate secretion in the motility of the spermatozoa (McNceal, 1988). The prostate is about (15-20 cm^3) in adult men. Based on predisposition to altered pathological processes in different parts of the gland, the prostate has been described as consisting of three zones:

a. Peripheral zone, from which more than 75% of cancers originate,
b. Transitional zone, which harbours the glandular tissue where excessive growth causes benign prostate hyperplasia (BPH), and

c. Central zone which contains about 25% of the glandular tissue and is resistant to both carcinoma and inflammation. Unlike the other zones, cells in the central zone have a number of distinctive morphological features including: more prominent and slightly basophilic cytoplasm as well as larger nuclei displaced at different levels in adjacent cells (McNceal, 1988; McNeal, 1981).

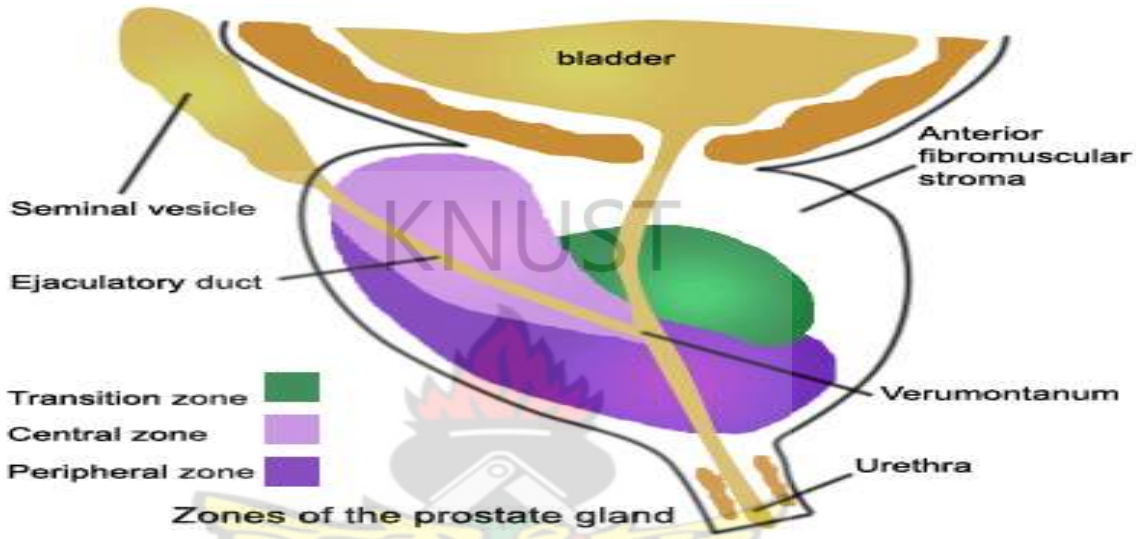

Fig. 2.2 Zones of the human prostate *(Source: www.infectiona2z.org)*

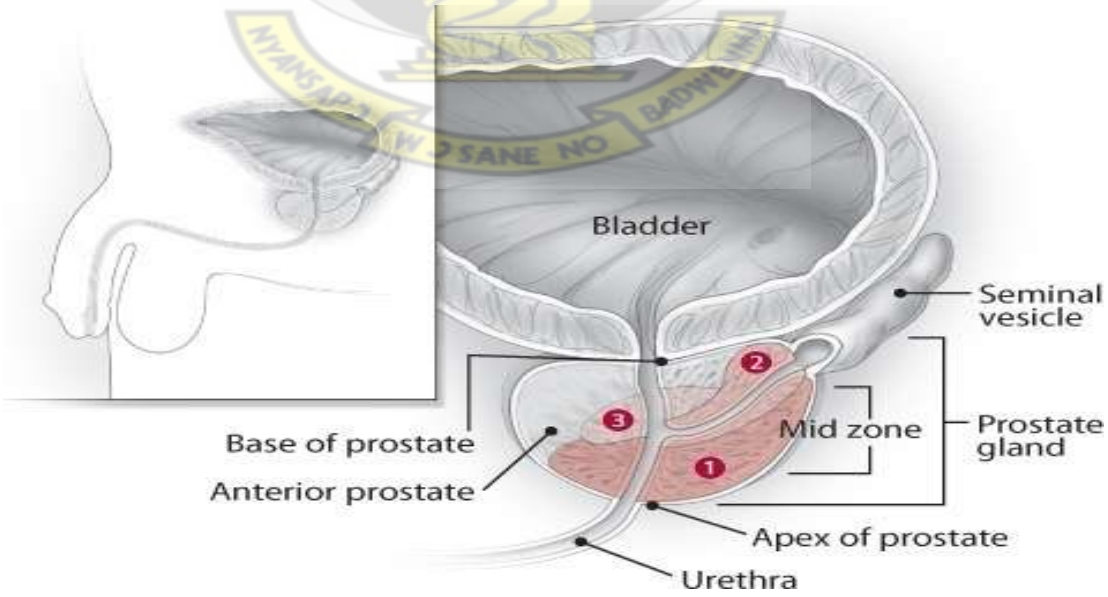

Fig. 2.3 Zones of the human prostate *(Source: www.infectiona2z.org)*

The base of the prostate is at the bladder neck and the apex at the urogenital diaphragm (Berry et al., 1984). The Denonvilliers' fascia, a thin, filmy layer of connective tissue, separates the prostate and seminal vesicles from the rectum posteriorly. Skeletal muscle fibers from the urogenital diaphragm extend into the prostate at the apex and up to the mid-prostate anteriorly (Kost and Evans, 1964).

In the twentieth century, several investigators maintained that the prostate gland was composed of diverse lobes by analogy with laboratory animals (Coffey, 1992; Berry et al., 1984). This concept became popular even though no distinct lobes can be seen in the human. Thereupon, McNeal established the current and most widely accepted concept of various zones rather than lobes of the prostate (McNeal, 1980; McNeal, 1981; McNeal, 1988). The peripheral zone comprises all the prostatic glandular tissue at the apex as well as all of the tissue located posteriorly near the capsule (Figure 1.2). In this zone, carcinoma, chronic prostatitis, and post inflammatory atrophy are relatively more common than in the other zones. The central zone is a cone-shaped area of the adult gland, with the apex of the cone at the confluence of the ejaculatory ducts and the prostatic urethra at the verumontanum (Figure 1.2). The transition zone consists of two equal portions of glandular tissue lateral to the urethra in the midgland. This portion of the prostate is involved in the development of age-related benign prostatic hyperplasia (BPH) and, less commonly, adenocarcinoma (McNeal, 1988).

The anterior fibromuscular stroma (AFMS) forms the convexity of the anterior external surface. The apical half of this area is rich in striated muscle, which blends into the gland and the muscle of the pelvic diaphragm (Figure 1.3). Toward the base, smooth muscle cells become predominant, blending into the fibers of the bladder

neck. The distal portion of the AFMS is important in voluntary sphincter functions, whereas the proximal portion plays a central role in involuntary sphincter functions (McNeal, 1972).

The histologic architecture of the prostate is that of a branched duct gland. Two cell layers; a luminal secretory columnar cell layer and an underlying basal cell layer, line each gland or duct. The lumens of otherwise normal prostatic glands and ducts frequently contain multilaminated eosinophilic concretions, termed corpora amylacea, that become more common in older men. Calculi are larger than those corpora with a predilection for the ducts that traverse the length of the surgical capsule, separating the transition and peripheral zones. The prostatic capsule is composed of fibrous tissue surrounding the gland. Although the term "capsule" is embedded in the current literature and common parlance, there is no consensus about the presence of a true capsule. This capsule is best appreciated posteriorly and posterolaterally as a layer more fibrous than muscular, between the prostatic stroma and extraprostatic fat (Ayala et al., 1989).

The seminal vesicles are located superior to the base of the prostate. They undergo confluence with the vas deferens on each side to form the ejaculatory ducts. The ejaculatory duct complex consists of the two ejaculatory ducts along with a second loose stroma rich in vascular spaces. The utricle (when present) is located between the ejaculatory ducts. The remnants of the utricle occasionally form cystic structures in the midline posteriorly. The seminal vesicles are resistant to nearly all of the disease processes that affect the prostate. Seminal vesicle involvement (SVI) by prostate cancer (PCa) is one of the most important predictors for PCa progression (Ohori et al., 1993). Metastatic PCa oftentimes involves pelvic lymph nodes. The prognostic

significance of this feature has been documented by several investigators (Cheng et al., 1998). In some individuals, periprostatic (PP) and periseminal vesicle (PSV) lymph nodes are present and, although uncommon, they may be involved by metastatic PCa as well, sometimes in the absence of pelvic lymph node metastases (Kothari et al., 2001).

2.2 PATHOLOGICAL CONDITIONS OF THE HUMAN PROSTATE

The human prostate gland is affected by a number of pathological conditions ranging from prostatitis (acute and chronic), prostatic abscesses, prostatic infarct, prostate calculi and prostatic tuberculosis and tumors which are either benign or malignant (Djavan et al., 2002).

2.2.1 BENIGN PROSTATIC HYPERPLASIA (BPH)

BPH simply refers to the enlargement of the prostate. It is non cancerous and occurs mostly in middle-aged and elderly men and it's usually confined to the transitional and periurethral zones. It is characterized by hyperplasia of prostatic stromal and epithelial cells which results in the formation of large, fairly discrete nodules in the periurethral region of the prostate. When sufficiently large, the nodules compress the urethral canal to cause partial or sometimes virtually complete, obstruction of the urethra thereby interfering with the urine flow. This can lead to a host of many complications such as urinary retention, urinary hesitancy, and frequent urination as well as increased risk of urinary tract infections (Djavan et al., 2002).

Benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are major health concerns that are likely to have an increasing impact in line with the gradual aging of the population. Benign prostatic hyperplasia (BPH) is the most prevalent of all

conditions in aging men, with the population prevalence in the 40- to 79-year age group estimated at 25% (Simpson et al., 1994). It is the most common benign adenoma in the male and represents a clinically significant cause of bladder outflow obstruction in up to 40% of men during their lifetime. As men in this age group account for 20% of the 727 million population of Europe, there are approximately 36 million European men with BPH. It is thus evidence that BPH constitutes a significant burden both on individuals and society at large (World Health Organization, 1991) as well as providing an important component of the workload for families and urologists.

2.2.2 PROSTATE CANCER - GLOBAL HEALTH MENACE

Cancer of the prostate (PCa) is now recognized as one of the most important medical problems facing the male population. In Europe for instance, PCa is the most common solid neoplasm, with an incidence rate of 214 cases per 1000 men, outnumbering lung and colorectal cancer (Boyle and Ferlay, 2004). Furthermore, PCa is currently the second most common cause of cancer death among men in Europe (Jemal et al., 2008). In addition, since 1985, there has been a slight increase in most countries in the number of deaths from PCa, even in countries or regions where PCa is not common (Quinn and Babb, 2002).

Prostate cancer affects elderly men more often than young men. It is therefore a bigger health concern in developed countries with their greater proportion being elderly men. Thus, about 15% of male cancers are PCa in developed countries compared to 4% of male cancers in undeveloped countries (Parkin et al., 2001). It is also worth mentioning that there are large regional differences in incidence rates of PCa. For example, in Sweden, where there is a long life expectancy and mortality from smoking-related diseases is relatively modest, PCa is the most common

malignancy in males, accounting for 37% of all new cases of cancer in 2004 (Swedish National Board of Health and Welfare, 2004).

Prostate cancer is the most common malignancy other than superficial skin cancer and the second leading cause of cancer death in American men. Unlike BPH, prostate cancer usually spread to other surrounding organs. The latest estimates of global cancer incidence show that prostate cancer has become the second most common cancer among men in the world, accounting for almost 11.7% of new cancer cases overall, which constitutes 19% of cancers in developed countries and 5.3% in developing countries (American Cancer, 2007). In some countries such as in the U.S., prostate surpassed lung as the most frequent cancer site among men (Dutta et al., 2005).

Prostate cancer is also increasing significantly worldwide. Clinical incidence is low in Asian men and highest in African-Americans and Scandinavians. Epidemiologic studies revealed that there are variations in the geographic and racial distribution of cancer of prostate. The incidence is low in Asia (3 – 8 per 100,000 men per year), intermediate in Africa and Eastern Europe, and high in Western Europe and North America (Kehinde et al., 2005).

In Europe, an estimated 2.6 million new cases of cancer are diagnosed each year. Prostate cancer constitutes about 11% of all male cancers in Europe (Bray et al., 2002) and accounts for 9% of all cancer deaths among men within the European Union (Black et al., 1997). Prostate cancer accounts for 33% of all newly diagnosed malignancies among men in the United States. In some countries such as in the U.S., prostate surpassed lung as the most frequent cancer site among men (Sadjadi et al., 2007).

Prostate cancer is the second most frequently diagnosed cancer in men, with 903,500 new cases estimated to have occurred in 2008. Nearly three-quarters of these cases were diagnosed in economically developed countries. Incidence rates of prostate cancer vary by more than 70-fold worldwide. The highest rates are recorded primarily in the developed countries of Europe, North America, and Oceania, largely because prostate specific antigen (PSA) testing is widely used and detects clinically important tumours, as well as other slow-growing cancers that might otherwise have escaped diagnosis. The lowest rates are in many parts of Asia (American Cancer Society, 2008).

2.2.3 ANATOMIC RELATIONSHIP

It appears that most PCa originates in the peripheral zone, but one in four arises in the transition zone: histological examination of radical prostatectomy specimens revealed that 68% of adenocarcinomas had arisen in the peripheral zone, and 24% had arisen in the transition zone (McNeal et al., 1988). The transition zone is also where most BPH originates; it may extend into the peripheral zone and occasionally originate from the peripheral zone. Interestingly, a histologic investigation has reported that about one-third of transition-zone PCa cases actually originate within BPH nodules (Bostwick et al., 1992).

2.2.4 AETIOLOGY OF PROSTATE CANCER

Extraordinarily, PCa is a heterogeneous disease with a variety of prognostic factors that influence the ultimate outcome for the patient. The most established risk factors involved in initiation and development of PCa include age, race, dietary or other environmental factors as well as family history of PCa. Age is considered the most prominent risk factor, with approximately 75% of all cases diagnosed in men between

50-70 years of age. There is also strong circumstantial evidence that androgens are implicated in the pathogenesis of PCa, yet there has so far been no conclusive evidence, despite numerous studies, that levels of circulating testosterone in individuals developing prostate cancer are higher than in controls (Slater and Oliver, 2000).

However, a recent analysis of previously published studies on hormonal predictors of risk for PCa by Shaneyfelt et al. (2000) did indicate that men with serum testosterone in the upper quartile of the population distribution have an approximately two-fold higher risk of developing prostate cancer. During the last few years, much effort has gone into determining molecular genetic mechanisms involved in the development of prostate malignancy. Hereditary prostate cancer is a subtype of familial prostate cancer in which the susceptible gene is inherited in a Mendelian fashion (Carter et al., 1990). A number of epidemiological studies indicate that dominantly inherited susceptible genes are able to cause nearly 10% of all prostate cancer cases and as much as 40% of early onset disease (<55 years of age) (Carter et al., 1990; Walsh and Partin, 1997).

