TITLE PAGE

EVALUATION OF DIAGNOSTIC POTENTIALS OF SOME TUMOUR MARKERS IN SUBJECTS WITH PROSTATE DISORDERS IN NNEWI, NIGERIA.

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DEDICATION

This research work is dedicated to God Almighty who gave me the strength, showed me mercies and provided for me the resources used to carry out this study.

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Abbreviations

5ARD-	5- alpha reductase
5ARIS -	5alpha-reductase inhibitors
AAS -	Atomic absorption spectrophotometer
ADT -	Androgen deprivation therapy
AR –	Androgen receptor
ATP –	Adenosine triphosphate
BMI -	Body mass index
BOO -	Bladder outlet obstruction
BPE -	Benign prostatic enlargement
BPH-	Benign prostate hyperplasia
CT -	Computed tomography
DHT-	dihydrotestosterone
DMSO -	Dimethyl sulfoxide
DNA –	Deoxyribonucleic acid
DRE - .	Digital rectal examination
ELISA -	Enzyme linked immunosorbent assay
EN2-	Engrailed-2 gene
FAD -	flavin adenine dinucleotide
FDA -	Food and Drug Administration
FPSA –	free prostate specific antigen
GLC -	Gas Liquid chromatography
GNMT-	Glycine N-methyl transferase

GnRH –	Gonadotrphic releasing hormone
Gpx -	Glutathione peroxidase
H_2O -	Water
H_2O_2 -	Hydrogen peroxide
HCIO -	Hypochlorous acid
hK3 -	human kallikrein 3
HRP -	Horseradish peroxidase
IFN –	Interferon-gamma
IGF-	Insulin growth factor
IL-2 -	Interleukin-2
ISUP -	International Society of Urological Pathology
IU –	International unit
LDL -	Low density lipoprotein
LHRH -	Luteinizing Hormone-Releasing Hormone
LUTS-	lower urinary tract symptoms.
Mg-	magnesium
MRI -	Magnetic Resonance Imaging
MT -	Metallothioneins
NAUTH -	Nnamdi Azikiwe University Teaching Hospital, Nnewi
NK -	Natural killer
O_2 -	Superoxide anion
OD -	Optical density
OH^- -	Hydroxyl radical
PAP--	Prostatic acid phosphatase
Pca –	Prostate cancer

PCA3 -	prostate cancer antigen 3
PIN -	prostatic intraepithelial neopalsia
PIPOX -	Pipecolic acid oxidase
PL-OH -	phospholipids hydroxide
PL-OOH -	phospholipids hydro peroxide
PSA-	Prostate-specific antigen
PUFA -	Polyunsaturated fatty acids
RDAs -	Recommended Dietary Allowances
RNA –	Ribonucleic acid
RNS	Reactive nitrogen species
ROH -	Hydroxyl fatty acid
ROS	Reactive oxygen species
RXR -	Retinoid X receptor
SAH -	S-adenosylhomocysteine
SAM -	S-adenosylmethionine
SARDH-	Sarcosine dehydrogenase
Se -	Selenium
SELECT -	Selenium and Vitamin E Cancer Prevention Trial
SOD-	Superoxide dismutase
SPF -	sun protection factor
SSRIs -	Selective Serotonin Reupdate Inhibitors
TAC -	Total Antioxidant Capacity
TNF -	Tumour necrosis factor-alpha
TNM -	Tumor/Nodes/Metastases
TPSA –	total prostate specific antigen

tRNA -	Transcription, ribonucleic aciad
TRUS -	Transrectal ultrasonography
TUMT-	Transurethral microwave thermotherapy
TUNA-	Transurethral needle ablation
TURP-	transurethral resection of the prostate
US -	Ultrasound
USPSA -	Ultrasensitive PSA
USPSTF -	United State Preventive Services Task Force
UTI -	Urinary tract infection
UV -	Ultraviolet
VDR -	Vitamin D receptors
VDRE -	Vitamin D response element
VITAL -	Vitamins And Lifestyle
XO -	xanthine oxidase
Zn -	Zinc
ZnT -	zinc transporter
%FPSA -	percentage free prostate specific antigen
$1,25(OH)_2D$ -	1,25-dihydroxyvitamin D

ABSTRACT

Introduction

Background. Prostate cancer is a major health problem worldwide and ranks as the second most common cause of cancer related death in men.Tumour markers are substances that aid in the detection, diagnosis and management of malignant growth. Prostate specific antigen (PSA) is one of the most widely used tumour marker and currently used for detection of prostate cancer (Pca). There is lack of consensus among researchers with respect to the benefits of PSA test. Evaluation of new biomarkers is therefore desirable for the diagnosis of prostate cancer.

Aim. This study evaluated the diagnostic potentials of some tumour markers in patients with prostate disorders in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria.

Methodology. The is a prospective study involving 60 male subjects with prostate cancer, 60 male subjects with benign prostate hyperplasia (BPH) and 60 male control subjects between the ages of 50 and 90 years. Sarcosine, total antioxidant capacity (TAC), superoxide dismutase (SOD) and creatinine were assayed using colorimetric methods. Engrailed -2 gene proteins (EN-2), 5-alpha reductase (5ARD), total PSA (TPSA) and free PSA (FPSA) were assayed using enzyme linked immunosorbent assay technique (ELISA). Vitamins C, D and E were assayed using gas chromatography mass spectroscopy (GC-MS), while Zn, Se and Mg were assayed using atomic absorption spectrophotometric (AAS) method. Statistical package for Social Sciences (SPSS) version 20 was used for data analysis.

Results. Sarcosine level (5.36±1.6nmol/l) in Pca group was statistically higher (p=0.0001) than in control (3±0.52 nmol/l). Sarcosine level in BPH (3.09±0.34nmol/l) did not differ significantly (p>0.05) from the controls. Sarcosine level was also significantly higher in the pca patients with metastasis than those without metastasis. The activity of 5ARD in BPH (6.55±1.9) and Pca (7.10±1.31ng/ml) was significantly higher than in in control (3.7±1.02ng/ml). Compared with control (15.23±7.3), the TAC was significantly lower (p=0.0001) in both BPH (7.40±2.60) and Pca (7.2±2.2nmol/l). The TPSA and FPSA (104±49.05 and 9.41±7.83 respectively) in Pca and BPH (42.80±49.15, 2.99±5.21 respectively) were significantly (p=0.0001) lower than in controls (5.24±4.97, 0.84±0.98). The SOD activity for Pca (1.41±0.63U/l) was significantly (P=0.0001) lower than the BPH (2.18±1.1) and control (2.11±0.96). The mean level of vitamin D for BPH and Pca were 10.91±13.49 and 8.82±14.66, these were significantly lower than the mean level of control (23.33±29.75mg/dl). Vitamin E level in controls (10.71±0.44mg/dl) was significantly higher than in BPH and Pca (6.17±7.87 and 6.07±0.48g/dl). There was no significant difference in the engrailed- 2 gene protein in Pca and BPH subjects compared with the controls. Selenium was significantly lower (p=0.02) in BPH (27.53±9.49) and in Pca (20.81±8.80) compared with control. Zinc was significantly lower in BPH (20.01±15.28) and in Pca (18.99±18.29) compared with control. Likewise, Mg was significantly lower (P=0.002) in BPH (3.18±0.22) and in Pca (1.89±1.79) compared (p=0.001) with control (5.17±1.06). The sensitivity (75%) and specificity (71%) of sarcosine as calculated using Bradley's formula was higher than that of SOD (68%, 65%), 5ARD (58%, 68%), TPSA (38%, 45%) and TAC (31%, 61%) respectively.

Conclusion. This study showed that sarcosine was a more reliable biomarker for the diagnosis of prostate cancer than all the other biomarkers studied.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Prostate cancer is a major health challenge throughout the world. It is the most common solid organ malignancy affecting men and a freqeuent cause of morbidity and mortality and in fact the second leading cause of cancer death in men. Globally, prostate cancer accounts for 9.7% of all cancers (Parkin et al, 2001). Early diagnosis remains a major problem. Precise diagnosis is based largely on the use of biomarkers called tumour markers. Tumour markers are substances that are produced by tumour cells themself or by the normal body cells in response to growth. These substances are produced at much higher levels in malignant conditions. They help to detect, diagnose and manage malignant growth. The prostate is an organ linked inextricably with the endocrine system. It is part of the male sex organ. During the development of the prostate, epithelium and mesenchyme are under the control of testicular androgens, and interact to form an organized secretory organ. Thus many of the disease processes affecting the prostate gland are attributed to, and therapies aimed at manipulation of endocrine system. Prostate gland is the male organ most commonly afflicted with benign or malignant neoplasms (Nathan and Mark, 2008). A man's risk of developing prostate disorders is related to his age, genetics, race, diet and lifestyle (Kathryn et al, 2012). The primary risk factor is age. In Nigeria, Osegbe (1997) reported prostate cancer hospital incidence rate of 127 per 100000 cases. More recent studies (Ejike, 2006) showed that 4% of apparently healthy Nigerian male population, aged 40 years and above, living in Nsukka (South East) exhibited prostate specific antigen (PSA) levels elevated beyond the normal range.

Prostate cancer is best diagnosed with tumour markers. However, prostate cancer is usually multifocal and more difficult to detect (Halpern, 2006) when compared with other cancers that present as a single mass. In addition prostate cancer symptoms do not typically present until the cancer has reached advance stage (Fuchsjager, 2008). Hence Surgeons have consistently relied on tumour markers as indicator of the presence and progression of cancer. An ideal biomarker would allow early and easy detection of cancerous tissues.

Various biomarkers used in the detection of prostate disorders include the established ones such as prostate specific antigen and the ones suspected to be potentially useful for diagnosis of prostate disorders such as sarcosine and engrailed 2 gene proteins (Brimelow, 2011: Lucarelli et al, 2012). Futhermore, some considered to be indirect biomarkers may be useful in the detection of prostate disorders, this include 5- alpha reductase enzyme. Several years, the use of biomarkers for prostate cancer screening, diagnosis and prognosis has revolutionized its clinical management. (Prensner et al, 2012). The first prostate cancer biomarker, prostatic acid phosphatase (PAP), was described in the 1930s and has since then been used as a clinical marker for prostate cancer progression, since serum PAP levels were found to be elevated in metastatic cases (Prensner et al, 2012 :). This biomarker was replaced by prostate-specific antigen (PSA) in the 1980s (Ercole et al, 1987) . Sarcosine is an amino acid derivative of N-methylglycine and is involved in the amino acid metabolism and methylation processes which are enriched during prostate cancer progression. Sarcosine is formed by the enzyme glycine N-methyl transferase (GNMT) or dimethylglycine dehydrogenase (DMGDH) and converted back to glycine via sarcosine dehydrogenase (SARDH). As prostate cancer progresses toward the metastatic disease, amino acid metabolism along nitrogen breakdown pathway increases (Sreekumar, et al 2009) Hence sarcosine may increase with increasing severity of disease.

Gene and protein expression have been extensively profiled in human tumors, little is known about the global metabolic alterations that characterize neoplastic progression. This test has been postulated to correlate with high probability of prostate cancer. It is a non- invasive test looking for the presence of the protein EN2 in blood or urine which may be more reliable and accurate than the existing biomarkers. The prostate gland is under androgenic control. The cells of the prostate require the androgen testosterone and its more potent reduced form dihydrotestosterone (DHT) for proper growth, differentiation. The key enzyme that converts testosterone to dihydrotetosterone is 5- alpha reductase (Gann et al, 1996). Inhibition of this enzyme results in decreased production of DHT, increased levels of testosterone; hence the elevation of this enzyme has been implicated in the pathogenesis of both benign prostate hyperplasia and prostate cancer (Andriole et al. 2004: Jason et al 2005).

Vitamin E functions as the lipid phase antioxidant in biological systems. Alpha tocopherols carry out this function by combining directly with radical species especially reactive oxygen and reactive nitrogen species (ROS/RNS). Nitrosamines are implicated in the etiology of cancers (Gupta et al, 2012). Nitrate radical produced in free radical mediated reactions may be quenched by alpha tocopherol resulting in the formation of tocopheryl radicals. Furthermore, report from Helzlsouer et al (2000) showed that vitamin E may also be protective against cancers by enhancing immune function.

Data relating to current biomarkers with respect to the detection and progression of prostate disorders appear sparse and available reports from various authors seem inconclusive.

1.2 Statement of the Problem

Prostate disorders are becoming an increasingly important public health problem Worldwide it has been estimated that men die of prostate cancer at the rate of 1 person every 18 minutes (Moya, 2010). In Nigeria, two percent of men have been reported to develop prostate cancer one of the common prostate disorders and 64% of them die after two years (Osegbe, 1997). This may be due to lack of screening facilities for diagnosis and poor management of the patients or even late reporting to hospital. It could also be as a result of inherent unreliability and limitations of the current markers, hence the intensive search for beter and more reliable markers for diagnosis of prostate cancer.

Among prostate disorders, prostate specific antigen is used currently for the diagnosis of prostate cancer. However, high PSA level may not be indicative of prostate cancer and it has been reported that some men with established cancer exhibit PSA levels lower than 4ng/ml (the upper limit of the reference range) which suggests that PSA exhibits low specificity (Bickers and Aukim 2009). Because owing to this limitation, there has been considerable debate over what PSA level demand concern and if PSA should be used at all (Mac Donald 2011). Between 2-10ng/ml described as the grey area, it is difficult to separate patient with prostate cancer from those without (Sreekumar, et al 2009). Despite the increasing current medical and surgical therapies available, the debate continues. It is in line with this current global problem that this study was designed to assess plasma sarcosine, engrailed -2 gene protein, 5- alpha reducatse, total antioxidant capacity, vitamin C, D and E in prostate disorders, in an attempt to improve understanding, early diagnosis and prognosis of these disorders.

1.3 Justification of the Study

The key limitations with PSA currently used as the standard detection test for prostate cancer stated above suggest the need for the evaluation of new tumour markers for prostate disorders.

1.4 Aim of the Study

This study was designed to explore for more sensitivity, specific and reliable panel of biomarkers of protate disorders (cancer).