As much as a 10-fold increase in life-time risk of developing prostate cancer is consequently reported in men with multiple first-degree relatives affected. Family-based linkage analysis has led to the identification of 7 different prostate cancer susceptible loci, located on several chromosomes (Karan et al., 2002). The possible existence of multiple prostate cancer genes may explain why there has been only limited confirmatory evidence of linkage for currently known highly susceptible loci or specific genes.

2.2.5 STAGING AND CLASSIFICATION OF PROSTATE CANCER

The stage of a cancer is a description of the extent the cancer has spread. The stage often takes into account the size of a tumour, how deeply it has penetrated, whether it has invaded adjacent organs, the number of lymph nodes it has possibly metastasized to, and whether it has spread to distant organs. Staging of cancer is the most important predictor of survival, and cancer treatment is primarily determined by staging. Thus, staging does not change with progression of the disease as it is usually used to assess prognosis. There is a uniform system for classifying prostate cancer based on its stage (Sobin et al., 2009).

The TNM classification describes the anatomic extent of cancer and is based on the premise that the choice of treatment and the chance of survival are related to the extent of the tumour at the primary site (T), the presence or absence of tumour in regional lymph nodes (N), and the presence or absence of metastasis beyond the regional lymph nodes (M). Tumours must be classified before treatment (ie, clinical staging [cTNM]) and after surgical intervention (ie, pathologic staging [pTNM]). T is usually divided into 4 major parts (T1 to T4), expressing increasing size or spread of the primary tumour. N and M comprise at least 2 categories each (0 and 1— absence or presence of tumour respectively). A number of sites have subcategories, with up to 4 subdivisions of T1 and T4 and 6 subdivisions of pN1 in breast carcinoma and 3 subdivisions of M in prostate carcinoma (Samowitz et al., 2001).

The TNM classification system for all solid tumours thus, takes into consideration the size and extension of the primary tumour, its lymphatic involvement, and the presence of metastases to classify the progression of cancer. Just like any other cancer, PCa staging can be divided into a clinical stage and a pathologic stage. In the TNM

(Tumour Node Metastasis) system for instance, clinical stage and pathologic stage are denoted by a small "c" or "p" before the stage. The clinical stage is based on all of the available information obtained before a surgery to remove the tumour. Thus, it may include information about the tumour obtained by physical examination, radiologic examination as well as endoscopy. The pathologic stage on the other hand adds additional information gained by examination of the tumour microscopically by a pathologist (Sobin et al., 2009). The TNM (Tumour Node Metastasis) classification for PCa using the European Association of Urology criteria for instance is shown below (Sobin et al., 2009).

2.2.5.1 Importance of Prostate Cancer Staging

The anatomic extent of disease is one of the three main axes of tumor classification, the others being topographic site and histological type. The importance of staging cannot be overemphasized in the management of patients. First and foremost, staging provides a format for the uniform exchange of information among clinicians regarding extent of disease and a basis for their selection of initial therapeutic approaches and consideration of the possible need for adjuvant treatment. For clinical investigators, staging allows the stratification of patients in observational and interventional therapeutic studies and facilitates the exchange of information through data sets and peer-reviewed communication. Meanwhile, staging also provides a means to evaluate non-anatomic prognostic factors at specific anatomic stages. For example, microsatellite instability in sporadic colon cancer is associated with improved prognosis, but this appears to apply to Stages III and IV but not Stages I and II carcinomas. Staging can also be used to measure early detection efforts (e.g., to see what impact screening could have on the stage distribution of disease at the time of diagnosis) (Samowitz et al., 2001).

2.2.5.2 Tumour Node Metastasis (TNM) classification of PCa using the European Association of Urology criteria.

According to Sobin et al. (2009), the PCa TNM is classified as follows using the European Association of Urology criteria.

T - Primary tumour

TX: Primary tumour cannot be assessed

T0: No evidence of primary tumour

T1: Clinically inapparent tumour not palpable or visible by imaging

T1a: Tumour incidental histological finding in 5% or less of tissue resected

T1b: Tumour incidental histological finding in more than 5% of tissue resected

T1c: Tumour identified by needle biopsy (e.g. because of elevated prostate-specific antigen [PSA] level)

T2: Tumour confined within the prostate

T2a: Tumour involves one half of one lobe or less

T2b: Tumour involves more than half of one lobe, but not both lobes

T2c: Tumour involves both lobes

T3: Tumour extends through the prostatic capsule

T3a: Extracapsular extension (unilateral or bilateral)

T3b: Tumour invades seminal vesicle(s)

T4: Tumour is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall

N - Regional lymph nodes

NX: Regional lymph nodes cannot be assessed

N0: No regional lymph node metastasis

N1: Regional lymph node metastasis

M - Distant metastasis

MX: Distant metastasis cannot be assessed

M0: No distant metastasis

M1: Distant metastasis

M1a: Non-regional lymph node(s)

M1b: Bone(s)

M1c: Other site(s)

2.3 THE GLEASON SCORE

The Gleason score is the most commonly used system for grading adenocarcinomas of the prostate (Gleason and Mellinger, 1974). The Gleason score can only be assessed using biopsy material (core biopsy or operative specimens). However, cytological preparations cannot be used. The Gleason score is the sum of the two most common patterns (grades 1-5) of tumour growth found. The Gleason score ranges between 2 and 10, with 2 being the least aggressive and 10 the most aggressive. In needle biopsy, it is recommended that the worst grade always should be included, even if it is present in < 5% of biopsy material (Amin et al., 2005).

Grading of conventional prostatic adenocarcinomas using the (modified) Gleason score system (Epstein et al., 2005) has been identified as the single strongest prognostic factor for clinical behaviour and treatment response. The Gleason score is therefore one of the parameters incorporated in nomograms that predict the risk of recurrence after prostatectomy (Amin et al., 2005).

2.3.1 INTERPRETATION OF THE GLEASON SCORE

The Gleason score is the sum of the most dominant and second most dominant (in terms of volume) Gleason grade. If only one grade is present, the primary grade is

doubled. If a grade comprises < 5% of the cancer volume, this grade is not incorporated in the Gleason score (5% rule). Both the primary and the secondary grade should be reported in addition to the Gleason score (e.g. Gleason score 7 [4 + 3]). A global Gleason score is given when there are multiple tumours, but a separate tumour focus with a higher Gleason score should also be mentioned. A tertiary Gleason grade 4 or 5, particularly if exceeding 5% of the prostate cancer volume, is an unfavourable prognosticator for biochemical recurrence. According to Harnden et al. (2007), the presence of the tertiary grade and its approximate proportion of the cancer volume should also be reported in addition to the Gleason score.

2.4 RISK FACTORS OF PROSTATE CANCER

2.4.1 AGE AND ETHNICITY

Prostate cancer has been known as a disease of elderly men. In the United States, over 70% of all cases of prostate cancer are diagnosed in men above 65 years of age (Weir et al., 2003). Diagnosis is rare before the age of 50 years, but after this age incidence and mortality rates both increase almost exponentially. The Surveillance, Epidemiology, and End Results (SEER) age-adjusted incidence rates in 1990 were 45.2 per 100,000 men age 50 to 55 years, 337.5 per 100,000 for those age 60 to 64 years, and more than 1,000 per 100,000 for men older than 65 years. The lifetime probability at birth of being diagnosed with prostate cancer in the United States has been estimated to be 8% (Haas and Sakr, 1997). Prostate cancer increases faster with age than does any other malignancy, and with an increase in the elderly population because of longer life expectancy, prostate cancer will continue to be a major health concern. The age-related increases in latent prostate cancer parallel the rising incidence of clinically detected cancer (Yatani et al., 1988). The probability of

developing prostate cancer increases from 0.005% among individuals aged >39 years to 2.2% (1 in 45) for those aged 40 to 59 years and 13.7% (1 in 7) for those aged 60 to 79 years. Overall, the lifetime risk of developing prostate cancer is 16.7% (1 in 6). The results of autopsy studies, however, suggest that the probability of developing histologic evidence of prostate cancer is even higher. A study done by Carter et al. (1990) showed that 20% of men aged 50 to 60 years and 50% of those aged 70 to 80 years had histologic evidence of malignancy.

It has been estimated that a 50-year-old man has a lifetime risk of 42% for developing histologic evidence of prostate cancer, a 9.5% risk of developing clinical disease, and a 2.9% risk of dying of prostate cancer. African Americans have among the highest rates of prostate cancer in the world (275.3 per 100,000 men) (Ward et al., 2004). The incidence among African Americans is nearly 60% higher than among whites (172.9 per 100,000), which, in turn, is higher than the rates for Hispanics (127.6 per 100,000) and Asians/Pacific Islanders (107.2 per 100,000). Moreover, for the period from 1992 to 1999, the mortality rate for African Americans was 2.3 times higher than for whites, 3.3 times higher than for Hispanics, and 5 times higher than for Asians/Pacific Islanders. Data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program indicate that a higher percentage of African Americans present with metastatic disease, but they are not more likely than whites to present with high-grade lesions (Clegg et al., 2002). For the last 3 decades, 5-year survival rates have improved significantly for African Americans, but they remain lower than for whites (93% vs. 98% for cases diagnosed from 1992 to 1998), although the gap between the 2 races appears to have narrowed (Bianco et al., 2002; Ward et al., 2004).

2.4.2 FAMILY HISTORY AND GENETIC SUSCEPTIBILITY

The risk of developing prostate cancer has been identified to double for men who have a father or brother affected by prostate cancer, and the risk increases further when multiple first-degree relatives are affected (Steinberg et al., 1990; Carter et al., 1990). Epidemiologic studies indicate that men with a positive family history are diagnosed at an earlier age—on average 6 to 7 years earlier—than those without affected first-degree relatives (Bratt, 2002). These studies estimate that 5% to 10% of all prostate cancer cases and up to 40% of those occurring at <55 years of age may have a hereditary basis (Bratt, 2002). Other than being diagnosed at an earlier age, hereditary prostate cancer does not differ clinically from disease that arises sporadically. The familial clustering of prostate cancer may be caused by inheritance of a susceptibility gene, but it may also be caused by exposure to common environmental factors or simply from chance alone because of the high incidence of this malignancy.

There have been 7 susceptibility loci for prostate cancer identified, but demonstrating linkage of currently known candidate genes has proved to be quite difficult (Simard et al., 2002). Using linkage analysis based on a genome-wide search, Smith et al. (1996) first mapped prostate cancer susceptibility to the hereditary prostate cancer HPC1 locus on the long arm of chromosome 1 in high-risk families from Sweden and the United States. In these families, prostate cancer developed at an early age, affected ≥5 family members, and spanned 2 generations. A subsequent pooled meta-analysis of 772 families with hereditary prostate cancer showed weak evidence of a genetic linkage to HPC1 in only 6% of the families (Xu, 2000). However, strong evidence of linkage was found in a subset of 8 families; in 2 of these families, a gene within the HPC1 locus—the 2′, 5′- oligoisoadenylate synthetase–dependent ribonuclease L

(RNASEL) gene—showed deleterious germ line mutations (Carpten et al., 2002). The RNASEL gene is believed to be a tumor suppressor gene that regulates cellular proliferation and apoptosis. Although RNASEL may represent a prostate cancer susceptibility gene, mutations of this gene will likely account for only a limited number of hereditary prostate cancer cases.

The familial clustering may also occur because of polymorphisms in genes that are important for prostate development and function. Among candidate genes, there is the transactivation domain encoded by exon 1 of the androgen receptor gene, which has 2 different nucleotide repeat variants (CAG and GGC) that affect gene transcription and activation (Simard et al., 2002). The CAG sequence varies in length from 11 to 31 repeats in healthy men, and the number of repeats is inversely related to the transcriptional activity of the androgen receptor.

Some studies have suggested that a shorter CAG repeat length is associated with increased prostate cancer risk, whereas other studies have failed to confirm this relation. Another candidate gene is SRD5A2, which encodes the 5 alpha-reductase type 2 that catalyzes the conversion of testosterone to the more active dihydrotestosterone. The Ala49Thr variant increases the catalytic activity of the enzyme and increases prostate cancer risk, particularly in African Americans and Hispanics (Makridakis et al., 1999).

2.4.3 DIET

A number of studies have identified diet as an important risk factor for prostate cancer. An influence of diet and environment on prostate cancer risk is suggested by studies of Japanese men who relocated to the United States (Shimizu et al., 1991; Whittemore et al., 1995; Cook et al., 1999). Instead of maintaining the low prostate

cancer incidence and mortality rates of their native land, their risk started to reflect the prevailing local rates. Notably, higher risk correlated with a younger age at the time of immigration and a longer time living in the new environment. The Western lifestyle, particularly the higher intake of fat, meat, and dairy products, may be responsible for conveying the higher prostate cancer risk. In a multicenter study of dietary factors, prostate cancer risk was associated with total fat intake in whites, African Americans, and Asian Americans About 10% to 15% of the difference in prostate cancer incidence among these ethnicities was attributed to differences in saturated fat intake (Shimizu et al., 1991; Whittemore et al., 1995; Cook et al., 1999).

Other studies have also linked consumption of diets rich in red meat with prostate cancer risk (Giovannucci et al., 1993). Beef and dairy products for instance are major sources of dietary branched fatty acids. An enzyme that plays a key role in the peroxisomal oxidation of these fatty acids (α-methyl-coenzyme-M-reductase) is upregulated in prostate cancer but not in the healthy prostate. The oxidation process generates hydrogen peroxide, which may be a source of carcinogenic oxidative damage to the prostate genome. Similarly, grilling or frying meats at high temperatures produces heterocyclic amines and other potent carcinogens that increase risk of certain malignancies, although a link with prostate cancer has not yet been established (Shirai et al., 2002). The lower incidence of prostate cancer in Japan than in the United States may be related to the difference in intake of soybean products that are rich in isoflavones, such as genistin and daidzin. Experimental studies suggest that these isoflavones can inhibit protein tyrosine kinases that are important in cell proliferation and transformation, as well as in angiogenesis, and thereby limit the development and metastasis of prostate tumors (Shirai et al., 2002). Alternatively, these isoflavones may reduce circulating androgen concentrations and increase the

concentration of sex hormone–binding globulin. Differences in diet may also help to explain the relation observed between plasma levels of insulinlike growth factor–1 (IGF-1) and prostate cancer risk. IGF-1 is primarily secreted by the liver; high fat and caloric diets stimulate growth hormone and insulin production and in turn IGF-1 production. This factor is known to regulate the proliferation and differentiation of cancer cells and to prevent them from undergoing apoptosis. In 3 prospective cohort studies, men in the highest quartile of IGF-1 concentrations had a 1.7- to 4.3-fold higher risk of prostate cancer than those in the lowest quartile (Chan et al., 1998).

2.4.4 HORMONAL AND OTHER FACTORS

The growth and differentiation of the prostate is controlled by androgen. Men who underwent castration before puberty and those with congenital abnormalities in androgen metabolism do not develop prostate cancer (Haas and Sakr, 1997). Moreover, androgen blockade with 5α-reductase inhibitors is also effective in causing involution of benign prostatic hyperplasia (BPH), whereas androgen ablation either surgically or with luteinizing hormone–releasing hormone agonists is an effective strategy in the treatment of advanced prostate cancer. Nevertheless, plasma testosterone or dihydrotestosterone concentrations determined either prospectively or at the time of cancer diagnosis have not been convincingly associated with increased risk of prostate cancer (Hsing et al., 2001). However, if androgens are indeed important in prostate cancer development, then measurement may need to be done in early adulthood, years before prostate cancer is actually detected.

Recent results from epidemiologic studies suggest that a high body mass index (BMI) and bone mass may be associated with prostate cancer. In the Cancer Prevention Study II, >400,000 men who were free of cancer were observed prospectively for 16

years (Clegg et al., 2002; Calle et al., 2003). Risk of prostate cancer mortality increased significantly in association with higher baseline BMI (P <0.001). Men with a BMI of 35.0 to 39.9 had a 34% greater risk of dying of prostate cancer than those with a normal BMI. However, this relation was less pronounced than for other malignancies. In contrast, BMI and other measures of body size at age 21 were unrelated to prostate cancer risk in an Australian case-control study (Giles et al., 2003). High calcium intake has also been suggested to relate to an increased prostate cancer risk in the Physicians' Health Study (Chan et al., 2001). Other factors including vasectomy, sexual activity, smoking, alcohol consumption, physical activity, and social class have not been shown to affect prostate cancer risk (Haas and Sakr, 1997; Albertsen and Gronberk, 2002; Chacko et al., 2002).

2.5 PROTECTIVE FACTORS

According to Miller et al. (2002) epidemiologic and case-control studies suggest that consumption of tomatoes and its products is associated with a lower risk of prostate cancer. It has also been pointed out that lycopene, an antioxidant in raw and processed tomato products, may be responsible for the lower risk, although other carotenoids and phytochemicals in these products may also contribute to the benefit. A report from Giovannucci et al. (2002) indicates that in a study of 2,481 men, high levels of lycopene consumption were associated with a 16% lower risk of prostate cancer as compared with consumption of its small amounts. However, a controlled dietary intervention study is required to substantiate the benefit of lycopene and tomato products as antioxidants. Moreover, the mechanism by which lycopene may reduce the prostate cancer risk has to be established.

Meanwhile, a number of studies including that of Vogt et al. (2003) and Clark et al. (1998) have suggested that selenium offers some level of protection against prostate cancer. Selenium is an essential trace element found mainly in fish, grains and meat. Vogt et al. (2003) reported that in a population-based, case-controlled study of white and African American men, serum selenium was inversely associated with prostate cancer risk and that men with the highest quartile of serum selenium had 29% lower risk than those in the lowest quartile. Their study results indicated that the pattern was similar for both whites and African Americans and that the risk reduction was found at selenium levels > 0.135 µg/mL. They pointed out that the strongest relation was found for men with low serum tocopherol concentrations, suggesting that the benefit may relate to the mechanism of antioxidant.

It has been reported by Weiderpass et al. (2002) that men with diabetes mellitus appear to have a lower risk of developing prostate cancer. According to their results from a population-based cohort study conducted in Sweden, men hospitalized for diabetes had a 9% lower risk of prostate cancer, and those hospitalized for a diabetic complication had an 18% lower risk than men in other population-based registers. In different hospital-based, case-control study, Rosenberg et al. (2002) reported that diabetes was associated with a 40% lower risk of prostate cancer overall and a 53% lower risk of regional or advanced prostate cancer and that this effect was found mainly in whites and Hispanics, but not in African Americans.

Obesity and hyperinsulinaemia are known to be associated with diabetes, and both may reduce IGF-1 levels and alter endogenous steroid metabolism. It however remains unclear, and yet to be determined, whether these effects actually contribute to

the observed inverse relation between diabetes and prostate cancer risk (Hammarsten and Högstedt, 2005).

2.6 DIAGNOSIS OF PROSTATE CANCER

The main diagnostic tools that are currently used to obtain evidence of PCa include direct rectal examination (DRE), serum concentration of prostate specific antigen (PSA) and transrectal ultrasonography (TRUS). Its definite diagnosis however, depends on the presence of adenocarcinomas in prostate biopsy cores or operative specimens. Histopathological examination also allows grading and determination of the extent of the tumour (Carvalhal et al., 1999).

2.6.1 DIGITAL RECTAL EXAMINATION (DRE)

Most prostate cancers are located in the peripheral zone of the prostate and may be detected by DRE when the volume is large. A suspect DRE is an absolute indication for prostate biopsy. In about 18% of all patients, PCa is detected by a suspect DRE alone, irrespective of the PSA level (Richie et al., 1993). A suspect DRE in patients with a PSA level of up to 2 $ngmL^{-1}$ has a positive predictive value of 5-30% (Carvalhal et al., 1999).

2.6.2 PROSTATE-SPECIFIC ANTIGEN (PSA)

The measurement of PSA level has revolutionized the diagnosis of PCa (Stamey et al., 1987). Prostate-specific antigen (PSA) is a kallikrein-like serine protease produced almost exclusively by the epithelial cells of the prostate. For practical purposes, it is organ-specific but not cancer-specific. Thus, serum levels may be elevated in the presence of benign prostatic hypertrophy (BPH), prostatitis and other non-malignant conditions. However, the level of PSA as an independent variable is a

better predictor of cancer than suspicious findings on DRE or TRUS (Catalona et al., 1994).

There are many different commercial test kits for measuring PSA, but no commonly agreed international standard exists (Semjonow et al., 1996). The level of PSA is a continuous parameter: the higher the value, the more likely is the existence of PCa (Table 2.1). This means there is no universally accepted cut-off or upper limit. The finding that many men may harbour PCa, despite low levels of serum PSA, has been underscored by the findings from a US prevention study (Thompson et al., 2004). Table 2.1 gives the rate of PCa in relation to serum PSA for 2,950 men in the placebo-arm and with normal PSA values.

Table 2.1 Risk of PCa in relation to low PSA values

PSA level ($ngmL^{-1}$)	Risk of PCa
0-0.5	6.6%
0.6-1	10.1%
1.1-2	17.0%
2.1-3	23.9%
3.1-4	26.9%

These findings in table 2.1 highlight an important issue about lowering the PSA-level threshold, which is how to avoid detecting insignificant cancers with a natural history unlikely to be life threatening (Stamey et al., 1987). As yet, there is no long-term data to help determine the optimal PSA threshold value for detecting non-palpable, but clinically significant, PCa. Several modifications of serum PSA value have been described, which may improve the specificity of PSA in the early detection of PCa. They include: PSA density, PSA density of the transition zone, age-specific reference

ranges and PSA molecular forms. However, these derivatives and certain PSA isoforms have limited usefulness in the routine clinical setting (Stamey et al., 1987).

2.6.2.1 Free/total PSA ratio (f/t PSA)

The free/total PSA ratio (f/t PSA) is the concept most extensively investigated and most widely used in clinical practice to discriminate BPH from PCa. The ratio is used to stratify the risk of PCa for men who have total PSA levels between 4 and 10 $ngmL^{-1}$ and a negative DRE. In a prospective multicentre trial, PCa was found on biopsy in 56% of men with an f/t PSA < 0.10, but in only 8% of men with f/t PSA > 0.25 (Catalona et al., 1998). Nevertheless, the concept must be used with caution as several pre-analytical and clinical factors may influence the f/t PSA. For example, free PSA is unstable at both 4°C and at room temperature. In addition, assay characteristics may vary and concomitant BPH in large prostates may result in a 'dilution effect' (Stephan et al., 1997). However, f/t PSA is clinically useless in total serum PSA values > 10 ng/mL and in follow-up of patients with known PCa.

2.6.3 PROSTATE BIOPSY

2.6.3.1 Baseline biopsy

The need for prostate biopsies is usually determined on the basis of the PSA level and/or a suspicious DRE. The patient's biological age, potential co-morbidities and the therapeutic consequences are also vital considerations.

The first elevated PSA level usually should not prompt an immediate biopsy. The PSA level should be verified after a few weeks by the same assay under standardized conditions (i.e. no ejaculation and no manipulations, such as catheterization, cystoscopy or TUR, and no urinary tract infections) in the same diagnostic laboratory,

using the same methods (Eastham et al., 2003; Stephan et al., 2006). It is now considered the standard of care to perform prostate biopsies guided by ultrasound. Although a transrectal approach is used for most prostate biopsies, some urologists prefer to use a perineal approach. The cancer detection rates of perineal prostate biopsies are comparable to those obtained of transrectal biopsies (Hara et al., 2008; Takenaka et al., 2008). The ultrasound-guided perineal approach is a useful alternative in special situations, e.g. after rectal amputation.

2.6.3.2 Repeat biopsy

The indications that usually call for repeat biopsy include:

• rising and/or persistent PSA, suspicious DRE;

• atypical small acinar proliferation (ASAP).

The optimal timing of a repeat biopsy is uncertain. It depends on the histological outcome of the baseline ASAP biopsy and the index of a persistent suspicion of PCa (high or dramatically rising PSA, suspect DRE, family history). The later the repeat biopsy is done, the higher the detection rate (Epstein and Herawi, 2006). High-grade prostatic intraepithelial neoplasia (PIN) as an isolated finding is no longer considered an indication for re-biopsy (Moore et al., 2005). A repeat biopsy should therefore be prompted by other clinical features, such as DRE findings and PSA level. It has however been known that if PIN is extensive (i.e. in multiple biopsy sites), it could be a reason for early re-biopsy as the risk of subsequent prostate cancer is slightly increased (Merrimen et al., 2009). Meanwhile, it has been suggested that if clinical suspicion for prostate cancer persists in spite of negative prostate biopsies, MRI may be used to investigate the possibility of an anterior located prostate cancer, followed by TRUS or MRI-guided biopsies of the suspicious area (Merrimen et al., 2009).

2.6.3.3 Sampling sites and number of cores

On baseline biopsies, the sample sites should be as far posterior and lateral as possible in the peripheral gland. Additional cores should be obtained from suspect areas by DRE/TRUS. These should be chosen on an individual basis. Sextant biopsy is no longer considered adequate. At a glandular volume of 30-40 mL, at least eight cores should be sampled. More than 12 cores are not significantly more conclusive (Moran et al., 2006). The British Prostate Testing for Cancer and Treatment Study has also recommended 10-core biopsies (Donovan et al., 2003).

2.6.3.4 Seminal vesicle biopsy

Indications for seminal vesicle biopsies are poorly defined. At PSA levels > 15-20 ng/mL, a biopsy is only useful if the outcome will have a decisive impact on treatment, i.e. if the biopsy result rules out radical removal for tumour involvement or radiotherapy with intent to cure. At PSA levels > 15-20 $ngmL^{-1}$, the odds of tumour involvement are 20-25% (Linzer et al., 1996).

2.6.3.5 Transition zone biopsy

Transition zone (TZ) sampling during baseline biopsies has been linked to the provision of a very low detection rate. TZ sampling has therefore been suggested to be confined to repeat biopsies (Pelzer et al., 2005).

2.6.4 DIAGNOSTIC TRANSURETHRAL RESECTION OF THE PROSTATE (TURP)

The use of diagnostic TURP instead of repeat biopsies is of minor importance. Its detection rate is no better than 8% and makes it a poor tool for cancer detection (Zigeuner et al., 2003).

2.7 LIPID PEROXIDATION

2.7.1 GENEREAL OVERVIEW

Lipid peroxidation is a reaction whereby molecular oxygen is incorporated into poly-unsaturated fatty acids (PUFA) to yield lipid peroxides. The process proceeds by a free radical chain reaction mechanism. During the lipid peroxidation process, lipids containing carbon-carbon double bonds undergo oxidative deterioration and a large number of toxic by-products are eventually formed which have effects at a site away from area of their generation. Hence they behave as toxic 'second messengers'. Membrane lipids are particularly susceptible to lipid peroxidation. Since membranes form the basis of many cellular organelles like mitochondria, plasma membranes, endoplasmic reticulum, lysosomes, peroxisomes etc. the damage caused by lipid peroxidation is highly detrimental to the functioning of the cell and its survival (Raha and Robinson, 2000).

Presence of polyunsaturated fatty acids (PUFAs) in the phospholipids of the bilayer of biological membranes is the basis of their critical feature of fluidity. Since lipid peroxidation attacks the components that impart these properties, it affects the biophysical properties of membranes. Lipid peroxidation not only decreases the membrane fluidity but also changes the phase properties of the membranes. In addition, cross-linking of membrane components restricts the mobility of membrane proteins. Meanwhile, peroxidative attack on PUFAs of a biological membrane will compromise one of its most important – its ability to act as barrier. As a result, lysosomes become fragile or 'leaky'. However, the leakage of cytosolic enzymes from whole cells may have some detrimental effects. For example, peroxidative attack

on the plasma membrane of hepatocytes causes extensive damage to the extent that molecules as large as enzymes are able to leak out (Raha and Robinson, 2000).

Similar to any radical reaction, the lipid peroxidation process typically involves three major steps: initiation, propagation and termination.

2.7.2 INITIATION

This is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH· and HO_2, which combines with a hydrogen atom to produce water and a fatty acid radical (Halliwell and Chirico 1993).

2.7.3 PROPAGATION

In this step, the fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This too is an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and lipid peroxide, or cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way (Halliwell and Chirico 1993).

2.7.4 TERMINATION

When a radical reacts with a non-radical, another radical is produced; this is why the process is called a 'chain reaction mechanism'. The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals. Living organisms have evolved different molecules that speed up termination by catching free radicals thereby protecting the cell membrane.

One important such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes: superoxide dismutase, catalase, and peroxidise (Halliwell and Chirico 1993).

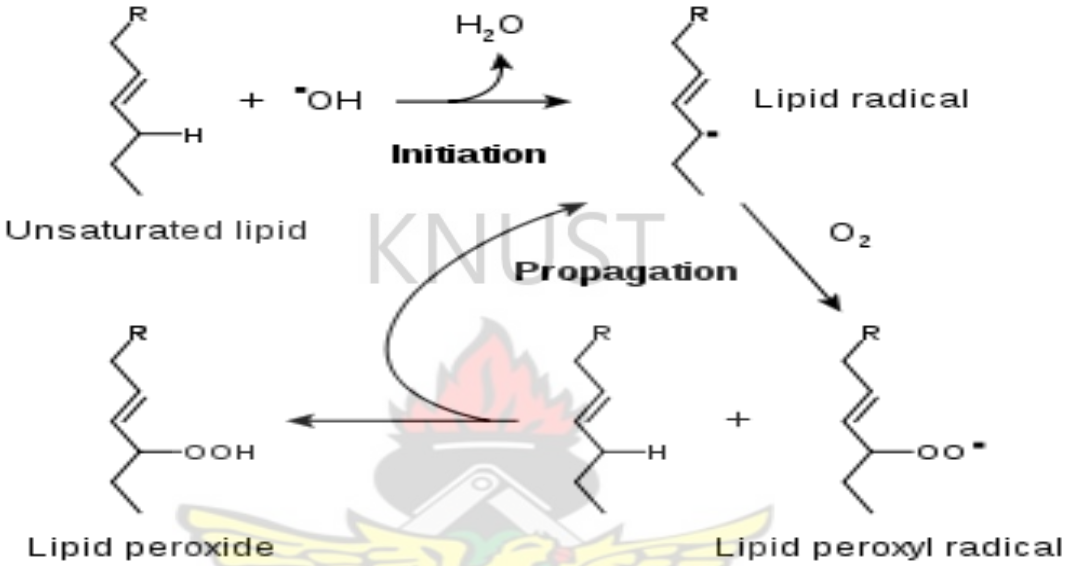

Figure 2.4 Mechanism of Lipid Peroxidation *(Source: www.wikipedia.org)*

2.7.5 EFFECTS OF LIPID PEROXIDATION

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage (Ozan et al., 2002). Oxidative stress is characterized by disequilibrium between oxidant and antioxidant forces in favour of oxidation. The lipid peroxidation process, initiated by the reaction of free radicals with polyunsaturated fatty acids (Hubel et al., 1989) is used as a marker of oxidant force. The lipid peroxidation products of major toxicological interest are malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and the various 2-alkenals.