1.5 Specific Objectives

1. To measure plasma sarcosine and engrailed-2 gen as a potential biomarkers in individuals with prostate disorders and controls.
2. To determine the activity of 5 alpha reductase, the plasma concentration of prostate specific antigen in prostate disorders and non-prostate diseased individuals.
3. To determine the levels of the antioxidants, vitamin E, C, D, selenium, Superoxide dismutase and total antioxidant capacity.
4. To assess zinc, magnesium in prostate disorders.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prostates

The prostate is a part of the male reproductive system that helps make and store seminal fluid. In adult men a typical prostate is about three centimeters long . The prostate is a small gland usually described as ‘’walnut-sized’’ that together with the seminal vesicles, produces the fluid that form part of the semen. It is located in the pelvis, under the urinary bladder and in front of the rectum. The prostate gland surrounds part of the urethra, the tube that carries urine from the bladder during urination and semen during ejaculation (Moore and Dalley, 1999). Due to its location prostate diseases often affect urination, ejaculation, and rarely defecation. The prostate gland contains many small glands which make about twenty percent of the fluid constituting semen (Steive, 1930).

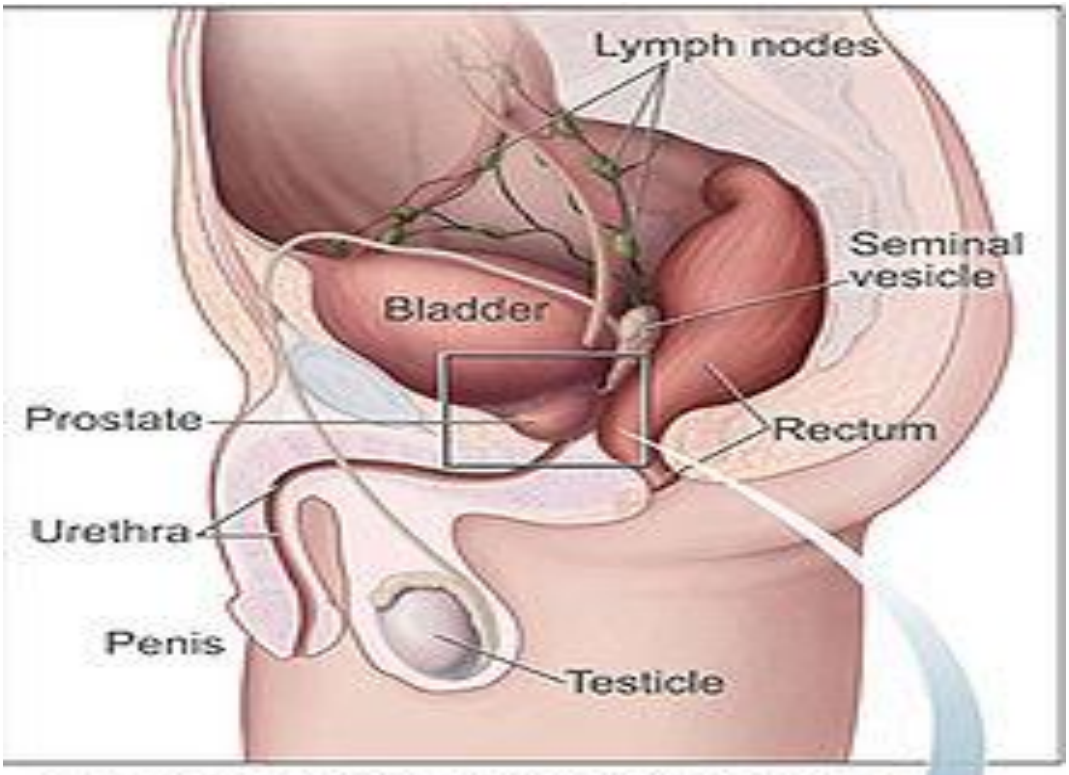
Prostate disorders are usually connected to age of the individual. Young men are rarely affected by these disorders. The most common prostate problem in men under 50 years is inflammation or infection, which is called prostatitis. Prostate enlargement also called benign prostate hyperplasia (BPH) is another prostate problem that is common in men over 50 years; this is as a result of constant growth of the gland as a man matures. Older men are at risk of Prostate cancer as well, but it is less common than benign prostate hyperplasia (NKUDIC, 2006). In other words the prostate can be afflicted with three major diseases, namely prostate cancer, benign prostatic hyperplasia, and prostatitis. In prostate cancer, the cells of this gland transform into cancer cells.

Apart from age, other factors including genetics and diet (lifestyle) have been implicated in the development of prostate disorders (Kathryn et al, 2012).. There is a significant relationship between lifestyle (including food consumption) and prostate disorders. Exercise (physical

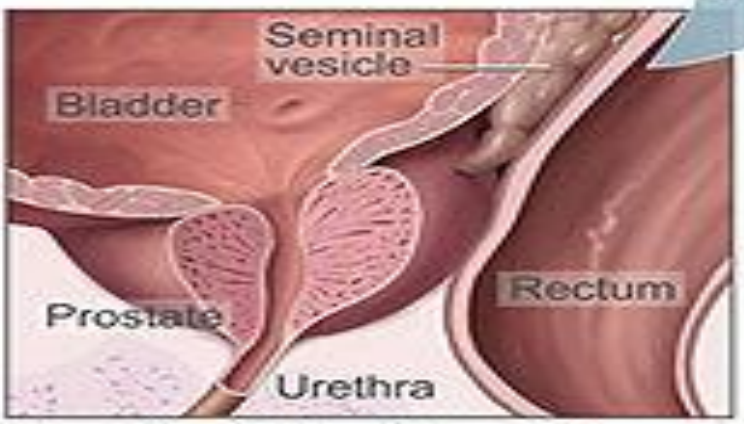
activity) may help**.** Observational studies (Kenfield et al, 2011) suggest that moderate to vigorous physical activity is associated with reduced risk of biochemical recurrence and mortality in men with prostate cancer. For example 3 hours of moderate to vigorous physical activity per week was associated with a 61 % decrease in prostate cancer mortality compared with less than 1 hour . Brisk walking is a potential alternative, as it provides exposure to moderate-intensity activity, and has been associated with anti-cancer cellular behaviour (Studenski et al, 2011). A higher body mass index has been associated with poorer prostate cancer survival suggesting that promotion of physical activity may reduce cancer progression through weight control.

The prostate glands require male hormones known as androgens to function properly. Androgens includes testosterone, which is made in the testes; dehydroepiandrosterone produced in the adrenal glands and dihydrotestosterone which is converted from testosterone within the prostate itself.

The prostate is from a Greek word which literally means ‘’one who stands before’’, ‘’protector’’, ‘’guardian’’. It is a compound tubule-alveolar exocrine gland of the male reproductive system in most mammals. The prostate was first described by Venetian anatomist in 1536 and illustrated by Flemish anatomist Andreas Vesalius in 1538. In 2002, the female paraurethral glands, or Skene’s glands were officially renamed the female prostate by the Federative International Committee on Anatomical Terminology (Flam, 2006). The paraurethral gland found in females is homologous to the prostate gland in male. However, evolutionarily, the uterus is in the same position as the prostate gland. The female prostate gland, like the male prostate gland secretes prostate specific antigen (PSA) and the levels of this antigen rise in the presence of carcinoma of the gland. The gland also expels fluid, like the male prostate gland during orgasm (Kratochvil, 1994).



This shows the prostate and nearby organs.



This shows the inside of the prostate, urethra, rectum, and bladder.

Myers, 2000

Fig 2.1 Anatomy of the Prostate Gland

2.2 Function of the Prostate gland

The function of the prostate is to secrete a slightly alkaline milky fluid, or white in appearance that usually constitutes 20-30% of the volume of the semen along with spermatozoa and seminal vesicle fluid. The alkalinity of semen helps neutralize the acidity of the vaginal tract, prolonging the life span of the sperm. The alkalinization of the semen is primarily accomplished through secretion from the seminal vesicles. The prostatic fluid is expelled in the first ejaculate fractions, together with most of the spermatozoa. In comparison with the few spermatozoa expelled together with mainly seminal vesicular fluid, those expelled in prostatic fluid have better motility, longer survival and better protection. The prostate also contains some smooth muscles that help expel semen during ejaculation. During male orgasm, sperm is transmitted from the ductus deferens into the male urethra via the ejaculatory ducts, which lie within the prostate gland. It is possible for men to achieve orgasm solely through stimulation of prostate gland such as prostate massage or receptive anal intercourse. Prostatic secretions vary among species. They are generally composed of simple sugars. In human prostatic secretions, the protein content is less than 1% and includes proteolytic enzymes, prostatic acid phosphate and prostate specific antigen. The secretion also contains zinc with the concentration 500-1000 times the concentration in the blood (Radhika and Ajay, 2016). Zinc in the body plays an important role in normal testicular development, spermatogenesis, and sperm motility (Madding et al 1986). Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa (Lin et al, 2000). Zinc copes up with excessive amount of superoxide anions, thus adequate amount of zinc in seminal plasma exerts protective effect by virtue of this antioxidant activity (Gavella et al, 1998). It appears to protect sperm from bacteria and chromosomal damage (O'Connor, 2001)

2.3 Types of Prostate Gland

The prostate can be classified by two ways: by zones or by lobes

Zones

The zone classification is more often used in pathology. The idea for zones was first proposed by Mc Neal in 1968 cited in Meyer (2000). The prostate gland has four distinct glandular regions, two of which arise from different segments of the prostatic urethra: The zones are as follows.

Peripheral Zone: This is a sub-capsular portion of the posterior aspect of the prostate gland that surrounds the distal urethra. It is from this portion of the gland that 70-80% of prostatic cancer originate (Harvey et al, 2012)

Central Zone: This zone surrounds the circulatory ducts. It accounts for roughly 2.5% of prostate cancers although these cancers tend to be more aggressive and more likely to invade the seminal vesicles.

Transitional Zone: About 10- 20% of prostate cancer originate in this zone. It surrounds the proximal urethra and is the region of prostate gland that grows throughout life and is responsible for the disease of benign prostatic hyperplasia.

Anterior Fibro-muscular Zone: This zone is usually devoid of glandular components, and is made of muscle and fibrous tissues.

The lobe classification is more commonly used in anatomy and it is as follows:

1. The anterior lobe which roughly corresponds to the part of transitional zone,
2. The posterior lobe which roughly corresponds to peripheral zone,
3. The lateral lobe that spans all the zones and

4. The median lobe (middle lobe) which roughly corresponds to part of central zone.

2.4 General Prostate Disorders

The three major diseases that afflict the prostate gland are

I. Prostatitis

II. Benign prostatic hyperplasia and

III. Prostate cancer.

Most experts agree that there is little men can do to prevent the big three prostate problems. However what one can do is to beware of the problems when they first develop and try to treat them before they get out of hand.

I. The symptoms of these disorders include:

II. Burning sensation while urinating

III. Pelvic pain

IV. Frequent urination

V. Dribbling or incontinence

VI. Impotence

VII. A sudden inability to urinate

VIII. Urinary tract infection,

IX. Haematuria and

X. Nocturia.

2.5 Prostatitis

Prostatitis refers to inflammation of the prostate gland. It could also mean that the prostate is irritated. In this type of disorder the patient feels burning sensation when urinating and may urinate more often, the patient may also have fever or just feel tired. Inflammation in any part of the body is usually a sign that the body is fighting infections or repairing an injury. Some kind of prostatitis is caused by bacteria.

There are primarily four different forms of prostatitis, each with different causes and outcomes. Two relatively uncommon forms, acute prostatitis and chronic bacterial postatitis, that are treated with antibiotics (categories I and II, respectively). Chronic non- bacterial prostatic or male chronic pelvic syndrome (category III), which comprises about 95% prostatitis diagnosis, is treated by a large variety of modalities including alpha blocker. The category IV is relatively uncommon in the general population; a type of leukocytosis. Patients with this type of disorder have to work with their physician to find a treatment that is good for them.

2.6 Benign Prostatic Hyperplasia

Benign prostatic hyperplasia is a condition in which the prostate gland becomes enlarged. However, the actual size of the gland does not necessarily predict symptom severity. Some men with minimally enlarged prostate glands may experience symptoms while other men with much larger glands may have few symptoms. It occurs in older men (Verhamme, et al 2002). The symptoms associated with BPH are collectively called lower urinary tract symptoms (LUTS). Benign prostatic hyperplasia is prostate disorder noncancerous cell growth of the prostate gland. It is the most common noncancerous form of cell growth in men and usually begins with microscopic nodules in younger men. Benign prostate hyperplasia is not a precancerous condition and does not lead to prostate cancer.

Although there is no precise reason for enlargement, many investigators think that the stimulation produced by male sex hormones play a key role (Jason et al, 2015). As a man advance toward middle age his prostate grows. It continues to grow in size as the man is alive. The prostate is a very small gland in young boys. It grows to the size of nickel in diameter as the boy turns into adult. Normally it remains stable for the next 25-30 years, and then begins to enlarge, sometimes to the size of an orange. This enlargement is believed to be due to hormonal effects. The problem with BPH is that the enlarged prostate can press on a man's urethra, causing difficulty in urination; other symptoms commonly associated with this condition mainly frequent urination or taking a while to get started (hesitancy). If the prostate grows too large, it may constrict the urethra and impede the flow of urine, making urination too difficult and painful and in extreme cases, unable to void urine (retention). Benign prostate hyperplasia is not a serious condition, unless the symptoms are so bothersome that it disrupts one from enjoying his life. One of the complications of BPH is urinary tract infection.

Benign prostatic hyperplasia can be treated with medication, a miniamally invasive procedure or in extreme cases by surgery that removes the prostate. Minimally invasive procedure include transurethral needle ablation of the prostate gland (TUNA) and transurethral microwave thermotherapy (TUMT). The surgery most often used is called transurethral resection of the prostate (TURP). In TURP, an instrument is inserted through the urethra to remove prostate tissues that press against the upper part of the urethra restricting the flow of urine. Transurethral resection of the prostate results in the removal of mostly transitional zone tissue in patient with BPH. Older men often have corpora amylacea (amyloid), dense accumulations of calcified proteinacious material, in the ducts of their prostates. The corpora amylacea may obstruct the lumens of the prostatic ducts and may underlie some cases of BPH. It is important to note that in

older men also, uninary frequency due to bladder spasm is common, this should not be confused with prostatic hyperplasia. Epidermological observations suggest that low in fat and red meat and high in protein and vegetables as well as regular alcohol consumption could protect against BPH (Kristal et al, 2008)

Hyperplasia is a common preneoplastic response to stimulus. Microscopically, cells resemble normal cells but are increased in numbers. Sometimes cells may also be increased in size (hypertrophy). Hyperplasia is different from hypertrophy in that the adaptive cell change in hypertrophy is an increase in the size of cells, whereas hyperplasia involves an increase in the number of cells.

Hyperplasia is considered to be a physiological (normal) response to a specific stimulus, and the cells of a hyperplastic growth remain subject to normal regulatory control mechanisms. This differs from neoplasia (the process underlying cancer and benign tumors), in which genetically abnormal cells manage to proliferate in a non-physiological manner which is unresponsive to normal stimuli.(MedlinePlus Medical Encyclopedia, 2014)

Hyperplasia may be due to any number of causes, including increased demand (for example, proliferation of basal layer of epidermis to compensate skin loss), chronic inflammatory response, hormonal dysfunctions, or compensation for damage or disease elsewhere. Hyperplasia may be harmless and occur on a particular tissue. An example of a normal hyperplastic response would be the growth and multiplication of milk-secreting glandular cells in the breast as a response to pregnancy, thus preparing for future breast feeding.

Perhaps the most interesting and potent effect of insulin growth factor (IGF) has on the human body is its ability to cause hyperplasia, which is an actual splitting of cells. By contrast, hypertrophy is what occurs, for example, to skeletal muscle cells during weight training and

steroid use and is simply an increase in the size of the cells. With IGF use, one is able to cause hyperplasia which actually increases the number of muscle cells present in the tissue. Weight training with or without anabolic steroid use enables these new cells to mature in size and strength. It is theorized that hyperplasia may also be induced through specific power output training for athletic performance, thus increasing the number of muscle fibers instead of increasing the size of a single fiber.

Whereas hypertrophy stems from an increase in cell size, hyperplasia results from an increase in cell number. Hyperplasia is increased cell production in a normal tissue or organ. Hyperplasia may be a sign of abnormal or precancerous changes. This is called pathologic hyperplasia

The term benign prostatic hyperplasia (BPH) has different connotations to the pathologist, urodynamicist, practicing urologist, and patient. To the pathologist, BPH is a microscopic diagnosis characterized by cellular proliferation of the stromal and epithelial elements of the prostate (Strandberg, 2000). To the practicing urologist, it represents a constellation of lower urinary tract symptoms (LUTS) that develop in the male population in association with aging and prostatic enlargement, presumably caused by bladder outlet obstruction (BOO) (Shapiro and Lepor, 2000). To the urodynamicist, the hallmark of BPH is the observation of synchronous elevated voiding pressure and a low urinary flow rate in the absence of other disease processes that cause BOO (Nitti, 2000). The patient is typically concerned about the impact of BPH on quality of life rather than the presence of cellular proliferation, prostatic enlargement, or elevated voiding pressures.

Because of the diverse connotations associated with the term, it is necessary to define BPH as microscopic BPH or clinical BPH. Microscopic BPH represents histologic evidence of cellular proliferation of the prostate. Macroscopic BPH refers to enlargement of the prostate resulting

from microscopic BPH. Clinical BPH represents the LUTS, bladder dysfunction, hematuria, and urinary tract infection (UTI) resulting from macroscopic BPH. Abrams (1994) has suggested using the more clinically descriptive terms benign prostatic enlargement (BPE), BOO, and LUTS to replace BPH.

Microscopic BPH describes a proliferative process of the stromal and epithelial elements of the prostate (Bartsch et al, 1979). The proliferative process originates in the transition zone and the peri-urethral glands (McNeal, 1983). It is rarely identified in men younger than 40 years (Berry, 1984). The autopsy incidence of BPH is age-dependent, with the proliferative process being present in approximately 70% and 90% of men in their seventh and ninth decades of life, respectively. The development of microscopic BPH requires aging and the testes as the source of androgens (Walsh, 1986). Androgens play a passive role in the proliferative process. The specific biochemical event that initiates and promotes microscopic BPH has yet to be identified and characterized. Growth factors presumably are involved through autocrine and paracrine stromal epithelial interactions (Steiner, 2000).

Macroscopic BPH denotes an "enlarged" prostate. Digital rectal examination (DRE) provides a relatively crude estimate of prostate size compared with measurements obtained using transrectal ultrasonography (TRUS) (Roehrborn et al, 1997). Although knowledge of prostate size may be clinically relevant in some cases, justifying the cost of obtaining a precise measurement of gland volume in all cases is questionable. A strong correlation exists between serum prostate-specific antigen levels and prostate volume (Roehrborn et al, 1999). There is no consensus regarding the extent of enlargement required to establish the diagnosis of macroscopic BPH. There is evidence that men with prostate volumes exceeding 40 cm^3 have a greater response to 5-alpa reductase

inhibitors (Boyle et al, 1996). Therefore, some experts limit the diagnosis of BPH to men with prostate volumes exceeding 40 cm^3.

The clinical manifestations of BPH include LUTS, poor bladder emptying, urinary retention, detrusor instability, UTI, hematuria, and renal insufficiency (JepsenBru and Skewitz, 2000). The overwhelming majority of men present with LUTS only. Historically, the pathophysiology of clinical BPH was attributed to BOO secondary to macroscopic enlargement of the prostate gland (Lepor, 2000: Roehrborn, 2005). This hypothesis was supported by epidemiologic data suggesting that the prevalence of microscopic BPH, macroscopic BPH, and clinical BPH is age-dependent and, therefore, causally related (Isaacs, 1989). This simplistic concept of the pathophysiology of BPH has been challenged by more recent reports demonstrating weak relationships among prostate size, severity of BOO, and severity of symptoms (Barry et al 1993, Yalla et al 1995).

2.7 Causes of Benign prostate hyperplasia

The prostate gland consists of glandular and stromal elements. The stroma contains smooth muscle and connective tissue. BPH involves an increase of all elements of the gland, but with a relatively greater increase of prostatic stroma. The prostate requires male hormones (testosterone and dihydrotestosterone) to grow. These hormones do not cause BPH, but are necessary for it to develop.

Aging and male hormones are the only proven risk factors for developing BPH. Any man with normal prostate and functioning testes will develop BPH if he lives long enough.

The testes produce 95% of testosterone found in the body. Testosterone is converted to dihydrotestosterone in the prostate gland. The prostate gland is much more sensitive to

dihydrotestosterone than testosterone. An enzyme called 5-alpha reductase mediates this conversion of testosterone to its active form. 5-Alpha reductase is specific to the prostate gland (Feldman and Feldman, 2001) and can be manipulated medically.

Dihydrotestosterone causes the formation of growth factors within the prostate gland, which in turn lead to an imbalance between cell growth and programmed cell death (apotosis).

The net effect of all this is a slow progressive enlargement of the prostate gland over time. While the majority of older men have clinically enlarged prostate glands, this per se does not necessarily lead to symptoms or complications.

BPH can cause symptoms due to its effect on the prostate itself, or due to its obstructive effect on the bladder outlet.

Proven Causes: Age, Hormones (testosterone)

Probable Causes: Genetics

Possible Causes

I. Western diet
II. Hypertension
III. Diabetes
IV. Obesity
V. Industrialised environment
VI. Increased androgen receptors
VII. Oestrogen/testosterone imbalance

2.8 Diagnosis of Benign prostate hyperplasia

Diagnosis of BPH is made based on medical history, physical examination and some confirmatory special tests.

History: Symptoms of BPH can be grouped as either obstructive or irritative. Diagnosis cannot be made on symptoms alone as many diseases can mimic the symptoms of BPH. A careful history will give clues to conditions other than BPH as the cause of symptoms.

Physical Examination: On physical examination the doctor will assess the patient's general health and examine the abdomen for the presence of a full bladder. A digital rectal examination will be performed to assess the size, shape and consistency of the prostate gland (Mistry and Cable, 2003)

This examination involves the insertion of a gloved finger into the rectum. The prostate gland is situated immediately adjacent to the anterior rectal wall and is easily palpable in this manner. The test is mildly uncomfortable, but should not be painful. BPH classically leads to smooth, rubbery enlargement, whereas prostate cancer causes hard irregular nodular enlargement of the prostate.

Unfortunately prostate size correlates poorly with symptoms or obstruction (Vesely, 2003). Many large prostates cause no symptoms or obstruction at all, and some very small prostates can lead to severe obstruction with symptoms and/or complications. An enlarged prostate per se is not an indication for treatment. In patients who do need treatment, the size of the gland can influence which treatment option is selected. A neurological examination is indicated if the history suggests a possible neurological cause for the symptoms.

2.9 Special Tests

Special tests are used to confirm diagnosis, rule out other causes of symptoms, prove or disprove obstruction and identify complications related to the obstruction.

Minimum recommended evaluation for BPH:

I. Medical history including symptoms index
II. Physical examination, including digital rectal examination
III. Urine analysis
IV. Urine flow rate
V. Assessment of renal function (serum creatinine)
VI. Pressure/flow urodynamic testing
VII. Serum PSA (prostate specific antigen)
VIII. Abdominal ultrasound of kidneys, ureter and bladder
IX. Transrectal ultrasound of prostate gland

Simple urine analysis can be performed in the office with dipstix. If this indicates possible infection a urine culture should be obtained. If the urine contains blood this should be further investigated to rule out other causes.

A urine flow rate is performed by asking the patient to pass urine into a machine, which measures urine flow rate. Most machines measure the volume of urine, the maximum flow rate and the time taken to empty the bladder. For a flow rate test to be of value the patient needs to pass at least 125-150 ml of urine at one time.

Serum creatinine is measured on a blood sample and is a fair reflection of renal function. Creatinine is one of the waste products excreted by the kidneys. If serum creatinine level is

elevated due to bladder outflow obstruction, it is prudent to drain the bladder with a catheter and allow the kidneys to recover prior to embarking on prostate surgery..

2.10 Treatment of Benign prostate hyperplasia

The main treatment options are watchful waiting, medication and surgery. In those patients who are totally unfit for surgery and for whom medication has failed, long-term indwelling catheters, self-intermittent catheterization or internal urethral stents can be used. The complications of BPH are generally regarded as indicators for surgery. Patients who have suffered complications related to BPH are not candidates for watchful waiting or medication.

Watchful waiting is a strategy of no immediate treatment with follow-up medical checks at regular intervals (Bill-Axelson et al, 2005) The natural history of BPH is not necessarily progressive. Symptoms remain stable or may even get better in many patients. Watchful waiting is suitable for patients with minimal symptoms and no complications. The patients can be reviewed yearly with symptom scores, physical examination and flow rate analysis. During watchful waiting patients should avoid tranquilisers and over-the-counter cold and sinus remedies, which can worsen symptoms and may even lead to urinary retention.

Several simple measures can improve symptoms related to BPH. Alcohol and caffeine should be taken in moderation, especially in the evening prior to going to bed. Tranquilisers and anti-depressants impair bladder muscle function and effective bladder emptying. Cold and flu remedies usually contain decongestants, which cause increased tone in smooth muscle fibres in the bladder neck and prostate, leading to worsening symptoms.

Phytotherapy refers to the use of plant extracts for medicinal purposes. These treatments for BPH-related symptoms have received attention in the popular press recently. Most widely known is the extract of serenoa repens (commonly known as Saw Palmetto). The mechanism of action

of these phytotherapies is unknown and their effectiveness unproven. Suggested modes of action include an anti-inflammatory effect to reduce prostate swelling and possible inhibition of hormones controlling the growth of prostatic cells. It is highly possible that their only action is as a result of the placebo effect.

2.11 Medication of Benign prostrate hyperplasia

Two types of medication are effective in the treatment of BPH, namely alpha-blockers and 5-alpha reductase inhibitors.

Alpha-blockers: The prostate and bladder neck contain large numbers of smooth muscle cells. The tone in these muscle cells is under sympathetic (involuntary) nervous system control. The receptors at the nerve endings are called alpha-receptors. Alpha-blockers are drugs that block these alpha-receptors, thus decreasing the tone in the prostate and bladder neck. The net effect is an increase in flow rate and an improvement in prostatic symptoms. Alpha-receptors are found elsewhere in the body, especially in blood vessels. The original alpha-blockers were designed to treat high blood pressure. Not surprisingly, the most frequent side-effect of alpha-blockers is orthostatic hypotension (dizziness upon standing due to a fall in blood pressure).

Commonly used alpha-blockers are prazosin (Minipress), doxazosin (Cardura), terazosin (Hytrin) and tamsulosin (Flomax) (Vincent et al, 1983: Sonders, 1986). Tamsulosin is a selective alpha 1A receptor blocker, specifically designed to block the sub-type of alpha-receptor found predominantly in the bladder and prostate

Alpha-blockers are effective in patients without absolute indications for surgery and post void residual volumes of less than 300ml. Most studies indicate a 30-60% reduction of symptoms and

a moderate increase in flow rate. All four alpha-blockers are effective at therapeutic dosages. The maximal effect is obtained within two weeks and the response is durable. Ninety precent of patients tolerates the treatment well. The main reasons for discontinuing treatment are dizziness due to hypotension and perceived lack of efficacy. No direct comparative studies between the various different alpha-blockers have been performed, and claims of relative superiority cannot be justified. Treatment usually needs to be life-long. A less common side effect is abnormal or retrograde ejaculation, which occurs in 6% of patients taking tamsulosin.

5-Alpha Reductase Inhibitors: The enzyme 5-alpha reductase converts testosterone to its active form, namely dihydrotestosterone within the prostate gland. Finasteride (Proscar) blocks this conversion. In some men finasteride can relieve BPH symptoms, increase urinary flow rate and shrink the size of the prostate gland. The improvements, however, are usually only modest and take up to six months to achieve. Recent studies indicate that finasteride may be more effective in men with bigger prostates and have little effect in men with smaller glands. Finasteride does reduce the incidence of urinary retention and the need for prostatic surgery by 50% over a four-year period.

Due to its cost, moderate efficacy and long time to achieve maximal benefit, finasteride is not widely used for BPH treatment in South Africa. Side-effects of finasteride include breast enlargement (0.4%), impotence (3-4%), decreased ejaculate volume.

Surgery (Prostatectomy): Prostatectomy is the most commonly performed urological procedure (Oleksandr et al 2016). About 200,000 prostatectomies are performed annually in the USA. A prostatectomy for benign disease (BPH) involves removal of only the inner portion of the prostate. This operation differs from radical prostatectomy for cancer in which all prostate tissue

is removed. Prostatectomy offers the best and fastest chance of improving BPH symptoms, but may not alleviate all irritative bladder symptoms. This is especially true for men over 80 years of age, where bladder instability is thought to account for a large proportion of symptoms.