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and is used as an indicator of oxidative stress in cells and tissues. It can also be generated during prostaglandin biosynthesis in cells. According to Marnette (1994), MDA reacts with amino groups on proteins and other biomolecules to form a variety of adducts, including adducts with DNA bases that have been found to be mutagenic and carcinogenic. Increased levels of lipid peroxidation products, by measurement of MDA, have also been found to be associated with benign prostatic hypertrophy (BPH) (Rosaria et al., 2003).

Lipid peroxidation has been implicated in the pathogenesis of a number of diseases and clinical conditions including fibrosis and cancer, atherosclerosis, a host of chronic inflammatory conditions, preeclampsia and eclampsia as well as diabetes. Experimental and clinical evidence suggest that aldehyde products of lipid peroxidation can also act as bioactive molecules in physiological and pathological conditions which can effect and modulate at very low and non-toxic concentrations several cell functions including cell proliferation, gene expression, signal transduction as well as target cell response (Rosaria et al., 2003).

Cell membranes are generally composed of lipid bilayers and thiol containing proteins. The unsaturated lipid component and thiol containing proteins of the cell membranes are vulnerable to free radical attack. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals, or oppose their actions (Sies, 1991). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Krishna Mohan and Venkataramana, 2007). Defense mechanisms of the body however, play an important role in the formation of

antioxidants, putting up remarkable attempt to minimize the damage, as an adaptation to stressful situations.

2.7.6 MALONDIALDEHYDE

2.7.6.1 Introduction

Malondialdehyde, MDA, is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation (Janero, 1990) and also during arachidonic acid metabolism for the synthesis of prostaglandins (Marnette, 1999). MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA. The monitoring of MDA levels in biological materials can be used as an important indicator of lipid peroxidation in vitro and in vivo for various diseases. This will mainly focus on MDA chemistry, biochemistry, routes of formation, detection, and biological health aspects (Marnette, 1999).

2.7.6.2 Routes of formation

Malondialdehyde is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, including MDA. MDA can be generated during cyclooxygenase (COX) catalysis in human platelets, formation from prostaglandin endoperoxide (PGH_2), catalyzed by thromboxane synthase (Diczfalusyet al., 1977) and in liver cells (Plastaras et al., 2000) by breakdown of PGH_2.

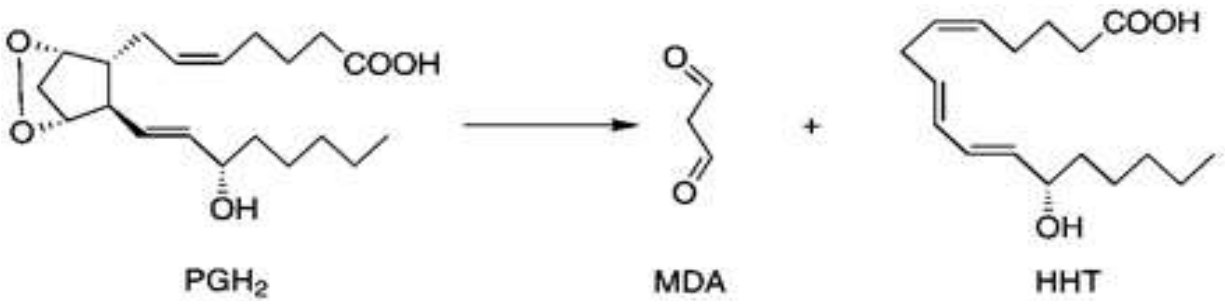

Figure 2.5 Conversion of prostaglandin endoperoxide (PGH_2) to 12-hydroxyheptadecatrienoate (HHT) and malondialdehyde (MDA). *(Source: www.wikipedia.org).*

2.7.6.3 Chemical and biological properties

MDA is also called malondialdehyde or bis (dimethyl acetal). It has the molecular formula $C_7H_{16}O_4$ and molecular weight 164.2. The boiling point is 183^0C, and the freezing point is 130^0C. It was suggested that the bulk of MDA in human plasma is bound to protein; this would help explain the very low levels of MDA in plasma as measured under standard assay conditions (Lefevre et al., 1996).

In addition to biological properties, DNA-protein cross-links are another result of the reaction between DNA and MDA. It has been reported that MDA reacts with DNA bases to form a series of adducts such as deoxyadenosine (M_1A), and deoxycytidine (M_1C) and deoxyguanosine (M_1G) (Marnette, 1994). The major adduct is a pyrimidopurinone, abbreviated M_1G or M_1dG. This adduct possesses a blocked Watson-Crick base-pairing region that has been shown to be mutagenic (Marnette, 1994). Another work has reported that it is the first endogenous DNA lesion found to be a target of nucleotide excision repair enzymes, and which is likely be a major endogenous DNA adduct that significantly contributes to cancer (Wang et al., 2004).

Recently, it has been reported that MDA adducts produced in mammalian cells may block enzyme RNA polymeraseII translocation and be subject to removal from DNA by transcription-coupled repair. An M_1dG formation result in two chemically distinct DNA adducts.

Meanwhile, both M_1dG and N^2-OPdG are highly mutagenic, inducing both frame shifts and base substitution, in bacteria and mammalian cells. Therefore, the efficient repair of MDA adducts is essential for genomic stability (Cline et al., 2004).

2.8 FREE RADICALS, REACTIVE OXYGEN SPECIES (ROS) AND ANTIOXIDANTS

2.8.1 GENERAL OVERVIEW

In order to obtain the full understanding of the effects of oxidative stress in prostate cancer, it is very essential to have a clear understanding of the relevant players of oxidative stress; free radicals, reactive oxygen species (ROS) and antioxidants.

The production of energy in the human body requires oxygen. According to Halliwell and Gutteridge (1989), cellular energy, at the molecular level, is produced by oxidative phosphorylation in mitochondria through electron transport from electron donors to electron acceptor such as oxygen; and that during these reaction steps, hydrogen [in the form of reducing equivalents (NADPH)] is produced and energy is also produced in the form of high-energy phosphates (ATP) whereas four- electron reduction of molecular oxygen to water produces free radicals and oxygen-derived reactive species (Halliwell and Gutteridge, 1989).

2.8.2 DEFINITION AND EFFECTS OF FREE RADICALS

The term free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell and Gutteridge, 1989). This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems (Miller et al., 2002). The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress (Valko *et al.*, 2004). Oxygen-free radicals (OFR), or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. It has been estimated that the average person has around 10000–20000 free radicals attacking each body cell each day. Despite the cell's antioxidant defense system to counteract oxidative damage from ORF, radical-related damage of DNA and proteins have been proposed to play a key role in the development of degenerative processes including amyotrophic lateral sclerosis, ischemic heart disease, Alzheimer disease, Parkinson disease, cancer, arthritis and aging. ROS are generated by mitochondria as the toxic by-products of oxidative phosphorylation, their energy generating pathway (Valko et al., 2004).

2.8.3 Classification of Free Radicals

There are two groups of free radicals; (1) a reactive oxygen species (ROS), such as super oxide anion radical $(O^{\cdot}{}_2)^{-}$, hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) as well as singlet oxygen ($O2^1$); and (2) reactive nitrogen species (RNS), such as nitrous oxide ($NO^{\cdot}$), nitric oxide ($NO_2^{\cdot}$), peroxynitrite ($OONO^{\cdot}$) (Kasapoglu and Ã–zben, 2001).

Because ROS are so reactive, they can inflict considerable damage on living cells if formed in significant amounts. These damage results primarily form enzyme inactivation, polysaccharide depolimerization, DNA breakage and membrane destruction. A lot of oxygenated compounds, particularly aldehyde such as malondialdehyde (MDA), are produced during the attack of free radicals to membrane lipoprotein and polyunsaturated fatty acid. Products of lipid peroxidation formed in the primary site reaching the other organs and tissue via the blood stream provoke lipid peroxidation and cause cellular and tissue damage. The increase of lipid peroxidation could possibly play a role in the complication of cardiovascular disease, chronic pulmonary disease, cataract, cancer (Kasapoglu and Ã–zben, 2001). MDA measurement is an indicator of lipid peroxidation and is used as a biomarker of oxidative stress.

2.8.4 Oxidative stress

Oxidative stress occurs when there is a disrupted balance by extreme production of reactive oxygen species and / or by insufficient anti oxidative defenses, including superoxide dismutase (SOD), catalase (CAT), vitamins (C,E), ß-carotene, uric acid, glutathione (GSH), and trace elements such as zinc (Zn), selenium (Se), magnesium (Mg), cupper (Cu), iron (Fe), which are Co factors for many biochemical reactions. ß-carotene is a member of a class of plant pigment molecules referred to as the carotenoids; it acts as provitamin A. It also performs a function as antioxidants, protecting the body from the potentially damaging effects of various oxidizing agents. ß-carotene can exert antioxidant effect in lipid systems by quenching singlet oxygen, peroxyl radical and inhibit lipid peroxidation. ß-carotene and other carotenoids offer protection against a variety of serious degenerative diseases especially cancer, cardiovascular and cataract (Pace and Leaf, 1995).

Apoptosis has been known as a physiological mechanism that preserves homeostasis in the turnover of normal tissue and can be initiated by a number of mechanisms including defects in activation signalling, cytokine imbalance or super-antigen stimulation. Usually, many agents that induce apoptosis are either oxidants or activators of cellular oxidative metabolism. This suggests that the generation of reactive oxygen species (ROS) could possibly induce apoptosis. It has been pointed out that oxidative stress mediated by the generation of ROS induces apoptosis (Pace and Leaf, 1995). ROS, namely: superoxide (O2•−), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO•), lipid peroxides (LOOH), nitric oxide (NO) and hypochloric acid (HOCl) are usually toxic and consequently oxidize all biological matter. Generally, the oxidants produced in the oxidative burst process by neutrophils and macrophages participate in the destruction of microorganisms and induce cellular injury of bystander cells (Dobmeyer *et al.*, 1995).

2.8.5 TYPES OF ROS

2.8.5.1 Singlet oxygen ($^1O_2$)

Singlet oxygen ($^1O_2$) is very reactive and is mostly involved in photochemical reactions. It does not contain unpaired electrons and is therefore not considered as a free radical. It is formed in vivo by enzymatic activation of oxygen. A classical example is the lipo-oxygenase activity during prostaglandin biosynthesis (Cadenas and Sies, 1984). Singlet oxygen has been identified as a very reactive ROS and has also been found to induce an amount of carcinogenic, genotoxic, and mutagenic effects through its action on polyunsaturated fatty acids (PUFAs) and DNA (Di Mascio *et al.*, 1994).

2.8.5.2 Peroxyl radicals (HOO•)

Peroxyl radicals are generally considered to be produced mainly during lipid peroxidation, a process which is initiated by abstraction of a hydrogen atom from unsaturated lipids. Despite many folds of useful roles played by lipid peroxidation in many biological processes, peroxidation of membrane PUFAs may adversely affect many paramount functional parameters including membrane fluidity, electrical potential and permeability as well as controlled transport of metabolites across the membrane (Halliwell, 1989).

2.8.5.3 Superoxide anion ($O_2^{\bullet -}$)

Superoxide is an anionic radical which is formed by the reduction of molecular oxygen through the acceptance of a single electron. It is formed when hydroperoxyl radical, which is unstable at physiological pH dissociates. It is primarily generated in vivo by the electron transport chains in the mitochondria and microsomes through electron leakage - a process which has been found to have a positive correlation with increase in oxygen utilization (McCord and Omar, 1993). They are also formed by metal ion-dependent oxidation of epinephrine and norepinephrine, and by the action of enzymes such as xanthine oxygenase, tryptophan hydroxylase as well as indoleamine dioxygenase. Quite interestingly, activated phagocytes have also been found to possess a considerable number of metabolic pathways for the production of superoxide radicals in response to bacterial infection (Curnutte and Babior, 1987).

2.8.5.4 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide and superoxide may at certain times, undergo further transformations in the presence of transition metals (especially copper and iron) which then results in the production of these highly reactive hydroxyl radicals, by the

Haber-Weiss or Fenton reactions. Eventually, this property when combined with the membrane permeability of hydrogen peroxide, results in superoxide and hydrogen peroxide the ability to affect the integrity of distant molecules within the cell (Cochrane, 1991).

2.8.5.5 Hydroxyl radical (HO•)

Within the ROS family, the hydroxyl radical is the most aggressive and can bring about extensive damage to different types of molecules including proteins, nucleic acids, and lipids. In DNA, the HO• can induce several effects which include: cross-linking between bases, cross-linking between DNA and proteins, base and sugar modifications, strand breaks, and formation of adducts (Cochrane, 1991). According to Stadtman (1992), the action of hydroxyl radicals on proteins has been identified to resulting in extensive protein–protein cross-linking. However, severe attacks on cellular integrity have been identified as one of the most serious outcomes of the role played by hydroxyl radicals in the peroxidation of polyunsaturated fatty acids (PUFAs) (Gutteridge, 1995).

2.9 ANTIOXIDANTS

Oxidative stress is characterized by disequilibrium between oxidant and antioxidant forces in favour of oxidation. Thus, oxidative stress results when there is an imbalance between the free radicals (mainly but not limited to ROS) production and their inactivation by antioxidants. Therefore, in order to help prevent oxidative damage to cellular components such as deoxyribonucleic acid (DNA), proteins and lipids (Sies, 1997) a very critical balance must always be maintained between free radicals production and antioxidant substances. By definition, antioxidants are substances which when present at low concentrations compared with that of an

oxidizable substrate, significantly inhibit or delays oxidation of the substrate. Examples of antioxidants include vitamin C (ascorbic acid), glutathione (GSH), glutathione peroxidase, N-acetylcysteine (NAC), vitamin E (tocopherol), selenium, and uric acid (Vertuani *et al.*, 2004).

2.9.1 Classification of antioxidants

Generally, antioxidants are classified into two major divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). The water-soluble antioxidants mostly react with oxidants in the cell cytosol and the blood plasma, whereas the lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). The synthesis of theses antioxidants may occur in the body or obtained from the diet. In body fluids and tissues, the antioxidants occur in different concentrations. Glutathione for instance, is mostly present within cells, while others like uric acid are more evenly distributed. Usually, the concentration of an antioxidant determines the degree of protection that can be provided to cells and tissues. However, other factors such as the reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts also affect the degree of protection to cells and tissues (Vertuani et al., 2004).