Indications for Prostatectomy

1. Retention of urine
2. Renal impairment secondary to obstruction
3. Recurrent urinary tract infections
4. Bladder stones
5. Large residual volumes (relative indication)
6. Failed medical treatment - ineffective or side-effects
7. Patient not keen on medical treatment

2.12 Transurethral Resection of Prostate (TURP)

This procedure is still considered the 'gold standard' of BPH treatments against which all other treatment options are measured. TURP is performed using a resectoscope, which is passed through the urethra into the bladder. A wire loop carrying an electrical current cuts the prostatic tissue away from the inside. A catheter is left in place for one to two days and hospital stay is usually about three days. TURP is associated with little or no pain and full recovery can be expected by three weeks after surgery. Marked improvement occurs in 93% of men with severe symptoms and 80% of those with moderate symptoms

2.13 Prostate Cancer

Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system. Prostate cancer is one of the most common cancers affecting older men in developed

countries and a significant cause of death for elderly men. Globally, about 9.7% of cancers in men are of the prostate. The figure rises to 15.3% in the developed world and drops to 4.3% in the under developed world (Parkin et al 2001). The prevalence of prostate cancer is highest in United States and Canada, but lowest in Asian countries (Quinn and Babb, 2002). It is the second leading cause of cancer death in the United State and United Kingdom after lung cancer. In Nigeria, 2% of men develop prostate cancer, and 64% of them die after two years (Osegbe, 1997). Moreover, it has been shown recently that 4% of an apparently healthy Nigeria male population (south-east) aged 40 years and above had their prostate specific antigen levels elevated beyond normal (Ejike, 2006). The specific causes of prostate cancer remain unknown (Hsing and Chokkalingam, 2006). The primary risk factors are age and family history. This disorder is uncommon in men younger than 45 years, but becomes more common with advancing age. Men who have first degree family members with prostate cancer appear to have double the risk of getting the disease compared to men without prostate cancer in the family. This risk appears to be greater for men with affected brother than for men with an affected father. Men with two first degree relatives affected have a fivefold greater risk compared with men with no family history.(Steinberg et al, 1990). No single gene is responsible for prostate cancer, many different genes have been implicated. Mutations in BRAC 1 and BRAC 2, important risk factors for ovarian and breast cancer in women, have also been implicated in proste cancer. (Struewing et al, 1997).

Some men with prostate cancer remain asymptomatic and die from unrelated causes rather than as a result of the cancer itself. This may be due to the advanced age of many men at the time of diagnosis, slow tumor growth, or response to therapy (Ruijter et al, 1999) The estimated number of men with latent prostate carcinoma (i.e., prostate cancer that is present in the prostate gland

but never detected or diagnosed during a patient's life) is greater than the number of men with clinically detected disease. A better understanding is needed of the genetic and biologic mechanisms that determine why some prostate carcinomas remain clinically silent, while others cause serious, even life-threatening illness.

Prostate cancer exhibits tremendous differences in incidence among populations worldwide; the ratio of countries with high and low rates of prostate cancer ranges from 60-fold to 100-fold (Stanford et al, 2014). Asian men typically have a very low incidence of prostate cancer, with age-adjusted incidence rates ranging from 2 to 10 cases per 100,000 men. Higher incidence rates are generally observed in northern European countries. African American men, however, have the highest incidence of prostate cancer in the world; within the United States, African American men have a 60% higher incidence rate than white men (Miller et al 1996).

These differences may be due to the interplay of genetic, diet, environmental, and social influences (such as access to health care), which may affect the development and progression of the disease. (Haas and Sakr, 1997) Differences in screening practices have also had a substantial influence on prostate cancer incidence, by permitting prostate cancer to be diagnosed in some patients before symptoms develop or before abnormalities on physical examination are detectable. An analysis of population-based data from Sweden suggested that a diagnosis of prostate cancer in one brother leads to an early diagnosis in a second brother using prostate-specific antigen screening (Hemminki et al, 2005). This may account for an increase in prostate cancer diagnosed in younger men that was evident in nationwide incidence data. A genetic contribution to prostate cancer risk has been documented, but knowledge of the molecular genetics of prostate cancer is still limited. Malignant transformation of prostate epithelial cells

and progression of prostate carcinoma are likely to result from a complex series of initiation and promotional events under both genetic and environmental influences (Hemminki, 2009).

2.14 An active Sex Life I s Good ForThe Prostate

Evidence suggests that ejaculation frequency may be inversely related to the risk of prostate cancer (Jennifer et al, 2016). An epidemiological study of 30,000 men (Leitzmann, 2004) showed that men who ejaculated 13 to 20 times monthly presented a 14% lower risk of prostate cancer than men ejaculating on average of 4 to 7 times monthly during most of their adul;t life. Those ejaculating over 21 times a month presented a 33% decreased risk of developing prostate cancer. Michael Leitzman, a cancer researcher and the author of the above of this study explains that there has been a suggested connction between greater sexual activity and increased incidents of prostate cancer in previous scientific data because of the link with the male hormone testosterone and its effect on promoting cancer cell growth. He was of the opinion that this theory has its shortcomings because testosterone levels alone do not predict prostate cancer risk. Ejaculation may protect the prostate through a variety of biological mechanisms which includes

I. Flushing out cancer-causing substances ie frequent ejaculation may help flush out retained chemical carcinogens.
II. Reducing tension. The release of psychological tension that accompanies ejaculation may lower nervous activity associated with stress and slow the growth of potentially cancerous cells.
III. Promoting rapid turnover fluids. Frequent ejaculation may prevent the development of mini-crystals that can block ducts within the prostate gland.

2.15 Risk Factors for Prostate Cancer

The three most important recognized risk factors for prostate cancer in the United States are: age, race, family history of prostate cancer.

Age: Age is an important risk factor for prostate cancer. Prostate cancer is rarely seen in men younger than 40 years; the incidence rises rapidly with each decade thereafter. For example, the probability of being diagnosed with prostate cancer is 1 in 298 for men 49 years or younger, 1 in 43 for men aged 50 through 59 years, 1 in 16 for men aged 60 through 69 years, and 1 in 9 for men aged 70 years and older, with an overall lifetime risk of developing prostate cancer of 1 in 7 (ACS, 2014)

Race: The risk of developing and dying from prostate cancer is dramatically higher among blacks, is of intermediate levels among whites, and is lowest among native Japanese (Bunker et al 2002, Altekruse et al, 2010) Conflicting data have been published regarding the etiology of these outcomes, but some evidence is available that access to health care may play a role in disease outcomes (Optenberg et al, 1995)

2.16 Family History of Prostate Cancer

As with breast and colon cancer, familial clustering of prostate cancer has been reported frequently (Steinberg et al, 1990) From 5% to 10% of prostate cancer cases are believed to be primarily caused by high-risk inherited genetic factors or prostate cancer susceptibility genes. Results from several large case-control studies and cohort studies representing various populations suggest that family history is a major risk factor in prostate cancer (Carter et al 1992) A family history of a brother or father with prostate cancer increases the risk of prostate cancer, and the risk is inversely related to the age of the affected relative (Ghadirian et al 1997).

Although many of the prostate cancer studies examining risks associated with family history have used hospital-based series, several studies described population-based series. The latter are thought to provide information that is more generalizable. The Massachusetts Male Aging Study of 1,149 Boston-area men found a relative risk (RR) of 3.3 (95% confidence interval [CI], 1.8–5.9) for prostate cancer among men with a family history of the disease.(Kalish et al 2000). This effect was independent of environmental factors, such as smoking, alcohol use, and physical activity. Further associations between family history and risk of prostate cancer were characterized in an 8-year to 20-year follow-up of 1,557 men aged 40 to 86 years who had been randomly selected as controls for a population-based case-control study conducted in Iowa from 1987 to 1989. At baseline, 4.6% of the cohort reported a family history of prostate cancer in a brother or father, and this was positively associated with prostate cancer risk after adjustment for age (Cerhan et al 1999).

The risk of prostate cancer may also increase in men who have a family history of breast cancer. Approximately 9.6% of the Iowa cohort had a family history of breast and/or ovarian cancer in a mother or sister at baseline, and this was positively associated with prostate cancer risk. Men with a family history of both prostate and breast/ovarian cancer were also at increased risk of prostate cancer. Other studies, however, did not find an association between family history of female breast cancer and risk of prostate cancer. (Damber et al 1998), A family history of prostate cancer also increases the risk of breast cancer among female relatives . The association between prostate cancer and breast cancer in the same family may be explained, in part, by the increased risk of prostate cancer among men with BRCA1/BRCA2 mutations in the setting of hereditary breast/ovarian cancer or early-onset prostate cancer. (Agalliu et al 2007)

Family history has been shown to be a risk factor for men of different races and ethnicities. In a population-based case-control study of prostate cancer among African Americans, whites, and Asian Americans in the United States (Los Angeles, San Francisco, and Hawaii) and Canada (Vancouver and Toronto), (Whittemore et al 1995), 5% of controls and 13% of all cases reported a father, brother, or son with prostate cancer. These prevalence estimates were somewhat lower among Asian Americans than among African Americans or whites. A positive family history was associated with a twofold to threefold increase in relative risk in each of the three ethnic groups.

2.17 Other Potential Modifiers of Prostate Cancer Risk

Endogenous hormones, including both androgens and estrogens, likely influence prostate carcinogenesis. It has been widely reported that eunuchs and other individuals with castrate levels of testosterone prior to puberty do not develop prostate cancer (Wu and Gu, 1991). Some investigators have considered the potential role of genetic variation in androgen biosynthesis and metabolism in prostate cancer risk, including the potential role of the androgen receptor (AR).

Some dietary risk factors may be important modulators of prostate cancer risk; these include fat and/or meat consumption (Kolonel, 2001), lycopene, and dairy products/calcium/vitamin D. (Chan and Giovannucc, 2001). Phytochemicals are plant-derived nonnutritive compounds, and it has been proposed that dietary phytoestrogens may play a role in prostate cancer prevention. (Barnes, 2001). For example, Southeast Asian men typically consume soy products that contain a significant amount of phytoestrogens; this diet may contribute to the low risk of prostate cancer in the Asian population. There is little evidence that alcohol consumption is associated with the risk of developing prostate cancer; however, data suggest that smoking increases the risk of fatal prostate cancer (Hickey et al, 2001). Several studies have suggested that vasectomy increases

the risk of prostate cancer,(Bernal-Delgado et al 1998) but other studies have not confirmed this observation (Stanford et al 1999). Obesity has also been associated with increased risk of advanced stage at diagnosis, prostate cancer metastases, and prostate cancer–specific death. Other nutrients have been studied for their potential influence on prostate cancer risk. The effect of selenium and vitamin E in preventing prostate cancer was studied in the Selenium and Vitamin E Cancer Prevention Trial (SELECT). This randomized placebo-controlled trial of selenium and vitamin E among 35,533 healthy men found no evidence of a reduction in prostate cancer risk (Lippman et al 2009)

2.18 Heredity in Prostate Cancer Risk

Many types of epidemiologic studies (Lichtenstein et al, 2000). (case-control, cohort, twin, family) strongly suggest that prostate cancer susceptibility genes exist in the population. An analysis of monozygotic and dizygotic twin pairs in Scandinavia concluded that 42% of prostate cancer risk may be accounted for by heritable factors .This is in agreement with a previous U.S. study that showed a concordance of 7.1% between dizygotic twin pairs and a 27% concordance between monozygotic twin pairs (Page et al, 1997). The first segregation analysis was performed in 1992 using families from 740 consecutive probands who had radical prostatectomies between 1982 and 1989. The study results suggested that familial clustering of disease among men with early-onset prostate cancer was best explained by the presence of a rare autosomal dominant, highly penetrant allele(s). Hereditary prostate cancer susceptibility genes were predicted to account for almost half of early-onset disease (age 55 years or younger). In addition, early-onset disease has been further supported to have a strong genetic component from the study of common variants associated with disease onset before age 55 years.

2.19 Genes Related to Prostate Cancer

Cancers occur when genetic mutations build up in critical genes, specifically those that control cell growth and division or the repair of damaged DNA. These changes allow cells to grow and divide uncontrollably to form a tumor. In most cases of prostate cancer, these genetic changes are acquired during a man's lifetime and are present only in certain cells in the prostate. These changes, which are called somatic mutations, are not inherited. Somatic mutations in many different genes have been found in prostate cancer cells. Less commonly, genetic changes present in essentially all of the body's cells increase the risk of developing prostate cancer. These genetic changes, which are classified as germline mutations, are usually inherited from a parent. In people with germline mutations, changes in other genes, together with environmental and lifestyle factors, also influence whether a person will develop prostate cancer.

Inherited mutations in particular genes, such as BRCA1, BRCA2 (Edwards et al, 2003) and HOXB13, account for some cases of hereditary prostate cancer. Men with mutations in these genes have a high risk of developing prostate cancer and, in some cases, other cancers during their lifetimes. In addition, men with BRCA2 or HOXB13 gene mutations may have a higher risk of developing life-threatening forms of prostate cancer.

The proteins produced from the BRCA1 and BRCA2 genes are involved in fixing damaged DNA, which helps to maintain the stability of a cell's genetic information. For this reason, the BRCA1 and BRCA2 proteins are considered to be tumor suppressors, which means that they help keep cells from growing and dividing too fast or in an uncontrolled way (Agalliu et al 2007). Mutations in these genes impair the cell's ability to fix damaged DNA, allowing potentially damaging mutations to persist. As these defects accumulate, they can trigger cells to grow and divide uncontrollably and form a tumor.

The HOXB13 gene provides instructions for producing a protein that attaches (binds) to specific regions of DNA and regulates the activity of other genes. On the basis of this role, the protein produced from the HOXB13 gene is called a transcription factor. Like BRCA1 and BRCA2, the HOXB13 protein is thought to act as a tumor suppressor. HOXB13 gene mutations may result in impairment of the protein's tumor suppressor function, resulting in the uncontrolled cell growth and division that can lead to prostate cancer.

Inherited variations in dozens of other genes have been studied as possible risk factors for prostate cancer. Some of these genes provide instructions for making proteins that interact with the proteins produced from the BRCA1, BRCA2, or HOXB13 genes. Others act as tumor suppressors through different pathways. Changes in these genes probably make only a small contribution to overall prostate cancer risk. However, it is suspected that the combined influence of variations in many of these genes may significantly impact a person's risk of developing this form of cancer. In many families, the genetic changes associated with hereditary prostate cancer are unknown. Identifying additional genetic risk factors for prostate cancer is an active area of medical research.

In addition to genetic changes, researchers have identified many other personal and environmental factors that may contribute to a person's risk of developing prostate cancer. These factors include a high-fat diet that includes an excess of meat and dairy and not enough vegetables, a largely inactive (sedentary) (Kathryn et al, 2012) lifestyle, obesity, excessive alcohol use, or exposure to certain toxic chemicals. A history of prostate cancer in closely related family members is also an important risk factor, particularly if the cancer occurred at an early age.

Prostate cancer is classified as an adenocarcinoma or glandular cancer, that begins when the normal semen secreting prostate gland mutate into cancer cells. The region of prostate where the adenocarcinoma is most common is the peripheral zone. Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma insitu or prostatic intraepithelial neopalsia (PIN). Although there is no proof that PIN is a cancer precursor, it is closely associated with cancer. Overtime, these cancer cells begin to multiply and spread to the surrounding prostate tissue (the stroma) forming a tumor. Eventually, the tumor may grow large enough to invade nearby organs such as the seminal vesicles or the rectum or the tumor cells may develop the ability to travel in the bloodstream and lymphatic system. Prostate cancer is considered a malignant tumor because it is a mass of cells that can infiltrate other commonly metastasizes to the bones, lymph nodes, may invade the rectum, bladder and lower ureters after local progression.

Early prostate cancer usually causes no symptoms. Often it is diagnosed during the workup for the elevated PSA noticed during a routine checkup. Prostate screening using PSA test is controversial at the moment (Marcione, 2011). Sometimes however prostate cancer does cause symptoms often similar to those of diseases such as BPH. An important part of evaluating prostate cancer is determining the stage which defines how far the cancer has spread. Knowing the stage helps define prognosis and is useful when selecting therapies. The most common system is the four stage TNM system (abbreviated from Tumor/Nodes/Metastases). Its components include size of the tumor, the number of involved lymph nodes and the presence of any other metastases. The most important distinction made by any staging system is whether or not the cancer is still confined to the prostate. In the TNM system, clinical T1 and T2 cancers are found only in the prostate while T3 and T4 cancers have spread elsewhere. Several tests can be

used to look for evidence of spread. These include computed tomography (CT) to evaluate spread within the pelvis, bone scan to look for spread to bones, and endorectal coil magnetic resonance imaging to closely evaluate the prostatic capsule and the seminal vesicles. Bone scan should reveal osteoblastic appearance due to increased bone density in the areas of bone metastasis.

2.20 Classification of Prostate Cancer

2.21 Grading System

The grading of prostate cancer is based on a system developed by Donald Gleason (1966). Over the years this grading system has been revised and in 2005 a major modification was developed by consensus and accepted for international implementation by the International Society of Urological Pathology (ISUP) (Epstein et al., 2005). In this modified system, tumours are graded from 1 to 5 on the basis of increasing architectural disorganisation. It is acknowledged that grades 1 and 2 should not be reported in thin core biopsies and thus grade 3 tumours represent the lowest grade of tumour, which is considered to be well differentiated. Given that many prostate cancers contain more than one grading pattern, a Gleason score for each core is reported. A Gleason score is the sum of the most common grade (primary pattern) and the second most common grade (secondary pattern). As a consequence, Gleason scores range from 6 (tumours containing pattern 3 only, i.e., 3+3=6) to 10 (tumours containing pattern 5 only, i.e., 5+5=10).

Although the criteria for each of the grades in the modified grading system are well described in the literature, there are currently issues relating to interpretation of grading by individual pathologists. In particular, several studies (Veloso et al., 2007) have demonstrated that interobserver reproducibility varies considerably in the reporting of tumour grade. For general pathologists, reproducibility has been shown to range from weak to moderate, while for

specialist urological pathologists it is either substantial or good to excellent (Allsbrook et al., 2001).

These findings imply that to achieve uniformity of grading in the pathology community, additional grading guidelines should be made available. This recommendation is supported by the observation that the provision of web-based images promotes uniformity of reporting and reduces interobserver variability between participating pathologists (Egevad, 2001).

2.22 Staging of Prostate Cancer

The process used to find out if cancer has spread within the prostate or to other parts of the body is called staging (Mack et al, 2006). There are several tests that the surgeon may use in the staging process. The biopsy and imaging tests help the Surgeon determine the stage and grade of the prostate cancer. This is also called the TNM system, tumor, node and metastases.

Stage I: In stage I, cancer is found in the prostate only. The cancer is found by needle biopsy (done for a high PSA level) or in a small amount of tissue during surgery for other reasons (such as benign prostatic hyperplasia). The PSA level is lower than 10 and the Gleason score is 6 or lower; or the cancer is found in one-half of one lobe of the prostate. The PSA level is lower than 10 and the Gleason score is 6 or lower; or it cannot be felt during a digital rectal examination and cannot be seen in imaging tests. Cancer is found in one-half or less of one lobe of the prostate.

Stage II: In stage II, cancer is more advanced than in stage I, but has not spread outside the prostate.

Stage II

Stage II is classified into stages IIA and IIB. In stage IIA, cancer is found by needle biopsy (done

for a high PSA level) or in a small amount of tissue during surgery for other reasons (such as benign prostatic hyperplasia). The PSA level is lower than 20 and the Gleason score is 7; or the cancer is found by needle biopsy (done for a high PSA level) or in a small amount of tissue during surgery for other reasons (such as benign prostatic hyperplasia). The PSA level is at least 10 but lower than 20 and the Gleason score is 6 or lower or it is found in one-half or less of one lobe of the prostate. The PSA level is lower than 20 and the Gleason score is 7 or it is found in more than one-half of one lobe of the prostate.

In stage IIB, cancer is found in opposite sides of the prostate. The PSA can be any level and the Gleason score can range from 2 to 10 or the cancer cannot be felt during a digital rectal exam and cannot be seen in imaging tests. The PSA level is 20 or higher and the Gleason score can range from 2 to 10 or it cannot be felt during a digital rectal exam and cannot be seen in imaging tests. The PSA can be any level and the Gleason score is 8 or higher.

Stage III: In stage III, cancer has spread beyond the outer layer of the prostate and may have spread to the seminal vesicles. The PSA can be any level and the Gleason score can range from 2 to 10.

Stage IV: In stage IV, the PSA can be any level and the Gleason score can range from 2 to 10. Also, cancer has spread beyond the seminal vesicles to nearby tissue or organs, such as the rectum, bladder, or pelvic wall or it may have spread to the seminal vesicles or to nearby tissue or organs, such as the rectum, bladder, or pelvic wall. Cancer has spread to nearby lymph nodes or the cancr has spread to distant parts of the body, which may include lymph nodes or bones. Prostate cancer often spreads to the bone

2.23 Early Detection of Prostate Cancer

It has been wildly accepted that the prostate-specific antigen (PSA) blood test is not a perfect test for finding prostate cancer early (Marcione, 2011). It misses some cancers, and in other cases it is elevated when cancer is not present. Researchers are working on two strategies to address this problem.

One approach is to try to improve on the test that measures the total PSA level, The percent-free PSA is one way to do this, although it requires two separate tests. Another option might be to measure only the "complexed" PSA (the portion of PSA that is not "free") to begin with, instead of the total and free PSA. This one test could give the same amount of information as the other two done separately.

The other approach is to develop new tests based on other tumor marker which is the main focus of this present study. Several newer blood tests seem to be more accurate than the PSA test, based on early studies. Early results have been promising, but these and other new tests are not yet available outside of research laboratories and will need more study before they are widely used to test for prostate cancer.

Other new tests being studied are urine tests. One test, called Progensa, looks at the level of prostate cancer antigen 3 (PCA3) in the urine (Wei et al, 2014: Yngve et al, 2016). The higher the level, the more likely that prostate cancer is present.

2.24 Controversies in PSA Testing

The American cancer Society's position regarding early detection is that research has not yet proven that the potential benefits of testing outweigh the harms of testing and treatment. The society believes that men should not be tested without learning about what is known and don't

know about the risks and possible benefits of testing and treatment. Hence one needs to talk with the doctor on the advantage and the disadvantage of testing so that the patient will decide the right choice to make.

While a majority of men undergo PSA screening for prostate cancer, a government panel recommends that healthy men of all ages avoid the testing. The United State Preventive Services Task Force (USPSTF) is expected soon to release an updated statement on PSA testing for men, recommending for the first time that healthy men avoid getting regular PSA tests. This is big news, as PSA test is one of the most common prostate cancer tests around. A panel of experts reviewed the existing research and concluded that PSA screening offers men over 50 and older little if any survival benefit, yet raises risks of harms caused by treatment, such as erectile dysfunction and incontinence (Moyer, 2012). The draft from the panel revealed that above recommendation applies in the U.S population that do not have symptoms that are highly suspicious for prostate cancer, regardless of age, race, or family history. However the task force did not evaluate the use of the PSA test as of a diagnostic strategy in men with symptoms that are highly suspicious for prostate cancer. This recommendation also did not consider the use of PSA test for surveillance after diagnosis and/ or treatment of prostate cancer. PSA testing is controversial and may lead to unnecessary, even harmful, consequences in some patients, it is quite expensive. Testing may lead to over diagnosis. Follow-up tests may lead to painful biopsies which can result in excessive bleeding and infection. The discoverer of PSA, Dr. Richard Ablin, concludes that the "tests popularity has led to a hugely expensive public health disaster". Since the test was introduced, PSA screening in U.S, more than one million additional men there have been diagnosed and treated for prostate cancer but it has been estimated that the vast majority (more than 95%) of these men receive no benefit from their positive diagnosis. Several other

ways of evaluating the PSA tests have been developed to avoid the shortcomings of simple PSA screening. The use of age-specific reference ranges improves the sensitivity and specificity of the test. The rate of rise of PSA over time, called the PSA velocity has been used to evaluate men with PSA between 4 and 10ng/ml, but it has not been proven to be an effective screening test (Roobol et al, 2004). Comparing the PSA level with size of the prostate as measured by ultrasound or magnetic resonance imaging has also been studied. This comparison called PSA density is both costly and of 1994, this was not considered to be an effective screening test.

After this news was posted on October 2011, reactions were fast and sometimes very furious. Some doctors and patient advocates defended the test, saying it is the only way to detect a cancer that seldom causes symptoms until it is advanced. Others countered that abnormal PSA does not always mean a man has cancer. Perhaps most crucially, the PSA does not differentiate the aggressive tumors from the far more common slow-growing, harmless ones that might never cause symptoms during a man's life. Also there is another type of prostate cancer called small cell carcinoma though very rare (Nutting et al 1997) that cannot be diagnosed using PSA. As of 2009 researchers have been trying to determine the best way to screen for this type of prostate cancer because it is relatively unknown and rare type of prostate cancer but very serious and quick to spread to other parts of the body.

2.25 Prevention of Prostate Cancer

Researchers continue to look for foods (or substances in them) that can help lower prostate cancer risk. Scientists have found some substances in tomatoes (lycopenes) and soybeans (isoflavones) that might help prevent prostate cancer. Studies are now looking at the possible effects of these compounds more closely. Scientists are also trying to develop related compounds that are even more potent and might be used as dietary supplements. So far, most research

suggests that a balanced diet including these foods as well as other fruits and vegetables is of greater benefit than taking these substances as dietary supplements.

Some studies have suggested that certain vitamin and mineral supplements (such as vitamin E and selenium) might lower prostate cancer risk. But a large study of this issue, called the Selenium and Vitamin E Cancer Prevention Trial (SELECT), found that neither vitamin E nor selenium supplements lowered prostate cancer risk after daily use for about 5 years. In fact, men taking the vitamin E supplements were later found to have a slightly higher risk of prostate cancer.

Another vitamin that may be important is vitamin D. Recent studies (Hendrickson et al, 2011) have found that men with high levels of vitamin D seem to have a lower risk of developing the more lethal forms of prostate cancer. Overall though, studies have not found that vitamin D protects against prostate cancer (Feldman et al 2014)

Many people assume that vitamins and other natural substances cause no harm, but recent research has shown that high doses may be harmful, including those supplements marketed specifically for prostate cancer. For example, one study found that men who take more than 7 multivitamin tablets per week may have an increased risk of developing advanced prostate cancer. Another study showed a higher risk of prostate cancer in men who had high blood levels of omega-3 fatty acids. Fish oil capsules, which some people take to help with their heart, contain large amounts of omega-3 fatty acids. Scientists have also tested certain hormonal medicines called 5-alpha reductase inhibitors as a way of reducing prostate cancer risk.

2.26 Diagnosis of Prostate Cancer

Prostate disorders screening is an attempt to identify individuals with prostate disorders, i.e those for whom there is no reason to suspect prostate disorders. There are currently two methods used: Digital rectal examination and prostate specific antigen.

Digital Rectal Examination (DRE): This examination is usually done first. DRE is part of routine physical examination for men over 50 years (Catalona at al, 1994). The patient is made to bend over a table or to lie on one side holding the knees close to the chest. The doctor slides a gloved, lubricated finger into the rectum and feels the part of the prostate that lies next to it. This test is slightly uncomfortable, but very brief. This examination tells the doctor whether the gland has any bumps, irregularities, soft spots or hard sports that require additional tests. If prostate infection is suspected, the doctor might massage the prostate during the DRE to obtain fluid for microscopic examination.