2.9.1.2 Tocopherol (vitamin E)

Vitamin E is the collective name for a set of eight related tocopherol which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001). Among them, α-tocopherol has been the most studied in detail and it has the highest bioavailability; the body preferentially also absorbs and metabolizes this form (Brigelius-Flohe and Traber, 1999). Studies have pointed out that the α-tocopherol form protects membranes from oxidation by reacting with lipid radicals produced in

the lipid peroxidation chain reaction which makes it the most important lipid-soluble antioxidant (Traber and Atkinson, 2007). This thus, helps remove the free radical intermediates and prevents the propagation reaction from continuing. Meanwhile, according to Wang and Quinn (1999), this reaction yields oxidized α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants including ascorbate, retinol or ubiquinol, etc. This is therefore in agreement with study findings by Seiler et al. (2008) which demonstrated that α-tocopherol rather than water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death. GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

2.9.1.3 Ascorbic acid

Ascorbic acid is a monosaccharide antioxidant found in both plants and animals. According to Smirnoff (2001), one of the enzymes required for the synthesis of ascorbic acid has been lost by mutation in humans; it must therefore be obtained from diet and this thus, justifies why it is considered as a vitamin. It has been demonstrated that, in cells, it is generally maintained in its reduced form by reaction with glutathione, which can be catalysed by protein and glutaredoxins (Meister, 1994). Ascorbic acid is widely considered as a reducing agent and can therefore reduce; and in the process it neutralizes reactive oxygen species such as peroxyl radical, hypochlorite, hydrogen peroxide, hydroxyl radical as well as singlet oxygen (Padayatty et al., 2003). A number of studies have also demonstrated in human plasma lipids that ascorbic acid is far more effective in inhibiting lipid peroxidation initiated by a peroxyl radical initiator than other plasma components such as protein thiols, bilirubin, urate and α-tocopherol by efficiently trapping peroxyl radicals in the aqueous phase before they can initiate lipid peroxidation. This awesome property of

ascorbic acid is thus very important in protecting bio-membranes against peroxidative damage (Frei et al., 1989).

2.9.1.4 Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is usually not obtained in diet. However, it is synthesized in cells from its constituent amino acids (Meister, 1994). Glutathione has been known to possess antioxidant properties owing to the fact that the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In almost all cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase. According to Meister (1994) glutathione is capable of not only reducing other metabolites and enzyme systems, such as glutathione peroxidases, ascorbate in the glutathione-ascorbate cycle and glutaredoxins, but also reacting directly with oxidants. As a result of its notably high concentration and its central role in maintaining the redox state of the cell, glutathione is known as one of the most important cellular antioxidants.

2.9.2 Enzyme Systems

The superoxide free radical is usually released during a number of processes, notably; oxidative phosphorylation which can be initailly converted to hydrogen peroxide and then further reduced to produce water. This detoxification pathway has been identified as the result of multiple enzymes, with superoxide dismutases (SOD) catalyzing the initial step followed by catalases (CAT) and various peroxidases (glutathione peroxidase) removing the hydrogen peroxide. The SODs, catalase and glutathione peroxidase thus, make up the main intracellular enzymic antioxidants, while the extracellular antioxidants are mostly of the preventive and scavenging types (Meister, 1994).

2.9.3 Uric acid and prostate cancer

Uric acid is a final enzymatic product in the degradation of purines in humans and higher primates. It is derived exclusively from the oxidation of xanthine and hypoxanthine by xanthine oxidase. Data from a number of prospective studies do suggest that serum uric acid is associated with PCa and other cancers, and that it may be used to predict the development of cancer. It has even been reported that though uric acid is increased by age, alcohol consumption, and other dietary factors, as a component of MetS, hyperuricaemia is associated with increased risk of PCa and other cancers (Hammarsten et al., 2010). Meanwhile, in their study of prospective analysis of Japanese men using the Cox Proportional Hazard Ratio (HR), Kolonel et al. (1994) identified an association of elevated serum uric acid with the risk for PCa development over a period of ten years following baseline measurement. Similarly, an increased in incident PCa observed in a Swedish cohort of MetS males was found to be associated with high serum uric acid. Furthermore, a study report by Hammarsten et al. (2010) did indicate that serum uric acid level above 358 μM has been found by binary regression analysis to be an independent and significant ($p<0.04$) prospective risk factor for incident PCa.

2.9.4 METABOLIC SYNDROME

2.9.4.1 Definition

In 1988, Reaven described Syndrome X as a cluster of clinical conditions that serve as risk factors for cardiovascular disease. For over a couple of decades now, much has been written about Syndrome X, which is now commonly called the metabolic syndrome, and its central component, insulin resistance. A working definition of the metabolic syndrome is presented in chapter three. To be classified with metabolic syndrome, individuals must have a number of the following 5 risk factors: 1)

abdominal obesity 2) hypertriglyceridaemia 3) low high-density lipoprotein (HDL) cholesterol 4) high blood pressure and 5) high fasting glucose. But the reference ranges or cut-offs for each risk factor may depend on the criteria to be used.

2.9.4.2 Metabolic Syndrome and prostate Cancer

It is recognized widely that certain racial and ethnic groups are predisposed to developing different features of the metabolic syndrome. Caucasians present most often with lipid abnormalities, including hypertriglyceridaemia and low HDL cholesterol (Ford et al., 2002); African Americans and Asians present with hypertension; whereas diabetes is diagnosed more frequently among Hispanics, Pacific Islanders, and Native Americans (Smith Jr et al., 2005). Abdominal obesity has increased drastically in all men over the past 40 years in the United States, regardless of race or ethnicity, and nearly 33% of adult men now have a waist circumference >102 cm (or approximately 40 inches). A number of recent reports have suggested that prostate cancer may be associated with features of the metabolic syndrome. Using data from a clinical study of men with prostate cancer in Sweden, Hammarsten and Hogstedt (2004) observed that certain features of the metabolic syndrome, including both hypertension and obesity, were more common in men who had stage T3 cancer compared with men who had stage T2 cancers. An association between BMI and PCa has also been reported in a number of studies. Engeland et al. (2003) for instance reported that BMI is related to higher mortality among PCa patients and that there may be a positive relationship between BMI and disease stage. Our modern society is characterized by a lifestyle with low level of exercise coupled with consumption of foods that are high in calorie, fat and sugar which may contribute significantly for one to obtain one or more of the features of the metabolic syndrome which has been linked to PCa.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA AND SITE

The study was conducted at Komfo Anokye Teaching Hospital (KATH), Kumasi. KATH is the second largest hospital in the country and the main referral hospital for the Ashanti, Brong Ahafo, Northern, Upper East and Upper Regions. It is affiliated to the medical school of the Kwame Nkrumah University of Science and Technology. The hospital is also accredited for postgraduate training by the West African College of Surgeons in surgery, obstetrics and gynaecology, otorhinolaryngology, ophthalmology and radiology. The urology department of KATH which serves as the main point of care for the middle and northern part of Ghana falls under the department of surgery. Cases presented to the urology department are usually in the form of referrals (out patients) as well as accident and emergency cases (*source: www.wikipedia.org*).

3.2 STUDY DESIGN

This cross-sectional study was conducted at the out-patient unit of the department of surgery, Komfo Anokye Teaching Hospital, Kumasi, between the period of November, 2010 and April, 2012. Prior to the study, ethics approval was sought from the KNUST School of Medical Science/KATH Committee on Human Research Publications and Ethics (CHRPE). In all, one hundred and twenty four adult males comprising 87 case subjects and 37control subjects aged at least forty two years were enrolled. The participation of the respondents was voluntary and informed consent was obtained from each of them.

Smokers were excluded from the study, since the constituents present in cigarette are potential sources of oxidative degradation of membrane lipids. Diabetics and hypertensive patients were also excluded from the control subjects. Medical history and biodata for the prostate cancer patients and the control subjects was solicited with a self-designed questionnaire. It contained the following: name, age, occupation, place of residence, hypertension, family hypertension history, diabetes and its duration of treatment, family prostate cancer history, duration and treatment of prostate cancer.

3.2.1 Case and Control Groups

The case group comprised those who presented with the clinical signs and symptoms and were diagnosed of prostate cancer at the outpatient department of the department of surgery, Komfo Anokye Teaching Hospital, Kumasi. The diagnosis of prostate cancer was through biopsy work-up and histological examination. Based on the Gleason scores, the prostate cancer was classified into mildly aggressive (5-6), moderately aggressive (7) and highly aggressive (8-10). The Gleason score is the sum of the two most common patterns (grades 1-5) of tumour growth found. The Gleason score ranges between 2 and 10, with 2 being the least aggressive and 10 the most aggressive. The control subjects were healthy normal adult males without diabetes, hypertension and prostate cancer.

3.2.2 Justification of sample size

The sample size was determined using the following formula (www.wikipedia.org)

$$N=z^2pq/d^2$$

Where N= sample size

Z= standard normal distribution

P= prevalence in the population

q= 1-p

d= delta (precision or degree accuracy)

3.2.3 Collection of Samples

Fasting venous blood specimens were collected from the study participants between 7 am and 11 am under aseptic conditions, at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. About 10 mL of blood was drawn from every study participant, 8 mL and 2 mL were dispensed into vacutainer plain-gel tubes and fluoride tubes respectively. The blood samples were then transported to the biochemistry laboratory at Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana within an hour. The blood samples were centrifuged together with the ones in fluoride tubes at 4000 r.p.m for 10 minutes. Sera and plasma obtained were separated and the concentrations of the various parameters such as malondialdehyde (MDA), prostate specific antigen (PSA), vitamin C, alkaline phosphates (ALP), uric acid, fasting and random blood sugars (FBS and RBS respectively), total cholesterol, triglycerides, HDL-cholesterol & LDL cholesterol as well as creatinine. The serum MDA and plasma vitamin C concentrations were then determined within four hours.

3.3 METHODS

3.3.1 Determination of Malondialdehyde (MDA) serum concentration

Principle and Method

Malondialdehyde (MDA) levels were determined by the MDA Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution based on the method described by Kamal et al., (1989). MDA, a secondary product of lipid

peroxidation, reacts with thiobarbituric acid (TBA) to generate a red coloured product, which was detected spectrophotometrically at 535 nm. This method is a fast, sensitive and low cost method that can be used to indicate the extent of lipid peroxidation in a variety of systems (Shlafer and Shepard, 1984).

A volume of 0.5 ml of the patient's serum sample was added to 2.5 ml of 20% trichloroacetic acid (TCA). Then 1 ml of 0.67% thiobarbituric acid (TBA) was added to the mixture. The resulting mixture at this point was then boiled in a water bath (at temperature of 100^{o}C for 30 minutes. The hot mixture was then allowed to cool using iced water bath and the sample was then extracted with 4 ml n-butanol and centrifuged at 4000 r.p.m for 10 minutes. The absorbances of supernatant were measured at 535 nm and the results were expressed as μmol L^{-1}, using the extinction coefficient of 1.56 x 10^{5} L mmol cm^{-1}.

Abs = **CεL**

Therefore, C = **Abs**/**εL**

Abs = absorbance of the test sample

C = concentration of the test sample

ε = extinction coefficient

L = light path (1 cm).

3.3.2 Determination of Vitamin C concentration

Vitamin C was determined by the method of Omaye et al. (1979).

Principle and Method

Ascorbic acid (vitamin C) in plasma is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4 dinitrophenylhydrazine to form a red dihydrazone

which is measured at 520 nm. Ascorbic acid concentrations were determined within 4 hours. The reagents used for the assay included:

- Ascorbic acid standard- 1 ml of glacial acetic acid was added to 100 mg of ascorbic acid and dissolved to 100 ml using distilled water.
- 5% Trichloroacetic acid (TCA) – 5 g of TCA and was dissolved with 100 ml of distilled water.
- DTC – a mixture of (0.4 g thiourea, 0.05 g $CuSO_4.5H_2O$ and 3 g of 2, 4 dinitrophenylhydrazine in 4.5 mol/L H_2SO_4).
- 65% H_2SO4 - 65 ml of concentrated H_2SO4 was added to 35 ml of distilled water.

To 0.5 ml of the test plasma an amount of 0.5 ml of distilled water and 1 ml of 5% TCA were added. The resulting mixture was then thoroughly mixed and centrifuged at 500g for 15 minutes. Then, 1 ml of the supernatant obtained was treated with 0.2 ml of DTC (which is a mixture of 0.4 g thiourea, 0.05 g $CuSO_4.5H_2O$ and 3 g of 2, 4 dinitrophenylhydrazine in 4.5 mol/L H_2SO_4) and the resulting mixture was incubated in a water bath at 37^oC for 3 hours. Then, 1.5 ml of 65% sulphuric acid (H_2SO_4) was added to the mixture which was thoroughly mixed. The resulting solution was then allowed to stand at room temperature for another 30 minutes. The standard and the test were then read against the blank at 520 nm and concentration of test determined using the following formula.

$\mathbf{T_{conc} = (T_{abs}/S_{abs}) X S_{conc}}$

$\mathbf{T_{conc}}$ = Concentration of the test sample

$\mathbf{T_{abs}}$ = Absorbance of the test sample

$\mathbf{S_{abs}}$ = Absorbance of the standard

$\mathbf{S_{conc}}$ = Concentration of the standard

3.3.3 Determination of serum total PSA

Prostate specific antigen levels were determined using Mindray MR-96A PSA semi-automated Analyzer ®, China. The principle and method adopted for the determination of serum total PSA was based on ELISA (Enzyme-Linked Immunosorbent Assay)

Principle

The ELISA for direct antigen makes use of matched highly specific monoclonal anti-PSA antibodies coated on the surface of microtitre wells, and covalently linked to enzyme respectively. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate are mixed to form the sandwich complex on the surface of the wells. At the end of incubation, excess conjugate and unbound antigen are washed out. In the second step, substrate is added and a blue colour develops which changes to yellow after stopping the reaction with a 'stop' reagent. The intensity of the colour is directly proportional to the PSA concentration in the test serum specimen. The absorbance of calibrators and test specimen is determined by using ELISA microplate reader. PSA concentration in patients test sera are calculated from a dose response curve generated by utilizing calibrators of known PSA concentration.

Twenty five microlitres (25µL) each of the patient's serum and calibrator were pipetted and dispensed into 2 separate microtitre wells. Then, 100 µL of the conjugate reagent was added to each of both wells. The microtitre wells were covered with adhesive strip, then mixed by votexing and incubated at room temperature (20^0C -25^0C) for 30 minutes.

The wells were then washed 5 times using the wash solution. 100 µL of the substrate reagent was added to each of both wells and incubated at room temperature for 15

minutes. Then, 100 µL of the stop reagent was also added to each of both wells. The resulting mixtures were thoroughly mixed. The absorbances of the calibrator and test serum were then measured using the microplate reader (Mindray MR-96A analyzer) and the corresponding PSA concentrations were displayed on the screen.

3.4 Biochemical assays

The biochemistry investigations were performed with Selectra E ® Chemistry Analyzer using ELITech reagents (Vital Scientific N.V, AC Dieren, Netherlands). Parameters that were determined include; alkaline phosphatase, uric acid, creatinine, fasting blood sugar, random blood sugar, total cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol which was calculated using the Friedwalds formula (Friedewald et al., 1972).

3.4.1 Creatinine

The method for this assay is based on the Jaffe (modified kinetic) method described by Fabiny et al. (1971). Creatinine reacts with picric acid in alkaline conditions to form a colour complex which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample.

$$Creatinine + Sodium\ Picrate \xrightarrow{alkali} Creatinine - Picrate\ complex$$

3.4.2 Uric Acid

Through the process of oxidation, uric acid is converted by uricase to allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzene-sulphonic acid (chlorophenol sulphonic acid) and 4-aminophenazone (4AP) to form a red-violet quinoneimine compound, which is proportional to the amount of uric acid present.

$$Uric\ acid + O_2 + 2H_2O_2 \xrightarrow{uricase} Allantoin + CO_2 + H_2O_2$$

$$2H_2O_2 + chlorophenol\ sulphonic\ acid + 4AP \xrightarrow{peroxidase} N - (4 - antiphyryl) - 3 - chloro - 5 - sulphonate - p - benzo - quinonei\min e$$

3.4.3 Alkaline Phosphatase

The method used for this assay is largely based on the kinetic photometric test according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). By this method, an enzyme, alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of p-nitrophenyl phosphate at a pH and temperature of 10.3 and 37^{o}C respectively, to liberate p-nitrophenol. The resulting absorbance increase is then measured at 405 nm.

$$p - nitrophenyl\ \ phosphate + H_2O \xrightarrow{ALP} p - nitrophenol + phosphate$$

3.4.4 Total Cholesterol

The method for this assay is based on that described by Trinder, (1969). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced as well as the preformed cholesterol is then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. However, the quinoneimine chromogen, which has its absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour produced is directly proportional to the total cholesterol concentration.

$$Cholesterol\ ester + H_2O \xrightarrow{cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{cholesterol\ oxidase} Cholest - 4 - en - 3 - one + H_2O_2$$

$$H_2O_2 + 4 - a\min ophenazone + Phenol \xrightarrow{peroxidase} H_2O + Quinonei\min e$$

3.4.5 Triglycerides

The method for this assay is based on a modified Trinder (Barham and Trinder, 1972) colour reaction to produce a fast linear endpoint reaction (McGowan et al., 1983).

Triglycerides in the test samples are hydrolyzed by the enzyme, lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by the enzyme, glycerol kinase. The glycerol-3-phosphate produced is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by an enzyme, peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglyceride + H_2 0 \xrightarrow{lipase} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{glycerol\ kinase} Glyerol - 3 - phosphate + ADP$$

$$Glycerol - 3 - phosphate \xrightarrow{glycerophosphate\ oxidase} DHAP + H_2O_2$$

$$2H_2O_2 + 4 - a\min oantipyrine + chlorophenol \xrightarrow{peroxidate} Quinonei\min e + 2H_2O$$

3.4.6 HIGH DENSITY LIPOPROTEIN– CHOLESTEROL

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg^{2+} ions. The cholesterol concentration in the HDL is then determined by the method described by Trinder for the assay of cholesterol.

3.4.7 Low Density Lipoprotein (LDL) – Cholesterol

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald et al., 1972).

$$LDL - Choresterol\ (mmolL^{-1}) = TC\,(mmolL^{-1}) - [TG(mmolL^{-1})/2.2] - HDL(mmolL^{-1})$$

3.4.8 Glucose

Glucose concentration in the samples was estimated with the glucose oxidase method according to the following reactions (Trinder, 1969).

$$Glu\cos e + O_2 \xrightarrow{Glu\cos e\ oxidase} Gluconic\ acid + H_2O_2$$

$$2H_2O_2 + Phenol + 4 - A\min oantipyrine \xrightarrow{peroxidase} Quinonei\min e + 4H_2O$$

3.5 Anthropometric Measurements

The measurements that were determined include hip and waist circumference, height, measured without shoes and weight to the nearest 0.1kg in light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd., Guangdong, China) and their heights measured with a wall-mounted ruler. Body Mass Index (BMI) for each subject was also calculated by dividing weight (kg) by height squared (m^2). Waist circumference (to the nearest centimeter) was measured with a Gulick II spring-loaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest. The hip circumference was however measured as the maximal circumference over the buttocks in centimetres and the waist to hip ratio (WHR) calculated by dividing the waist circumference by the hip circumference.

3.6 Blood Pressure

Blood pressure measurements were taken by qualified nurses at the urology department using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for more than 5 minutes in accordance with the recommendation of the American Heart Association. Triplicate measurements were taken with at least, a 5 minute rest interval between measurements and the mean value was recorded.

3.7 Classification of Metabolic Syndrome

The metabolic syndrome was classified based on a set of criteria from three different institutions: The National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) Criteria, International Diabetes Federation (IDF) Criteria as well as the World Health Organization (WHO) Criteria.

3.7.1 National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) Criteria

The NCEP ATP III criteria mandates that individuals with metabolic syndrome should have three or more of the following five components of metabolic syndrome: (1) Abdominal obesity (waist circumference >102 cm for men or >88 cm for women); (2) Raised triglyceride (≥1.7 mmol L-1); (3) Low HDL-cholesterol (<0.9 mmol L-1 in men or <1.0 mmol L-1 in women); (4) High Blood Pressure (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of hypertension) and (5) Raised fasting glucose (≥6.1 mmol L-1).

3.7.2 INTERNATIONAL DIABETES FEDERATION (IDF) CRITERIA

The IDF criteria mandates that metabolic syndrome be diagnosed if Central obesity (waist circumference >90 cm for men or >80 cm for women) is accompanied by any

two (2) of the following four (4) factors: (1) Triglyceride level ≥1.7 mmol L-1; (2) HDL cholesterol <1.03 mmol L-1 for men or <1.29 mmol L-1 for women; (3) Blood pressure ≥130/85 mmHg or treatment of previously diagnosed hypertension and (4) Fasting blood glucose (FBG) ≥5.6 mmol L-1 or previously diagnosed type 2 diabetes.

3.7.3 THE WORLD HEALTH ORGANIZATION (WHO) CRITERIA

The WHO criteria mandates the presence of diabetes mellitus, impaired glucose tolerance or insulin resistance and any two (2) of the following: (1) Body mass index (BMI) ≥30 kg m-2 and/or waist to hip ratio >0.90 for males or >0.85 for females; (2) Blood pressure ≥140/90 mmHg or on medication; (3) Triglyceride ≥1.7 mmol L-1 and (4) HDL cholesterol <0.91 mmol L-1 in males or <1.01 mmol L-1 in females.

3.8 STATISTICAL ANALYSIS

Data were entered on Microsoft Excel. Graph Pad Prism version 5.00 for windows was used for the statistical analysis (Graph Pad software, San Diego California USA). One-way ANOVA and Fisher exact test were used for comparison of variables proportions and one-way ANOVA used to test differences in means for continuous variables. The results are expressed as Mean ± SD. A level of $p<0.05$ was considered as statistically significant.

CHAPTER 4

RESULTS

4.1 GENERAL CHARACTERISTICS

Table 4.1 presents the general characteristics of the study population. With the exception of the highly aggressive PCa group who were significantly older than the control subjects ($p<0.05$), there was no significant difference in the mean age of the mildly aggressive, moderately aggressive PCa patients and control subjects ($p>0.05$). The mean concentrations of prostate specific antigen (PSA) in all the PCa groups were significantly higher than the control group ($p<0.001$). The highest concentration of ALP was observed among the highly aggressive PCa group ($484.3\pm242.5\ \mu molL^{-1}$; $p<0.001$). However, creatinine concentration did not show any significant difference among all the study population ($p>0.05$).

4.1.1 CARDIOVASCULAR, LIPID PROFILE, OXIDATIVE STRESS AND ANTIOXIDANT MARKERS

Compared to the controls, the mean measured levels of waist to hip circumference ratio was significantly higher among the prostate cancer patients. The mean measured levels of fasting and random blood sugars, low density lipoprotein cholesterol and coronary risk among the entire study population ($p<0.05$) were significantly higher in the highly aggressive PCa group compared to the control subjects ($p<0.05$). However, there was no significant difference in the mean measured levels of waist and hip circumferences, body mass index, total cholesterol, triglycerides, HLD-cholesterol, VLDL-cholesterol as well as systolic and diastolic blood pressure among all the study population ($p>0.05$) as shown in table 4.1. The study observed a significant higher mean concentrations of malondialdehyde and uric acid among the prostate cancer

patients compared to the control subjects ($p<0.0001$). However, the mean concentration of vitamin C was significantly lower among the prostate cancer patients compared to the control subjects (Table 4.1).

Table 4.1General characteristics of the study population stratified by Gleason score

Parameter	Control (n=37)	PROSTATE CANCER CASES			P value
		Mildly Aggressive (n=8)	Moderately Aggressive (n=36)	Highly Aggressive (n=43)	
Age (years)	62.49±8.4	60.3±5.2	63.78±7.3	68.1±7.8**	0.003
PSA (ngmL^{-1})	1.726±0.9	32.9±16.1**	29.11±25.7***	37.4±28.8***	< 0.001
ALP (µmolL^{-1})	268.1±64.5	285.0±64.9	324.5±178.8	484.3±242.5$^{***\#\,ttt}$	< 0.001
CRE (µmolL^{-1})	143.0±39.	137.5±43.8	152.7±57.6	185.0±16.4	0.237
Cardiovascular and lipid profile					
WC (cm)	91.5±7.1	90.4±6.1	94.0±9.8	94.0±9.6	0.422
HC (cm)	102.3±7.4	97.1±6.3	101.3±9.7	101.6±9.8	0.527
WHR	0.9±0.0	0.9±0.0***	0.9±0.0***	0.9±0.0***	0.023
BMI (kgm^{-2})	24.2±1.5	24.4±1.2	25.3±2.8	25.3±2.9	0.161
CHO (mmolL^{-1})	4.7±3.3	5.0±1.6	4.6±1.3	4.7±1.7	0.967
TG (mmolL^{-1})	1.8±0.8	1.6±0.3	1.5±0.7	1.7±01.0	0.455
HDL (mmolL^{-1})	1.4±0.4	1.2±0.6	1.2±0.4	1.2±0.4	0.136
LDL (mmolL^{-1})	2.1±0.7	3.1±1.2	2.8±1.0	2.8±1.5*	0.013
VLDL (mmolL^{-1})	0.8±0.4	0.7±0.1	0.7±0.3	0.8±0.4	0.476
FBS (mmolL^{-1})	4.2±0.4	4.3±0.6	4.5±0.8	5.1±1.7**	0.005
RBS (mmolL^{-1})	6.1±0.6	6.3±0.8	6.4±1.4	7.8±3.7$^{**\,t}$	0.006
SBP (mmHg^{-1})	122.8±6.2	118.5±5.7	122.5±11.0	129.2±14.7	0.015
DBP (mmHg^{-1})	73.05±4.2	70.3±0.7	73.1±6.8	77.4±8.6$^{\,t}$	0.009
CR	3.3±0.9	4.9±1.9**	4.1±1.0	4.5±2.1**	0.003
Oxidative stress markers					
MDA (µmolL^{-1})	3.2±0.9	8.0±2.7***	8.5±2.9***	8.7±3.3***	< 0.001
VIT C (µmolL^{-1})	30.4±7.8	10.0±3.8***	12.9±6.4***	11.4±7.5***	< 0.001
UA (µmolL^{-1})	293.4±66.5	574.6±204.9	470.7±256.2	694.5±478.5$^{***\,t}$	< 0.001

WC: Waist circumference. HC: Hip circumference. BMI: Body mass index. CHO: Cholesterol. TG: Triglyceride. HDL: High density lipoprotein. LDL: Low density lipoprotein. VLDL: Very low density lipoprotein. FBS: Fasting blood sugar. RBS: Random blood sugar. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. CR: Coronary risk. MDA: Malondialdehyde. VIT C: Vitamin C. UA: Uric acid. PSA: Prostate specific antigen. ALP: Alkaline phosphatase. CRE: Creatinine. WHR: Waist to hip circumference ratio. Data are presented as mean±SD. One way ANOVA used to test mean differences between groups (* prostate cancer cases vs. control; ¥ mildly aggressive vs. moderately aggressive; # mildly aggressive vs. highly aggressive; t moderately aggressive vs. highly aggressive) *P<0.05. **P<0.001. ***P<0.0001. tP<0.05. ttP<0.001. tttP<0.0001.

4.2 PREVALENCE OF METABOLIC SYNDROME AND ITS SCORES

Table 4.2 presents the general overview of metabolic syndrome (MetS) in the study population defined by three different classification criteria. The overall prevalence of MetS was 9.2%, 18.4% and 12.6% using NCEP-ATP III, IDF and WHO criteria respectively for the PCa population. The prevalence was highest among the highly aggressive PCa group (i.e. 18.6%, 23.3% and 20.9% for NCEP-ATP III, IDF and WHO respectively) as compared to the mildly aggressive PCa group (0.0%, 12.5% and 0.0%) and moderately aggressive PCa population (0.0%, 13.9% and 5.6%) for the same criteria mentioned above respectively (Table 4.2). By the NCEP-ATP III and IDF criteria, the proportion of individuals without any MetS risk factor (i.e. Zero metabolic score) was highest among the moderately aggressive PCa population whereas individuals with only one metabolic score were mostly associated with the mildly aggressive PCa population. However, IDF and WHO criteria, there were a number of individuals who possessed at least three metabolic score yet did not have metabolic syndrome. This kind of population was mostly observed among the highly aggressive PCa group as depicted in Table 4.2.

Whereas the highest prevalence of hypertriglyceridaemia was mostly associated with the mildly aggressive PCa group, the prevalence of diabetes, hypertension, and low HDL-cholesterol were highest among the highly aggressive PCa population for all the three criteria. These contributed to the highest prevalence of MetS observed among the highly aggressive PCa patients (Table 4.3). The significantly increased level of low density lipoprotein cholesterol observed among the highly aggressive PCa group most probably contributed to the higher coronary risk observed among them.

Table 4.2 Prevalence of metabolic syndrome and metabolic score among the population stratified by Gleason score

Parameters	Control (n=37)	Total PCa Cases (n=87)	Mildly Aggressive (n=8)	Moderately Aggressive (n=36)	Highly Aggressive (n=43)
NCEP-ATP III	0(0.0)	8(9.2)	0.(0.0)	0(0.0)	8(18.6)
IDF	1(2.7)	16(18.4)	1(12.5)	5(13.9)	10(23.3)
WHO	0(0.0)	11(12.6)	0(0.0)	2(5.6)	9(20.9)
Prevalence of clustering of one, two or more components of metabolic syndrome					
NCEP-ATP III					
0	12(32.4)	33(37.9)	3(37.5)	17(47.2)	13(30.2)
1	23(62.2)	29(33.3)	4(50.0)	9(25.0)	16(37.2)
2	2(5.4)	17(19.5)	1(12.5)	10(27.8)	6(14.0)
Individuals with >2 metabolic scores but without MetS	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0 (0.0)
IDF					
0	7(1.9)	16(18.4)	1(12.5)	10(27.8)	5(11.6)
1	13(35.1)	23(26.4)	4(50.0)	6(16.7)	13(30.2)
2	16(43.2)	17(19.5)	2(25.0)	2(5.6)	13(30.2)
Individuals with >2 metabolic scores but without MetS	1(2.7)	21(24.1)	1(12.5)	8(22.2)	12(27.9)
WHO					
0	12(32.4)	36(41.4)	3(37.5)	14(38.9)	19(44.2)
1	20(54.1)	24(27.6)	4(50.0)	9(25.0)	11(25.6)
2	5(13.5)	15(17.2)	1(12.5)	8(22.2)	6(14.0)
Individuals with >2 metabolic scores but without MetS	0(0.0)	12(13.8)	0(0.0)	5(13.9)	7(16.3)

Data are presented as absolute counts and percentages.