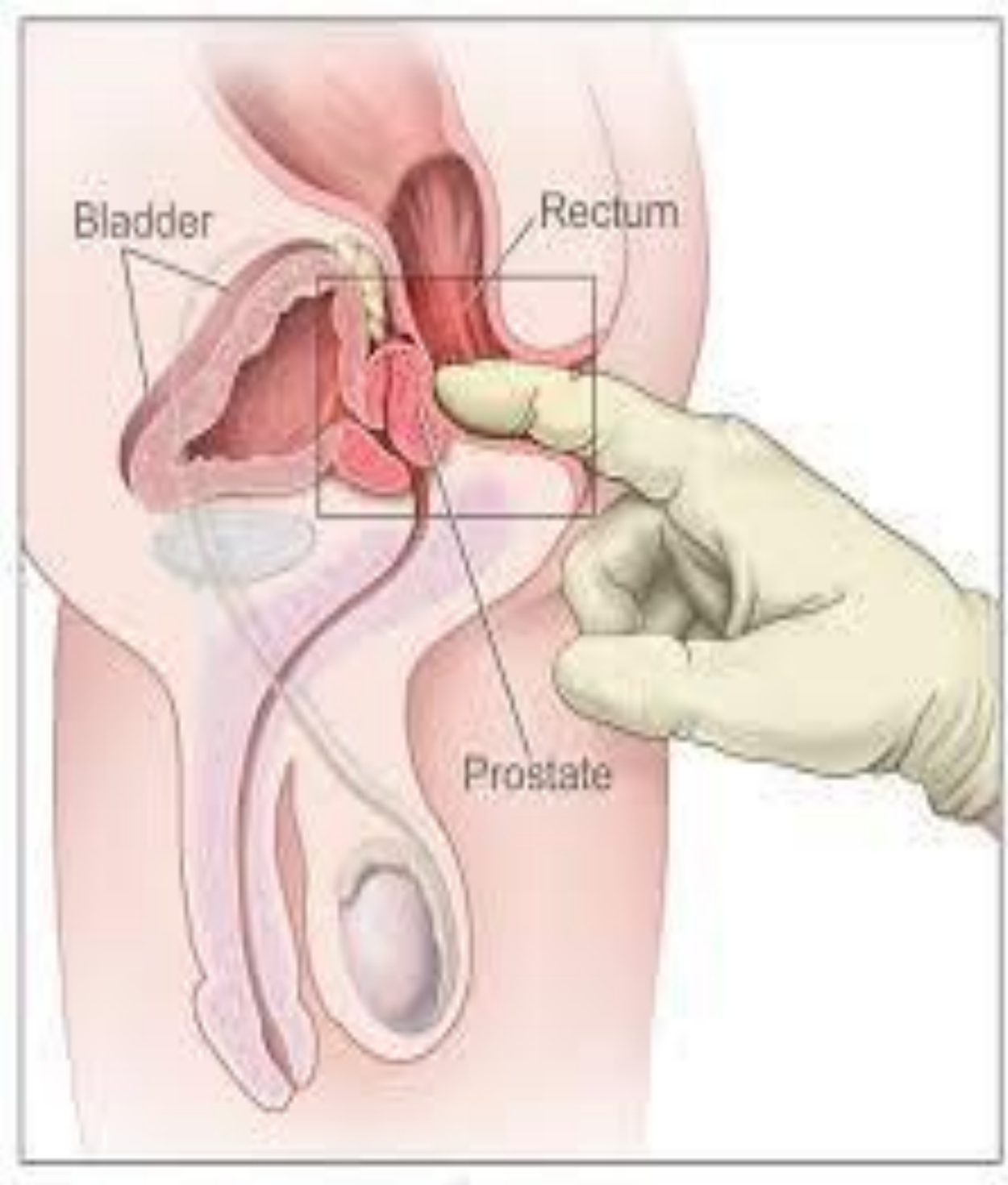


Fig 2.2 Diagram illustrating digital rectal examination

Catalona at al, 1994

PSA Blood Test: The prostate specific antigen is an enzyme (protein) secreted by the epithelial prostatic cells and is often higher in blood of men who have prostate disorders (Catalona et al, 1994). Prostate specific antigen is a serine protease similar to kallikrein. It is also known as gamma-seminoprotein or kallikrein-3. Its normal function is to liquefy gelatinous semen after ejaculation, allowing spermatozoa to more easily navigate through the uterine cervix.

Urinalysis: This test is required to rule out the presence of blood in urine.

Prostate Imaging: Ultrasound (US) and Magnetic Resonance Imaging (MRI) are the two main imaging methods used for prostate disorders. Urologists use transrectal ultrasound during prostate biopsy. But US has poor tissue resolution. Prostrate MRI has superior soft tissue resolution. It uses magnetic fields to locate and characterize prostate cancer.

Prostate Biopsy: If cancer is suspected, the doctor obtains tissue samples from the prostate via the rectum. A biopsy gun inserts and removes special hollow-core needle (usually three to six on each side of the prostate) in less than a second. After a prostate biopsy, the pathologist looks at the samples under the microscope. If cancer is present, the pathologist reports the grade of tumor. The grade tells how much the tumor tissue differs from normal prostate tissue and suggests how fast the tumor is likely to grow. The Gleason system is used to grade prostate tumors from 2 to 10, Where a Gleason score of 10 indicates the most abnormalities. The pathologist assigns a number from 1 to 5 for most common pattern observed under the microscope. Suspected prostate cancer is typically confirmed by the microscopic examination of the biopsy samples from the prostate gland. Further tests, such as computed tomography (CT) scans may be required

Cystoscopy: Another way to see a problem from the inside is with a cystoscope, which is a thin tube with lenses like microscope. The tube is inserted into the bladder through the urethra while the doctor looks through the cytoscope.

Urine Flow Study: The patient is asked to urinate into a special device that measures how quickly the urine is flowing. A reduced flow or weak stream and difficulty emptying the bladder may be signs of blockage caused by enlarged prostate.

2.27 Prostate Specific Antigen

Prostate specific antigen is a substance produced almost exclusively by certain cells within the prostate gland. Biochemically, it belongs to the protease family of kallikrein and is also known as human kallikrein 3 (hK3). PSA is secreted by the prostate in the semen where its role is to liquefy the semen following ejaculation. Most of the PSA produced by the prostate gland is carried out of the body in semen, but a very small amount escapes into the blood stream, so PSA is normally found in low amounts (nanograms per milliliter or ng/mL) in the blood. The blood level of PSA is often elevated in men with prostate cancer, and the PSA test was originally approved by the Food and Drug Administration (FDA) in 1986 to monitor the progression of prostate cancer in men who had already been diagnosed with the disease. In 1994, the FDA approved the use of the PSA test in conjunction with a digital rectal exam (DRE) to test asymptomatic men for prostate cancer. Men who report prostate symptoms often undergo PSA testing (along with a DRE) to help determine the nature of the problem. PSA is measured by a blood test. Since the amount of PSA in the blood is very low (Catalona et al, 1994), detection of it requires a very sensitive type of technology (monoclonal antibody technique). The PSA protein can exist in the blood by itself (known as free PSA), or bound with other substances (known as

bound or complexed PSA). Total PSA is the sum of the free and the bound forms. The total PSA is what is measured with the standard PSA test.

In addition to prostate cancer, a number of benign (not cancerous) conditions can cause a man's PSA level to rise. The most frequent benign prostate conditions that cause an elevation in PSA level are prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate). There is no evidence that prostatitis or BPH leads to prostate cancer, but it is possible for a man to have one or both of these conditions and to develop prostate cancer as well.

However, more recent studies (Thompson et al, 2004) have shown that some men with PSA levels below 4.0 ng/mL have prostate cancer and that many men with higher levels do not have prostate cancer. In addition, various factors can cause a man's PSA level to fluctuate. For example, a man's PSA level often rises if he has prostatitis or a urinary tract infection. Prostate biopsies and prostate surgery also increase PSA level. Conversely, some drugs including finasteride and dutasteride, which are used to treat BPH, lower a man's PSA level. PSA level may also vary somewhat across testing laboratories.

Another complicating factor is that studies to establish the normal range of PSA levels have been conducted primarily in populations of white men. Although expert opinions vary, there is no clear consensus regarding the optimal PSA threshold for recommending a prostate biopsy for men of any racial or ethnic group. In general, however, the higher a man's PSA level, the more likely it is that he has prostate cancer. Moreover, continuous rise in a man's PSA level over time may also be a sign of prostate cancer.

2.28 Causes of PSA Elevation in the Blood

It is believed that elevation of PSA in the blood is due to its liberation into the circulation because of disruption of the prostate cellular architecture (structure). This can occur in the setting of different prostate diseases including prostate cancer. It is important to note that PSA is not specific to prostate cancer but to prostatic tissue and therefore PSA elevations may indicate the presence of any kind of prostate disease (Mac Donald 2011). The most common cause of PSA elevation includes benign prostatic hyperplasia (BPH = enlargement of the prostate, secondary to a noncancerous proliferation of prostate gland cells) and prostatitis (inflammation of the prostate). In fact, PSA elevation can also occur with prostate manipulation such as ejaculation, prostate examination, urinary retention or catheter placement, and prostate biopsy. As such, men choosing to undergo PSA testing should be aware of these important factors, which may influence results. Age and prostate volume may also influence PSA test results. The "normal" PSA serum concentration ranges between 1.0 and 4.0 ng/mL. However, since the prostate gland generally increases in size and produces more PSA with increasing age, it is normal to have lower levels in young men and higher levels in older men. The PSA level also depends on ethnicity and family history of prostate cancer.

2.29 The Limitations and Potential Harms of the PSA Test for Prostate Cancer Screening

Detecting prostate cancer early may not reduce the chance of dying from prostate cancer**.** When used in screening, the PSA test can help detect small tumors that do not cause symptoms. Finding a small tumor, however, may not necessarily reduce a man's chance of dying from prostate cancer. Some tumors found through PSA testing grow so slowly that they are unlikely to

threaten a man's life. Detecting tumors that are not life threatening is called "overdiagnosis," and treating these tumors is called "overtreatment."

Overtreatment exposes men unnecessarily to the potential complications and harmful side effects of treatments for early prostate cancer, including surgery and radiation therapy. The side effects of these treatments include urinary incontinence (inability to control urine flow), problems with bowel function, erectile dysfunction (loss of erections, or having erections that are inadequate for sexual intercourse), and infection. In addition, finding cancer early may not help a man who has a fast-growing or aggressive tumor that may have spread to other parts of the body before being detected.

The PSA test may give false-positive or false-negative results. A false-positive test result occurs when a man's PSA level is elevated but no cancer is actually present. A false-positive test result may create anxiety for a man and his family and lead to additional medical procedures, such as a prostate biopsy, that can be harmful. Possible side effects of biopsies include serious infections, pain, and bleeding. Most men with an elevated PSA level turn out not to have prostate cancer; only about 25 percent of men who have a prostate biopsy due to an elevated PSA level actually have prostate cancer (Barry, 2001).

A false-negative test result occurs when a man's PSA level is low even though he actually has prostate cancer. False-negative test results may give a man, his family, and his doctor false assurance that he does not have cancer, when he may in fact have a cancer that requires treatment.

Scientists are investigating ways to improve the PSA test to give doctors the ability to better distinguish cancerous from benign conditions and slow-growing cancers from fast-growing, potentially lethal cancers. Some of the methods being studied include:

Free (% free) versus total PSA: The amount of PSA in the blood that is "free" (not bound to other proteins) divided by the total amount of PSA (free plus bound). Some evidence suggests that a lower proportion of free PSA may be associated with more aggressive cancer. (Catalona et al, 1998: Alden et al, 2016)

PSA density of the transition zone: The blood level of PSA divided by the volume of the transition zone of the prostate. The transition zone is the interior part of the prostate that surrounds the urethra. Some evidence suggests that this measure may be more accurate at detecting prostate cancer than the standard PSA test. (Benson et al, 1992: Catalona et al, 1994)

Age-specific PSA reference ranges. Because a man's PSA level tends to increase with age (ACS, 2014), it has been suggested that the use of age-specific PSA reference ranges may increase the accuracy of PSA tests. However, age-specific reference ranges have not been generally favored because their use may delay the detection of prostate cancer in many men.

PSA velocity and PSA doubling time: PSA velocity is the rate of change in a man's PSA level over time, expressed as ng/mL per year. PSA doubling time is the period of time over which a man's PSA level doubles. Some evidence suggests that the rate of increase in a man's PSA level may be helpful in predicting whether he has prostate cancer (Loeb et al, 2007).

Pro-PSA: Pro-PSA refers to several different inactive precursors of PSA. There is some evidence that pro-PSA is more strongly associated with prostate cancer than with BPH. One recently approved test combines measurement of a form of pro-PSA called [-2] proPSA (Na et al, 2014: Peter et al, 2016) with measurements of PSA and free PSA. The resulting "prostate

health index" can be used to help a man with a PSA level of between 4 and 10 ng/mL decide whether he should have a biopsy.

A relatively new test called "ultrasensitive PSA" (USPSA) has been reported. It has been suggested that this test may be useful in monitoring for persistence or recurrence of cancer after treatment. This test detects PSA at much lower levels than the traditional test. It has been suggested that increases in PSA due to the persistence or return of cancer can be identified much sooner with this test. However, results of this test must be interpreted with caution. Because the test is very sensitive, there can be an increase in PSA levels from one time to the next even when no cancer is present (false positive).

2.30 Treatment of Prostate Cancer

2.31 Active Surveillace

Active Surveillance: This concept means monitoring the cancer closely with prostate-specific antigen blood tests, digital rectal examinations (DREs), and ultrasounds at regular intervals to see if the cancer is growing. Prostate biopsies may be done as well to see if the cancer is becoming more aggressive. With active surveillance, the cancer will be monitored carefully. Usually this approach includes a doctor visit with a PSA blood test and DRE about every 3 to 6 months. Transrectal ultrasound-guided prostate biopsies may be done every year as well.

Treatment can be started if the cancer seems to be growing or getting worse, based on a rising PSA level or a change in the DRE, ultrasound findings, or biopsy results. On biopsies, an increase in the Gleason score or extent of tumor (based on the number of biopsy samples containing tumor) are both signals to start treatment (usually surgery or radiation therapy).

2.32 Watchful Waiting

Observation is sometimes used to describe a less intensive type of follow-up that may mean fewer tests and relying more on changes in a man's symptoms to decide if treatment is needed. Not all clinicians agree with these definitions or use them exactly this way. In fact, some clinicians prefer to no longer use the term watchful waiting. They feel it implies that nothing is being done, when in fact a man is still being closely monitored.

Watchful waiting and active surveillance are reasonable options for some men with slow-growing cancers because it is not known whether treating the cancer with surgery or radiation will actually help them live longer. These treatments have definite risks and side effects that may outweigh the possible benefits for some men. Some men are not comfortable with this approach, and are willing to accept the possible side effects of active treatments to try to remove or destroy the cancer.

2.33 Surgery for Prostate Cancer

A radical prostatectomy is the surgical removal of the prostate gland. This treatment is an option for curing localized prostate cancer and locally-advanced prostate cancer (Oleksandr et al 2016). Like any operation, this surgery carries some risks, and there may be some side effects. These are outlined below.