Table 4.3 Prevalence of metabolic syndrome and its components among study population stratified by Gleason score

Parameter	Control (n=37)	Cases (n=87)	PCa Category			P value
			Mildly Aggressive (n=8)	Moderately Aggressive (n=36)	Highly Aggressive (n=43)	
NCEP III						
Criteria						
WC	3(8.1)	14(16.1)	0(0.0)	5(13.9)	9(20.9)	0.3917
TG	17(45.9)	30(34.5)	4(50.0)	11(30.6)	15(34.9)	0.2339
HDL	7(18.9)	21(24.1)	2(25.0)	6(16.7)	13(30.2)	0.6414
BP	0(0.0)	19(21.8)**	0(0.0)	6(16.7)	13(30.2)	0.0008
FBS	0(0.0)	9(10.3)	0(0.0)	1(2.8)	8(18.6)	0.0565
MetS	**0 (0.0)**	**8(9.2)****	**0(0.0)**	**0(0.0)**	**8(18.6)**	**0.0020**
IDF Criteria						
WC	20(54.1)	49(56.3)	4(50.0)	21(58.3)	24(55.8)	0.8454
TG	17(45.9)	30(34.5)	4(50.0)	11(30.6)	15(34.9)	0.2339
HDL	10(27.0)	37(42.5)	3(37.5)	13(36.1)	21(48.8)	0.1115
BP	0(0.0)	21(24.1)**	0(0.0)	8(22.2)	13(30.2)	0.0004
FBS	0(0.0)	22(25.3)**	0(0.0)	8(22.2)	14(32.6)	0.0002
MetS	**1(2.7)**	**16(18.4)***	**1(12.5)**	**5(13.9)**	**10(23.3)**	**0.0215**
WHO Criteria						
Diabetes Mellitus	0(0.0)	16(18.4)*	0(0.0)	3(8.3)	13(30.2)	0.0028
BMI	0(0.0)	8(9.2)	0(0.0)	3(8.3)	5(11.6)	0.1038
WHR	5(13.5)	12(13.8)	0(0.0)	8(22.2)	4(9.3)	1.000
BP	0(0.0)	17(19.5)	0(0.0)	6(16.7)	11(25.6)	0.1038
TG	17(45.9)	30(34.5)	4(50.0)	11(30.6)	15(34.9)	0.2339
HDL	7(18.9)	21(24.1)	2(25.0)	6(16.7)	13(30.2)	0.6414
Met S	**0(0.0)**	**11(12.6)***	**0(0.0)**	**2(5.6)**	**9(20.9)**	**0.0328**

WC: Waist circumference. TG: Triglyceride. HDL: High density lipoprotein. BP: Blood pressure. FBS: Fasting blood sugar. Met S: Metabolic syndrome. BMI: Body mass index. WHR: Waist to hip circumference ratio. Data are presented as absolute counts and percentages. NCEP III Criteria: WC > 102cm; TRG ≥ 1.7mmol/L; HDL< 0.9mml/L; BP ≥ 130/85mmHg; FBS ≥ 6.1 mmol/L. IDF Criteria: WC > 90cm; TRG ≥ 1.7mmol/L; HDL < 1.03mml/L; BP ≥ 130/85mmHg; FBS ≥5.6mmol/L. WHO Criteria: BMI(kg/m^2) ≥ 30; WHR(waist to hip circumference ratio) > 0.9; BP ≥ 140/90mmHg; TRG ≥ 1.7mmol/L; HDL < 0.9mml/L. One way ANOVA was used for mean differences between groups (* PCa cases vs. control). *P<0.05. **P<0.001. ***P<0.0001.

4.3 THE GLEASON SCORE

Based on the Gleason scores, the PCa patients were classified into mildly aggressive (5-6), moderately aggressive (7) and highly aggressive (8-10). The Gleason scores for the prostate cancer patients ranged from 5 to 9 with a mean score of 7.56±1.13 corresponding to moderately aggressive cancer. Almost half (49.4%) of the prostate cancer patients had highly aggressive PCa whereas 41.4% and 9.2% had the moderate and mild PCa respectively **(Figure 4.1)**.

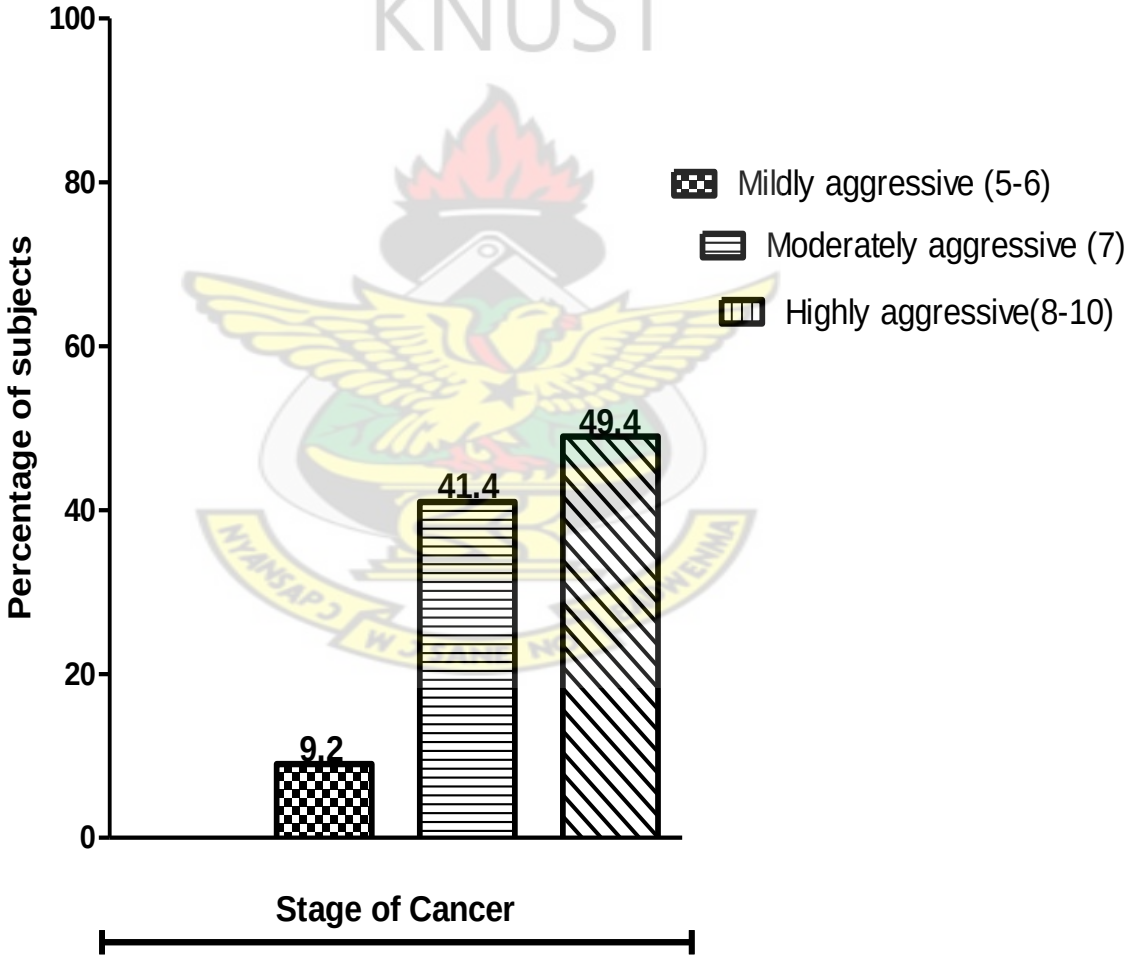

Figure 4.1 Distribution of stage of prostate cancer based on Gleason score

4.4 CORRELATION AMONG VARIABLES

The correlations among the various analytes of the study population are presented in Table 4.4. Strongly significant positive correlation was observed between fasting blood sugar and blood pressure (r = 0.001). Among the indicators, total cholesterol had a significantly strong positive correlation with coronary risk across the study population whereas a significantly strong positive correlation was also observed for body mass index with coronary risk, malondialdehyde, prostate specific antigen as well as abdominal obesity (waist and hip circumference) (r = 0.001).

Another significantly strong positive correlation identified was between prostate specific antigen and blood pressure (r = 0.001). However, few significantly strong negative correlations were also observed among the characteristic variables in the study population. These include coronary risk versus high density lipoprotein; vitamin c versus blood pressure as well as vitamin c versus body mass index (r = 0.001).

Table 4.4 Pearson correlation coefficient of clinical variables in prostate cancer (upper right-hand side) and control group (lower left-hand side)

	AGE	FBS	CHOL	TRG	HDL	LDL	VLDL	CR	PSA	MDA	VIT C	SBP	DBP	WC	HC	BMI
AGE		0.364**	0.129	0.093	-0.029	0.120	0.091	0.156	0.278*	0.395**	-0.220*	0.495***	0.533***	0.330*	0.339**	0.318**
FBS	-0.070		-0.036	0.026	-0.056	-0.047	0.026	0.010	0.335*	0.323*	-0.091	0.574***	0.562***	0.057	0.086	0.122
CHOL	-0.255	0.193		0.240*	0.438***	0.936***	0.239*	0.445***	0.056	0.302*	-0.073	0.263*	0.262*	0.342*	0.346**	0.446***
TRG	0.008	0.030	0.338*		-0.157	0.028	0.999**	0.402***	0.174	0.208	-0.136	0.140	0.253	0.157	0.130	0.179
HDL	0.113	0.273	0.611***	0.068		0.227*	-0.159	-0.542***	-0.018	0.171	-0.092	0.040	0.056	0.032	0.003	0.072
LDL	-0.411*	0.077	0.794***	-0.105	0.186		0.028	0.565	0.011	0.230*	-0.008	0.242*	0.204	0.334*	0.356**	0.438***
VLDL	0.007	0.029	0.339*	1.000**	0.069	-0.105		0.402	0.171	0.210	-0.139	0.143	0.254*	0.156	0.129	0.178
CR	-0.342*	-0.127	0.058	0.258	-0.711***	0.365*	0.256		0.033	0.108	-0.067	0.215*	0.167	0.225*	0.250*	0.293**
PSA	-0.108	-0.059	0.131	0.354*	0.116	-0.075	0.352*	0.056		0.269*	-0.215*	0.502***	0.642***	0.315**	0.324**	0.347**
MDA	-0.281	0.116	0.180	0.053	-0.130	0.289	0.051	0.369*	0.068		-0.348*	0.522***	0.514****	0.219*	0.233*	0.285**
VIT C	-0.206	0.280	0.093	-0.100	-0.134	0.254	-0.101	0.212	-0.019	0.361*		-0.380**	-0.323**	-0.104	-0.108	-0.225*
SBP	0.025	0.198	0.001	0.164	0.053	-0.117	0.164	-0.112	-0.084	0.031	0.076		0.887***	0.378**	0.407***	0.493***
DBP	0.041	0.156	-0.012	0.103	0.070	-0.114	0.103	-0.119	0.026	0.062	0.079	0.873***		0.464***	0.482***	0.562***
WC	0.024	0.134	-0.040	-0.280	0.125	0.016	-0.279	-0.220	-0.065	-0.270	-0.076	0.107	0.134		0.987**	0.885***
HC	0.008	0.192	-0.016	-0.205	0.067	0.043	-0.203	-0.122	-0.081	-0.194	-0.059	0.118	0.128	0.945***		0.884***
BMI	0.000	0.171	0.206	-0.156	0.284	0.186	-0.154	-0.198	0.004	-0.112	0.043	0.087	0.065	0.713***	0.739***	

*Correlation is significant at 0.05 level (2 tailed). **Correlation is significant at 0.01(2 tailed). *** Correlation is significant at 0.001(2 tailed).

CHAPTER 5

DISCUSSION

5.1 PREVALENCE OF METABOLIC SYNDROME AND ITS COMPONENTS

The current study assessed the prevalence of the metabolic syndrome (MetS) among the PCa population using the NCEP-ATP III, IDF and WHO criteria. Generally, the prevalence of MetS differs from place to place for ethnicity, race, lifestyle (diet, physical activity, etc.), genetics, etc. (Roberts and Barnard, 2005). In this study, the MetS prevalence obtained for the PCa population were 9.2%, 18.4% and 12.6% using NCEP-ATP III, IDF and WHO criteria respectively. The highest prevalence was generally associated with the highly aggressive PCa group as compared to the controls and other PCa groups.

A study done by Grundmark et al. (2010) in Uppsala, Sweden, reported a MetS prevalence of 14.1% and 13.7% using the NCEP-ATP III and IDF criteria respectively among their PCa patients compared to a lower 9.2% and a higher 18.4% found in our study using the same respective criteria. Using the IDF criteria, the MetS prevalence among our prostate cancer patients was twice higher compared with the NCEP-ATP III criteria (18.4% and 9.2% respectively). By the IDF criteria, men are considered to have abdominal obesity if they have waist circumferences greater than 90cm unlike NCEP-ATP III criteria whose waist circumferences need to be greater than 102cm. Many of the PCa patients in our study had waist circumferences between 90 cm and 102cm. This may explain the higher prevalence of abdominal obesity observed among the PCa patients using the IDF criteria which were at least three times higher compared with the NCEP-ATP III (56.3% and 16.1% respectively). Meanwhile, unlike the NCEP-ATP III criteria by which diabetes is defined as fasting blood glucose ≥6.1mmol/L compared with ≥5.6mmol/L or diabetes history for the IDF criteria. It was realised that the PCa patients had a significantly higher prevalence (25.3%) of diabetes

using the IDF criteria compared with 10.3% using the NCEP-ATP III criteria. The difference in the diabetes prevalence is a reflection of the difference in the reference definition by the two criteria.

Unlike our study's PCa population, all the PCa patients in the study done by Grundmark et al., (2010) had the advanced stage of the disease. Besides, all their patients had the highly aggressive and moderately aggressive form of the cancer (Gleason score ≥ 7). However, irrespective of the criteria used the highly aggressive PCa group in our study had the highest prevalence of MetS compared to the other PCa groups. This suggests that the nature of the disease may have an effect on the prevalence of MetS. This is evidenced by a study done by Kwon et al. (2013) which reported a high MetS prevalence of 82.8% among their PCa patients with Gleason scores of at least 7. Meanwhile, factors such as variation in race, ethnicity, and lifestyle cannot be completely ruled out for the differences in the MetS prevalence for the two studies. Our study population for instance was entirely blacks compared to theirs which was a combination of black and white people. It has been reported that the causes of metabolic syndrome are complex and are thought to involve metabolic, hormonal, genetic, and lifestyle interactions. It has been reported that genetic factors usually predispose a person to a disease whereas lifestyle factors determine whether (and when) the disease will develop (Roberts and Barnard, 2005; Lin et al., 2005; Cossrow and Falkner, 2004).