I. Some men have problems with urinary incontinence. This can range from leaking small drips of urine, to leaking larger amounts. However, for most men, this usually clears up within three to six months of the operation. About two in every 10 men have long-term problems requiring the use of pads.

II. Some men have problems getting an erection (erectile dysfunction). For some men, this improves with time, but around half of men will have long-term problems.

III. In extremely rare cases, problems arising after surgery can be fatal. For example, one in 1,000 men under 65 years old and one in 200 men over 65 will die following a radical prostatectomy.

For many men, having a radical prostatectomy will get rid of the cancer cells. However, for around one in three men, the cancer cells may not be fully removed, and the cancer cells may return some time after the operation.

The application of radiotherapy after prostate removal surgery may increase the chances of a cure of prostate cancer, although research is still being carried out as regards when it should be used after surgery. After a radical prostatectomy, the person will no longer ejaculate during sex. This means that the person will not be able to have a child through sexual intercourse. There is no age threshold for radical prostatectomy and a patient should not be denied this procedure on the grounds of age alone (Droz et al., 2010). Increasing co- morbidity greatly increases the risk of dying from non-prostate cancer -related causes (Albertsen et al., 2011) and an estimation of life expectancy is paramount in counselling a patient about surgery (Walz et al, 2007).

2.34 Radiation Therapy for Prostate Cancer

Radiation therapy uses high-energy rays or particles to kill cancer cells. Radiation may be used:

I. As the first treatment for low-grade cancer that is still just in the prostate gland. Cure rates for men with these types of cancers are about the same as those for men getting radical prostatectomy.

II. As part of the first treatment (along with hormone therapy) for cancers that have grown outside of the prostate gland and into nearby tissues.

III. If the cancer is not removed completely or comes back (reocurs) in the area of the prostate after surgery.

IV. If the cancer is advanced, radiation helps to reduce the size of the tumor and to provide relief from present and possible future symptoms.

The National Institutes of Health (NIH) stated that "external irradiation offers the same long-term survival results as surgery". Moreover, external beam radiotherapy (EBRT) provides a quality of life at least as good as that following surgery (Bolla et al, 2002). A more recent systematic review has provided a more sophisticated overview of outcomes from reports that meet the criteria for stratifying patients by risk group, standard outcome measures, numbers of patients, and minimum median follow-up period.

2.35 Brachytherapy

Brachytherapy is a form of radiotherapy where the radiation dose is delivered inside the prostate gland. It is also known as internal or interstitial radiotherapy.

The radiation can be delivered using a number of tiny radioactive seeds that are surgically implanted into the tumour. This is called low dose-rate brachytherapy. The radiation can also be delivered through hollow, thin needles placed inside the prostate. This is called high dose-rate brachytherapy. This method has the advantage of delivering a high dose of radiation to the prostate, while minimising damage to other tissues. However, the risk of sexual dysfunction and urinary problems is the same as with radiotherapy, although the risk of bowel problems is slightly lower.

2.36 Hormone (Androgen Deprivation) Therapy for Prostate Cancer

Hormone therapy is also called androgen deprivation therapy (ADT) or androgen suppression therapy. The goal is to reduce levels of male hormones, called androgens, in the body, or to stop them from affecting prostate cancer cells.

The main androgens are testosterone and dihydrotestosterone (DHT). Most of the body's androgens come from the testicles, but the adrenal glands also make a small amount. Androgens stimulate prostate cancer cells to grow (Gann et al, 1996). Lowering androgen levels or stopping them from getting into prostate cancer cells often makes prostate cancers shrink or grow more slowly for a time. But hormone therapy alone does not cure prostate cancer.

Hormone therapy may be used:

I. If the cancer has spread too far to be cured by surgery or radiation.
II. If the cancer remains or comes back after treatment with surgery or radiation therapy
III. Along with radiation therapy as initial treatment if a patient is at higher risk of the cancer coming back after treatment (based on a high Gleason score, high PSA level, and/or growth of the cancer outside the prostate)
IV. Before radiation to try to shrink the cancer to make treatment more effective

Several types of hormone therapy can be used to treat prostate cancer. Some lower the levels of testosterone or other androgens (male hormones). Others block the action of those hormones.

2.37 Treatments to Lower Androgen Levels

2.38 Orchiectomy (surgical castration)

Even though this is a type of surgery, its main effect is as a form of hormone therapy. In this operation, the surgeon removes the testicles, where most of the androgens (testosterone and

DHT) are produced. With this source removed, most prostate cancers stop growing or shrink for a time. This is probably the least expensive and simplest way to reduce androgen levels in the body. But unlike some of the other methods of lowering androgen levels, it is permanent, and many men have trouble accepting the removal of their testicles. Some men having the procedure are concerned about how it will look afterward. If wanted, artificial silicone sacs can be inserted into the scrotum. These look much like testicles. Bilateral orchiectomy which can be total or subcapsular (i.e. with preservation of tunica albuginea and epididymis), is a simple and virtually complication-free surgical procedure. It is easily performed under local anaesthesia and is the quickest way to achieve a castration level, usually within less than 12 hours. (Desmond et al., 1988)

2.39 Luteinizing Hormone-Releasing Hormone (LHRH) Analogs

These drugs lower the amount of testosterone produced by the testicles. Treatment with these drugs is sometimes called chemical castration or medical castration because they lower androgen levels just as well as orchiectomy.

Even though LHRH analogs (also called LHRH agonists or GnRH agonists) cost more than orchiectomy and require more frequent physician visits, most men choose this method. These drugs allow the testicles to remain in place, but the testicles will shrink over time, and they may even become too small to feel.

LHRH analogs are injected or placed as small implants under the skin. Depending on the drug used, they are given anywhere from once a month up to once a year. The LHRH analogs available are leuprolide (Lupron, Eligard), goserelin (Zoladex), triptorelin (Trelstar), and histrelin (Vantas).

When LHRH analogs are first given, testosterone levels go up briefly before falling to very low levels. This effect is called flare and results from the complex way in which LHRH analogs work. Men whose cancer has spread to the bones may have bone pain. If the cancer has spread to the spine, even a short-term increase in tumor growth as a result of the flare could compress the spinal cord and cause pain or paralysis. Flare can be avoided by giving drugs called anti-androgens for a few weeks when starting treatment with LHRH analogs.

Chronic exposure to LHRH agonists eventually results in down-regulation of LHRH-receptors, suppressing pituitary LH and FSH secretion and testosterone production. Testosterone levels decrease to castration levels usually within 2-4 weeks (Klotz et al., 2008). However, about 10% of patients treated with LHRH agonists fail to achieve castration levels (Crawford et al., 2011). However, LHRH antagonists (i.e. degarelix) rapidly and directly inhibit the release of androgens, unlike LHRH agonists that initially stimulate LHRH receptors before leading to hypogonadism

2.40 Chemotherapy

Chemotherapy is mainly used to treat prostate cancer that has spread to other parts of the body (metastatic prostate cancer) and which is not responding to hormone therapy. Chemotherapy destroys cancer cells by interfering with the way they multiply. Chemotherapy does not cure prostate cancer, but can keep it under control and reduce symptoms (such as pain) so everyday life is less affected. The main side effects of chemotherapy are caused by their effects on healthy cells, such as immune cells. They include infections, tiredness, hair loss, sore mouth, loss of appetite, nausea and vomiting.

2.41 Sarcosine

Sarcosine, also known as N-methylglycine with the chemical formula CH_3NHCH_2COOH, was firstly isolated and named by German chemist Justus von Liebig in 1847. It is a non-proteinogenic amino acid that occurs as an intermediate product in the synthesis and degradation of amino acid glycine (Issaq et al, 2011). Sarcosine is an amino acid that is an intermediate and byproduct in glycine synthesis and degradation with potential anti-depressant and anti-schizophrenic activities. Sarcosine is a product of dietary consumption of choline and creatine and is rapidly converted into glycine. Sarcosine is synthesized by the glycine N-methyltransferase (GMNT) enzyme which uses a methyl group from S-Adenosyl Methionine to donate to Glycine, creating sarcosine and S-adenosylhomocysteine. It is also metabolized by either the sarcosine dehydrogenase (SARDH) enzyme or pipecolic acid oxidase (PIPOX).

S-adenosylmethionine (SAM) dependent methyltransferases, a common group of enzymes in the mammalian cell, are involved in numerous reactions of small molecule biosynthesis as well as DNA, RNA and protein methylation. One such enzyme, glycine N-methyltransferase (GNMT), catalyzes the transfer of a methyl group from SAM to the amino group of glycine producing sarcosine (N-methylglycine) and S-adenosylhomocysteine (SAH) . Sarcosine, an intermediate in the pathway of degradation of choline and betaine, might be important in the regulation of osmosis and protein stabilization. It can be rapidly converted to glycine by folate-dependent sarcosine dehydrogenase. In combination with this reaction, GNMT catalysis constitutes a metabolic cycle, which degrades SAM and simultaneously incorporates the active methyl group into the intracellular folate pool. In this regard, this cycle counterbalances the activated folate methyl cycle, which supplies methyl groups from the folate pool to SAM through the biosynthesis of methionine. It has been proposed that the functional significance of the GNMT

reaction is associated with the control of SAM/SAH ratio and the overall methylation potential of the cell.

DNA methylation is the best known epigenetics alteration in prostate cancer. DNA methylation can regulate gene expression and can function in favor of malignancy-associated phenotypes such as cellular differentiation, growth, migration and invasion, metastasis, apoptosis, hormonal regulation of steroid receptors, and DNA repair. DNA methylation appears to be very sensitive to external stimuli or influences such as diet and oxidative stress.

The dynamics of DNA methylation are controlled by the availability of methyl groups (methyl donors), the utilization of these groups by DNA methyl transferase (DNMT), DNA demethylation processes–whether active or passive, cell responsiveness to DNA methylation defects, as well as abundance of methyl group acceptors and enzymes in the cell (Ross, 2003). Dietary sources of methyl donors include folate, methionine, vitamin B_{12}, betaine, and choline (Blusztajn, 1998). The "ultimate" methyl donor, *S*-adenosylmethionine (SAM) is eventually derived from these dietary sources and also from cellular recycling of *S*-adenosylhomocysteine (SAH). In some studies, insufficient dietary methyl donors resulted in a decreased availability of SAM *in vivo* (Hoffman, 1981: Shivapurkar and Poirier, 1983) whereas a diet proficient in the same compounds consequently resulted in an increase in SAM. Another example of this dependence was seen in rats in which dietary betaine caused increased synthesis of betaine–homocysteine methyltransferase–the enzyme that transfers methyl groups to SAH to produce SAM (Finkelstein et al, 1983). The effect of methyl donor enriched diets on one of the end products, methylation of DNA or histones is not so straight forward. Studies have reported that disruption of folate metabolism resulted in global hypomethylation of DNA (Alonso-Aperte et al, 1999), and in another study in mice, an excess of methionine intake resulted in methylation of

genes in the mouse frontal cortex (Tremolizzo et al, 2005). On the other hand, Kovacheva and colleagues have shown that when choline is in short supply in the diet, a reverse adaptive epigenetic response occurs that causes an upregulation of DNMT-1 and increase in global DNA methylation in rat fetal liver and brain (Kovacheva et al, 2007).

2.42 Metabolism of Sarcosine

Metabolites play essential role in an understanding the biological reactions and thereby the changes in their levels contribute to the development of new diagnostic and therapeutic methods to diagnose specific diseases (Metallo, 2012: Burton et al, 2012). Biochemical pathways of formation and oxidation of sarcosine occur in mitochondria and are provided by two basic pathways. Phosfatidylethylamine is methylated repeatedly by S-adenosylmethionine (SAM) during transulfuration to phosphatidylcholine in the first pathway with the resulting intermediate product betaine. This reaction forms dimethylglycine and regenerates methionine from homocysteine. Dimethylglycine is subsequently converted to sarcosine via dimethylglycine dehydrogenase (DMGDH) (Montrose et al, 2012). The second metabolic pathway creates sarcosine during the transformation of the methyl group of S-adenosylmethionine catalysed by the enzyme glycine-N-methyltransferase (GNMT), that is a tetramer of identical 32 kDa subunits (Luka et al, 2012). These two reactions ultimately produce 5, 10-methylenetetrahydrofolate and are dependent on oxidized flavoproteins (e.g., flavin adenine dinucleotide (FAD^+). This pathway also includes an oxidative phase, which removes the methyl group from sarcosine to create glycine and an active one-carbon unit by sarcosine dehydrogenase (SARDH), a mitochondrial flavoprotein (Bar-joseph et al. 2012) which, in a study by Montrose *et al.*, was observed within the tumour tissue with GNMT and other enzymes and can explain the increases in sarcosine

levels . The absence of the activity of SARDH involves also conversion of choline to glycine in humans; this is transmitted genetically and represents a disorder in metabolism of amino acid that is manifested as sarcosinemia, a surplus of sarcosine accompanied by high concentrations of sarcosine in blood and urine (Bergeron et al, 1998).