Meanwhile, a study conducted by Santana et al., (2008) on MetS among PCa patients in Brazil obtained a prevalence of 68.7% (11 out of 16) using the IDF criteria compared to a lower prevalence of 18.4% obtained in this study using the same criteria. More than half (56.3%) of the PCa patients in our study had abdominal obesity compared with 42.5% for low HDL cholesterol, 34.5% for hypertriglyceridaemia, 25.3% for diabetes and 24.1 % for

hypertension. With the exception of abdominal obesity whose prevalence was highest among the moderately aggressive PCa group, the prevalence of all the other parameters was highest among the highly aggressive PCa patients. However, the mildly aggressive PCa patients were neither diabetic nor hypertensive. The significant difference in the prevalence rate for these two studies could be due to a number of factors including nature of the PCa, genetics, ethnic difference, lifestyle (diet, physical activity, etc.). Most of our PCa patients were the 'freshly' and early diagnosed PCa group who were active and were not on any medication such as chronic use of systemic corticosteroids which has possible severe side effects including hyperglycaemia, insulin resistance, hypertension, diabetes mellitus, etc. Besides, about 90.8% of the PCa patients had their Gleason scores ranging from 7 to 10 representing the highly aggressive and moderately aggressive forms of PCa. It is well established that the higher the Gleason score, the faster the growth of the cancer and the greater the probability of spreading to other organs in the human body resulting in early death (Harnden et al., 2007). In their study, PCa patients with Karnofsky performance status (KPS) lower than 70% and chronic use of systemic corticosteroids were excluded. Karnofsky performance status basically refers to a measure of cancer patient's general well-being and activities of daily life. It is also established that the higher the KPS score, the higher the level of physical activity and greater well-being. A 70% score for instance implies caring for self but not capable of normal activity or work whereas 90% score implies being capable of normal activity with few symptoms or signs of disease. It is possible that the level of physical activity among our PCa patients was higher than those patients in their study. This in addition to difference in diet among the two study populations may have contributed to the difference in the prevalence of the individual components of metabolic syndrome most of which are lifestyle related. Our PCa population had a significantly lower prevalence of 24.1% compared to their 75.0% for hypertension. Also, the prevalence of type 2 diabetes was significantly higher among their

PCa population compared to ours (31.3% vs. 25.3%). Secondly, our study population was entirely blacks compared to theirs which was a combination of black and white people. The ethnic difference may also have contributed to the difference in the prevalence of metabolic syndrome. A study done by Cossrow and Falkner (2004) reported that the prevalence of the metabolic syndrome varies between ethnic groups.

Also, a study conducted by Braga-Basaria et al. (2006) on metabolic syndrome involving 38 PCa patients in John Hopkins, USA, reported metabolic syndrome prevalence of 39.5% (15 out of 38) using the NCEP-ATP III criteria compared to a significantly lower prevalence of 9.2% obtained in this study. Most of the PCa patients in our study were 'freshly' diagnosed and were not on any medication. Secondly, our PCa patients were all blacks. The usual treatment approach for PCa includes prostatectomy and/or radiation therapy for localized cancers and androgen deprivation therapy for metastasized or recurrent cancers. Most of our PCa patients had not undergone any of these therapies since they were diagnosed during the study and sampling was done before commencement of any therapy. The study done by Braga-Basaria et al. (2006) however involved 38 PCa patients, 52.6% (20 out of 38) of who were on androgen deprivation therapy (ADT) whereas the remaining 47.4% (18 out of 38) had undergone prostatectomy or radiation therapy. ADT has been identified to be associated with a number of possible side effects including obesity, increased fat mass, reduced lean body mass, osteoporosis, sexual dysfunction as well as poor quality of life. It is reported that increased fat mass results from increased deposition of both subcutaneous and visceral fats which result in abdominal obesity (Basaria et al., 2002). It was reported in their study that the prevalence of abdominal obesity and hyperglycaemia among the ADT group were significantly higher than the non ADT group. This most likely resulted in the significantly higher MetS prevalence for the ADT group (about two and a half times) compared to the non ADT group. It is therefore most likely that the significantly high prevalence of MetS

reported in their study may have been influenced by the ADT (Diamond et al., 2004). It has also been reported that people from different countries particularly those from different continents may vary in race, lifestyle, genetics as well as ethnicity which affects metabolic syndrome prevalence. Therefore, differences in measured levels of waist circumference for instance which may arise due to variation in diet and physical activity whereas that of diabetes and hypertension due to genetics, diet etc. may have partly accounted for the difference in the MetS prevalence obtained in the two studies (Roberts and Barnard, 2005).

Furthermore, a study conducted by Beebe-Dimmer et al. (2007) concerning features of the metabolic syndrome and prostate cancer in African American men using the NCEP ATP III criteria identified the presence of any 2 of the features (i.e., abdominal obesity and hypertension, abdominal obesity and diabetes, or diabetes and hypertension) in 139 subjects which were significantly associated with a diagnosis of prostate cancer (adjusted OR, 1.8; 95% CI, 1.1–2.8). Of the 3 possible combinations of factors, they also found out that abdominal obesity and hypertension exhibited the strongest association with prostate cancer (adjusted OR, 1.9; 95% CI, 1.1–3.3). By the same criteria, the prevalence of diabetes and hypertension were significantly higher among the PCa patients compared with the control. Meanwhile, the highest prevalence of diabetes and hypertension was observed among the highly aggressive PCa patients. However, the mildly aggressive PCa patients were neither diabetic nor hypertensive. The prevalence of abdominal obesity, hypertriglyceridaemia and low HDL cholesterol, though statistically insignificant were higher among the PCa patients compared to the controls. Similarity in race may possibly account for some of the similarities in these two studies. The study populations for these studies were entirely blacks. It was observed that the mean measured levels of low density lipoprotein cholesterol were significantly higher among the highly aggressive PCa patients compared to the other study population. This may have contributed to the higher coronary risk observed among the highly

aggressive PCa population. The cause of hypertension in metabolic syndrome is multifactorial and likely includes all the elements of the syndrome, including obesity, insulin resistance, and dyslipidaemia. During the study, urine retention among a number of the PCa patients particularly those with the highly aggressive type was observed. It is however possible that the urine retention may have activated the rennin-angiotensin system which may have also triggered increased renal sodium retention in the kidney thereby causing a compensatory increase in fluid volume and thus ultimately initiating a rise in blood pressure as observed among the highly aggressive PCa patients (Navar and Hamm, 1999).

5.2 OXIDATIVE STRESS AS A RISK FACTOR FOR PROSTATE CANCER

In the present study, the mean levels of MDA among the PCa patients were significantly increased compared to the control. Conversely, the mean levels of vitamin C were significantly reduced among the PCa patients compare to the control. Meanwhile, a study conducted by Ozmen et al. (2006) in Turkey reported similar results. Though statistically insignificant, the MDA level in our study was highest among the highly aggressive PCa patients followed by the moderately and mildly aggressive PCa patients (8.7 ± 3.3 $\mu molL^{-1}$, 8.5 ± 2.9 $\mu molL^{-1}$ and $8.0\ \pm2.7$ $\mu molL^{-1}$ respectively). It is possible that PCa patients with higher aggressiveness of the disease were subjected to higher levels of oxidative stress and that may possibly explain why the MDA levels were observed to increase with increasing Gleason score (Woźniak et al., 2012; Yossepowitch et al., 2007). However, the highest and least levels of vitamin C were observed among the moderately aggressive and mildly aggressive PCa populations respectively. Unlike their study which used high performance liquid chromatography (HPLC) for the measurement of both MDA and vitamin C, colorimetric and spectrophotometric procedures were used in our study for MDA and vitamin C measurement. Both methods are sensitive, accurate and specific though HPLC is known to

be superior in terms of the above mentioned indices. It has been reported that in the course of PCa, imbalance of oxidant-antioxidant processes occur and that oxidative stress usually occurs when there is an imbalance between extreme production of reactive oxygen species and insufficient antioxidative defences, including superoxide dismutase (SOD), catalase (CAT), vitamins (C,E), ß-carotene and glutathione (GSH) (Woźniak et al., 2012).

In this study, uric acid and malondialdehyde levels were significantly elevated among the PCa patients compared to the controls. However, reduced level of uric acid has been reported by Yossepowitch et al. (2007). Compared to control subjects in their study, patients with localized prostate cancer had no difference in oxidative stress indices, whereas those with metastatic disease had an increased malondialdehyde concentration ($p < 0.05$) but a decreased uric acid concentration ($p < 0.04$). In their study, it was reported that subjects with the metastasized PCa had an increased malondialdehyde concentration but a decreased uric acid concentration compared to increased concentrations of both malondialdehyde and uric acid observed among the PCa population in this study. Though statistically insignificant, the highest malondialdehyde levels in our study were observed among the highly aggressive PCa patients followed by the moderately and mildly aggressive PCa patients. Therefore, PCa patients with fastest growth of the disease tumour were subjected to highest levels of oxidative stress and that may possibly explain why the malondialdehyde levels were observed to increase with increasing Gleason score (Woźniak et al., 2012; Yossepowitch et al., 2007). Both studies also used Thiobarbituric acid (TBA) test for the measurement of malondialdehyde and this in addition to similar disease patterns may have played a key role for the similar malondialdehyde results.

The uric acid levels among our PCa patients were significantly higher compared to the controls. Among the PCa patients, the highest uric acid levels were observed among the

highly aggressive PCa group (694.5±478.5 $\mu molL^{-1}$) followed by the mildly aggressive (574.6±204.9 $\mu molL^{-1}$) and moderately aggressive PCa patients (470.7±256.2 $\mu molL^{-1}$). Generally, uric acid can act as either a pro-oxidant or antioxidant and it likely that the uric acid levels measured in our study was acting as a pro-oxidant (Hille, 2005). According to Hammarsten et al. (2010), uric acid is increased by alcohol consumption and other dietary factors, age, etc. The PCa patients in both studies were of similar age. However, PCa patients with alcohol consumption were not excluded from our study. Besides, their dietary data were not collected to help ascertain the influence of diet on the uric acid levels. It is therefore possible that alcohol consumption and dietary factors may have also contributed to the elevated levels of uric acid observed among the PCa patients.

Uric acid is the final enzymatic product in the degradation of purines in humans and higher primates. It is derived exclusively from the oxidation of xanthine and hypoxanthine by xanthine oxidase. Ames et al. (1982) hypothesized the ability of uric acid to provide a primary defense against human cancer based on its capacity to scavenge singlet oxygen, its capacity to inhibit lipid peroxidation, as well as its high serum concentration in humans. A study done by Becker (1993) for instance, identified the protective antioxidant properties of uric acid in many different organ systems. Nonetheless, Petersson and Trell (1983) as well as Becker (1993) have reported that increased serum urate, the dominant monosodium form of uric acid at physiological pH, was found to exhibit strong statistical association with increased premature cancer death in both men and women, and thus acting as a pro-oxidant. This therefore suggests a rather more complex role of uric acid in cancer biology than that of a general antioxidant.

5.3 PROSTATE SPECIFIC ANTIGEN (PSA) AND PROSTATE CANCER

A significantly higher mean concentration of PSA was found among the PCa patients compared to the controls ($p < 0.0001$). According to Thompson et al. (2004) the level of PSA is a continuous parameter: the higher the value, the more likely is the existence of PCa. However, an individual may have PCa despite low PSA level. For instance, a US study done by Thompson et al. (2004) on the prevalence of prostate cancer among men with normal PSA, underscored reports that many men harbour PCa despite low levels of serum PSA.

In their study on metabolic syndrome involving 38 PCa patients in John Hopkins, USA, Braga-Basaria et al. (2006) reported a significantly increased PSA level (6.4±10.9 $ngmL^{-1}$) in their PCa patients compared to controls. In this study however, a significantly much higher levels were obtained for the PCa patients in our study. The levels were highest among the highly aggressive PCa group (37.37±28.84 $ngmL^{-1}$) 90followed by the mildly aggressive (32.9±16.11 $ngmL^{-1}$) and moderately aggressive (29.11±25.68 $ngmL^{-1}$) PCa patients. Their PCa patients (68.1±8.9 years) were significantly older than the mildly aggressive (60.3±5.2 years) and moderately aggressive (63.8±7.3 years) but comparable with the highly aggressive PCa group (68.1±7.8 years) in age. It is reported and widely accepted that a man's PSA level naturally increases as he ages. According to Richardson and Oesterling (1997), the rise in PSA level is attributed to the growth of the prostate with time.

A number of factors may account for the variation of PSA levels obtained in these two studies. It has been reported by Link et al. (2004) that age, race/ethnicity, medications, prostate gland inflammation, benign enlargement of the prostate gland, laboratory variability, and body mass index are among the factors other than prostate cancer that can influence PSA level. First of all, our study population was entirely blacks compared to theirs who were predominantly white people. In 1997, Richardson and Oesterling reviewed multiple studies

and determined age-specific reference ranges for serum PSA in men of different races. During the study, men were grouped into 4 decades and the determined acceptable ranges were found to be lower for Asians and African Americans than for Caucasians. Asians for example, are known to have a smaller prostate size, which would contribute to the lower PSA value.

Secondly, most of our PCa patients were 'freshly' diagnosed and were not on any medication such as androgen deprivation which is reported to be associated with hypogonadism and low levels of PSA and testosterone. In their study, about 52.5% of their PCa patients had undergone androgen deprivation resulting in a significantly lower PSA levels compared to the non androgen deprived men. Therefore, the overall significantly lower levels of PSA among their PCa patients compared to ours may have possibly resulted from the androgen deprivation therapy (Basaria et al., 2002).

CHAPTER 6

6.1 CONCLUSIONS

It has been established by this study that increased lipid peroxidation (oxidative stress) with reduced antioxidant levels are common in PCa patients. Malondialdehyde (lipid peroxidation index) and uric acid were significantly increased among the PCa groups compared to the control whereas the measured antioxidant (vitamin C) was significantly reduced among the PCa patients. Therefore oxidative stress may have a significant role in prostate cancer as other studies have reported. Meanwhile, based on the findings, it may seem reasonable to propose that therapeutic regimens aimed at beefing up the antioxidant defences could offer some degree of protection for PCa patients against oxidative stress.

The prevalence of metabolic syndrome (MetS) in the PCa population was 9.2%, 18.4% and 12.6% using NCEP-ATP III, IDF and WHO criteria respectively. The highest prevalence was generally associated with the highly aggressive PCa group (i.e. 18.6%, 23.3% and 20.9% for NCEP-ATP III, IDF and WHO respectively) as compared to the control and other PCa groups. This points out that metabolic syndrome is associated with prostate cancer as other studies have reported. However, the role of metabolic syndrome in PCa development cannot be ascertained by this study's findings. It can only point out that metabolic syndrome may have a role in the progression of PCa.

6.2 RECOMMENDATION

There is the need for further detailed investigation into the relationship between prostate cancer and oxidative stress that will factor in multiple assessments of many different pro-oxidants and antioxidants as a case control study among prostate cancer patients. This can also be applied to the metabolic syndrome features in order to help affirm further their association with prostate cancer for which if proven suggests that prevention or control of

these conditions eventually may lead to a significant reduction in the deaths of PCa patients worldwide.

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