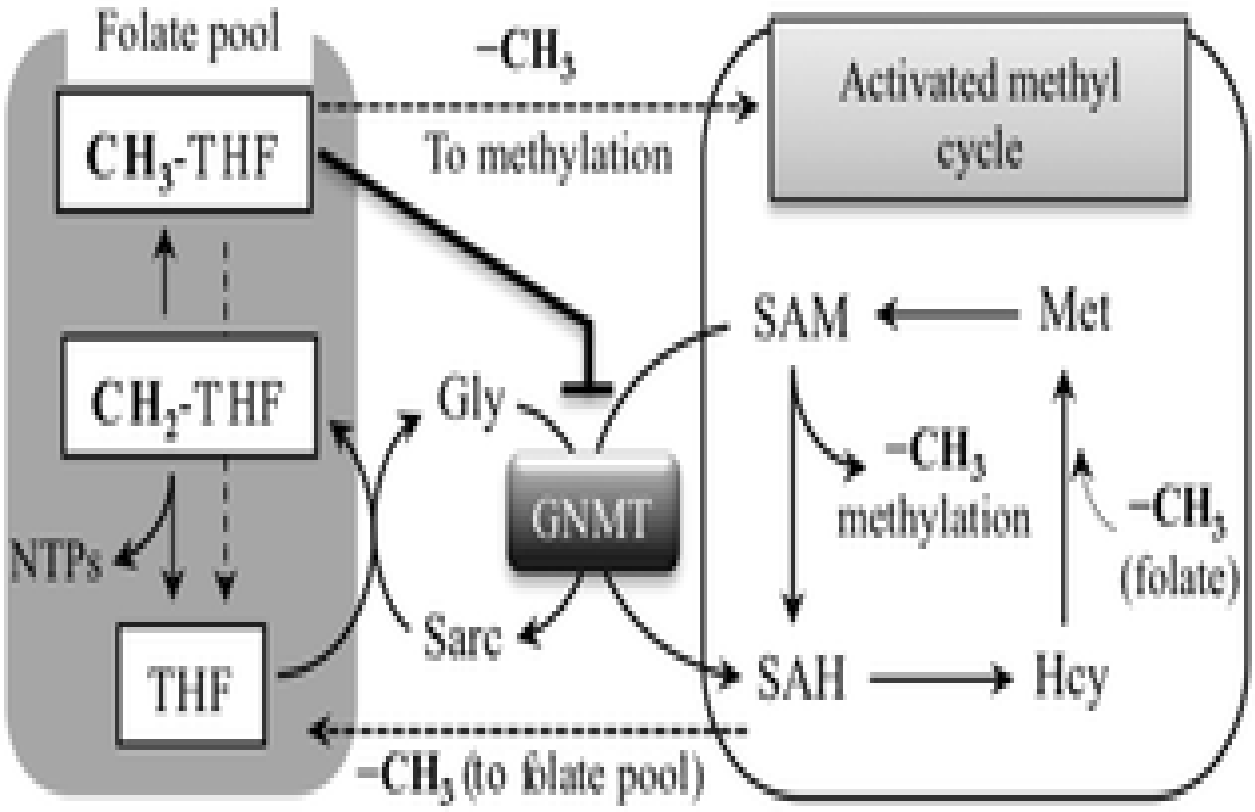


Fig 2.3 Sarcosine Metabolism

DebRoy et al, 2013

2.43 Molecular Biology of Sarcosine

Glycine N-methyltransferase (GNMT) acts as an essential component that influences synthesis of sarcosine (Wang et al, 2011). Synthesis of GNMT is controlled by the same gene named *GNMT*. It has recently been reported that the *GNMT* gene is located on the short (p) arm of chromosome 6 at position 12 and acts as a tumour-susceptible gene (Song et al, 2011). According to the study by Ianni *et al.* (2013) T allele of the rs9462856 SNP in the promoter region of the *GNMT* gene is overexpressed in patients suffering from cancer of the prostate and its overexpression significantly increases the risk of the disease (Ianni et al 2013). Phenotypic analysis of three *GNMT* haplotypes (A, B, and C) indicated that haplotype C had the highest promoter activity and haplotype B had significantly higher activity compared to the haplotype A. The difference between the haplotypes B and C is due to the T allele of SNP1 that exerts a strong disequilibrium. The *GNMT* gene contains in TATA-less core promoter region the Sp1 site and a CCAAT-box that is one of the most ubiquitous elements being present in 30% of all eukaryotic promoters (Nardini et al, 2013). This region represents a binding site for the transcriptional factor, NF-Y, a trimer with histone-like subunits NF-YB/NF-YC and the sequence-specific NF-YA (Dolfini et al, 2012). NF-Y is a sequence-specific transcription factor with nucleosome-like properties in nonspecific DNA binding that helps to establish permissive chromatin modifications at CCAAT promoters.

The expression of the *GNMT* induced in this manner leads to synthesis of GNMT that contributes to the regulation of the level of S-adenosylmethionine (SAM) and influences gene expression by affecting the DNA methylation. DNA methylation is an essential process in the body. Methyl groups transferred by SAM are used to synthesize many essential compounds

including creatine and/or phosphatidylcholine. In addition, DNA methylation is essential for regulation of gene expression. Deficiency of donors of methyl group (e.g., choline and methionine) or coenzymes of metabolism of methyl group (e.g., folate and vitamin B12) disturbs the intracellular levels of S-adenosylmethionine, triggers the DNA hypomethylation, and promotes cancers of the liver, prostate, and other organs . Patients suffering from cancer of the prostate have been revealed to show a decreased methylation of DNA, whereas patients positive for the *GNMT* T allele had a lower level of methylated DNA than controls with the same allele (Ianni et al, 2013). GNMT also binds a number of polycyclic aromatic hydrocarbons and inhibits the formation of DNA adducts.

Due to properties of GNMT, its excessive production causes an excess cleavage of glycine to sarcosine and elevates the presence of sarcosine in blood. Stabler *et al.* (2011) reported that increased flux through the GNMT gene results in the increased formation of homocysteine and sarcosine through increased utilization of SAM. This makes sarcosine interesting in the field of non-invasive cancer biomarkers. Supplementation of sarcosine to prostate cancer cell lines induced a selection of invasive phenotype in culture. Dahl *et al.* (2011) reported for the first time on a significant upregulation of a potent oncoprotein human epidermal growth factor receptor 2 (HER2/neu) in androgen-dependent prostate cancer cells upon exposure to exogenous sarcosine.

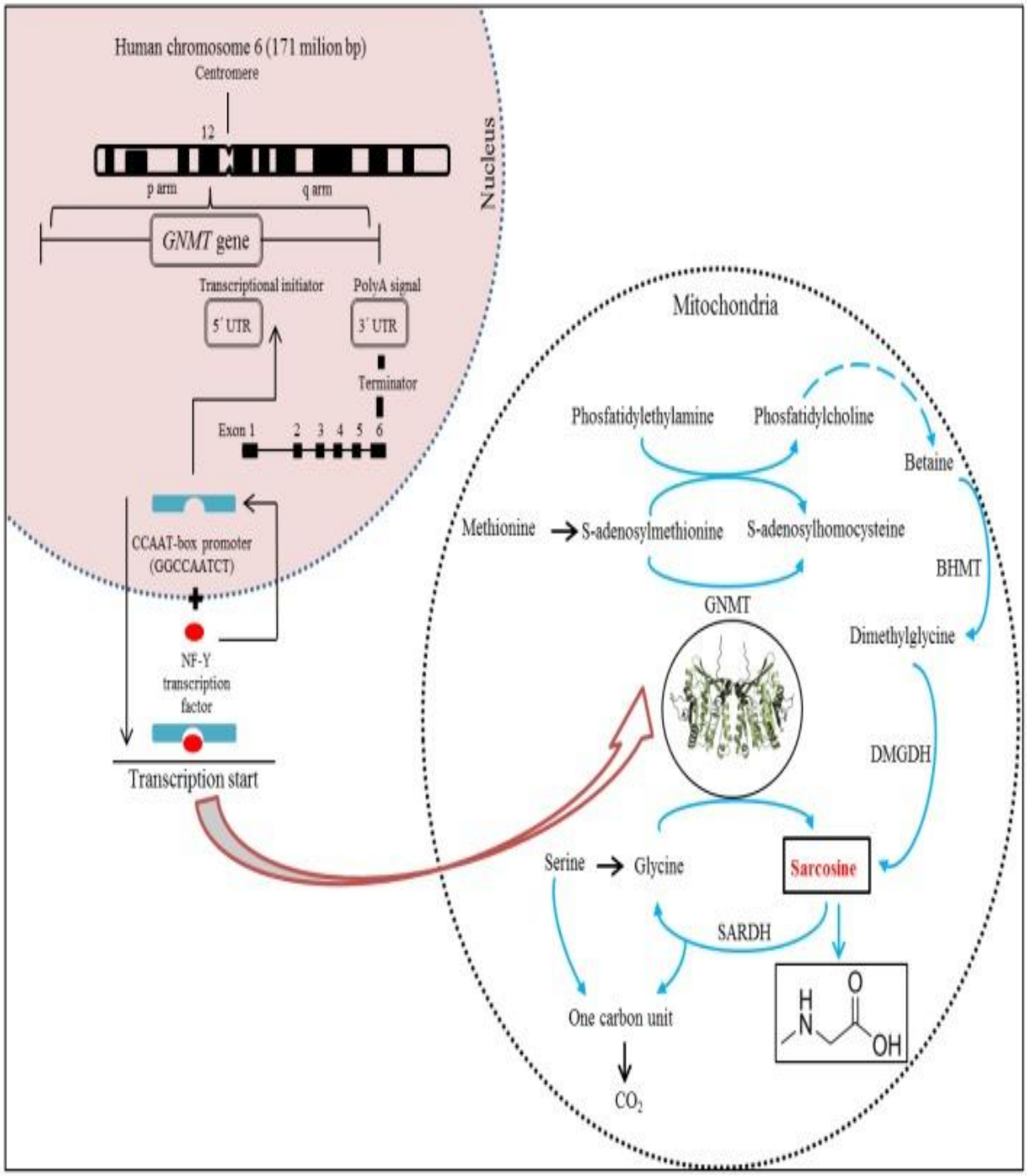


Fig 2.4 Structure of human cine-N-methyltransferase (GNMT) gene in connection with scheme of sarcosine genesis in biochemical pathway.

Sunipa, 2011

2.44 Creatinine

Creatinine is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine (Carl et al, 2006). Virtually all filtered creatinine by the glomerulus of the kidney is excreted in the urine, hence, a good diagnostic indicator of renal function. The serum creatinine concentrations are influenced by age, sex, muscle mass, and intake and absorption of dietary creatine and creatinine present in meat (Perrone et al., 1992).

Potential correlates of serum creatinine includes ethnicity, weight, body mass index (BMI), lean mass, and upper arm circumference, serum triglycerides and total cholesterol, physical activity, blood pressure, diseases such as diabetes, hypertension, and heart disease, and use of antihypertensive medication, statins, cimetidine, or diuretics (Vikse et al., 2004).

Serum creatinine, a measure of renal function is also influenced by other factors and therefore not a specific or sensitive indicator of renal disease within normal ranges (Coresh et al., 2001). Serum creatinine could be a marker for homocysteine status and one carbon metabolism, as synthesis of the fomer results in removal of a methyl group from S-adenosylmethionine, creating homocysteine, and potentially reducing availability of one-carbon groups for DNA methylation, synthesis, and repair (Eto and Krumdieck, 1986, Stead, 2006). In other words the synthesis of creatine, which is converted to creatinine in muscle cells, requires a methyl group from S-adenosylmethionine, which in turn is converted via adenosylhomocysteine to homocysteine. Previous studies (Jac ques et al, 2001: Elshorbagy et al, 2007), have shown that serum creatinine correlated with serum homocysteine. Creatinine could also be considered a proxy for meat consumption, where lower levels are associated with reduced intake (Levey et al 1988). Studies among prostate cancer patients have also shown that serum creatinine is associated with more

advanced disease (Chiong et al, 2005) and with decreased survival, although this relationship is not supported by some studies (Merseburger et al., 2001). Elevated serum creatinine was also associated with reduced survival in a group of men with hormone-resistant prostate cancer (Fossa et al., 1992). In other studies, significant relationships between elevated serum creatinine and disease stage, recurrence, progression, or mortality were attenuated when adjusted for other factors (e.g., age, stage, race, or PSA), or were only marginally significant or not associated (Merseburger et al., 2001).

2.45 Engrailed -2 Gen Protein

Engrailed 2 gene protein or homeobox protein that in humans is encoded by the EN2 gene. It is a member of the HOX gene family. Homeobox-containing genes are thought to have a role in controlling development. In Drosophila, the 'engrailed' (en) gene plays an important role during development in segmentation, where it is required for the formation of posterior compartments. Different mutations in the mouse homologs, En1 and En2, produced different developmental defects that frequently are lethal. The human engrailed homologs 1 and 2 encode homeodomain-containing proteins and have been implicated in the control of pattern formation during development of the central nervous system (Brunet et all, 2005).

2.46 5-Alpha Reductase

The 5-alpa reductase enzyme is involved in processing androgens, which are hormones that direct male sexual development. Specifically, the enzyme is responsible for a chemical reaction that converts the hormone testosterone to a more potent androgen, dihydrotestosterone (DHT), in male reproductive tissues. Testosterone and DHT are essential for the normal development of male sex characteristics. Before birth, testosterone is responsible for the formation of internal

male genitalia, including the tubes that collect sperm and carry it out of the testes (the epididymis and vas deferens) and glands that help produce semen (the seminal vesicles). Dihhdrotestosterone directs the development of the external genitalia, including the penis and scrotum, and the prostate gland. During puberty, these two hormones also play an important role in the development of male secondary sex characteristics such as the growth of facial and body hair, increased muscle mass, and deepening of the voice.

The 5- alpha-reductase is an enzyme that is present in highest concentration in the male reproductive tissues, the skin, especially that overlying the genitalia, and the liver. Two human isoenzymes have been identified as 5-alpha reductase 1 and 2. The enzyme 5 alpha-reductase 2 is the predominant form in the prostate (Steers, 2001). The biological role of 5- alpha reductase 1 has not yet been ascertained, but at present it cannot be ruled out that some of the actions ascribed to testosterone are indeed in cells producing DHT via this enzyme. The activity of 5-alpha-reductase is also implicated in benign prostatic hypertrophy, hirsutism and possibly male-pattern baldness, recent evidence discounts the role of 5 alpha reductase 2 in sebaceous glands and acne. Specific inhibitors of both enzymes are available and finasteride, a 5 alpha-reductase 2 inhibitor has been used successfully in clinical trials of benign prostatic hypertrophy.

2.47 Function of the 5ARD2 Gene

The SRD5A2 gene provides instructions for making an enzyme called steroid 5-alpha reductase 2. This enzyme is involved in processing androgens, which are hormones that direct male sexual development. Specifically, the enzyme is responsible for a chemical reaction that converts the hormone testosterone to a more potent androgen, dihydrotestosterone (DHT), in male reproductive tissues.

Testosterone and DHT are essential for the normal development of male sex characteristics. Before birth, testosterone is responsible for the formation of internal male genitalia, including the tubes that collect sperm and carry it out of the testes (the epididymis and vas deferens) and glands that help produce semen (the seminal vesicles). DHT directs the development of the external genitalia, including the penis and scrotum, and the prostate gland. During puberty, these two hormones also play an important role in the development of male secondary sex characteristics such as the growth of facial and body hair, increased muscle mass, and deepening of the voice.

2.48 Changes in the 5ARD Gene in Related Health Conditions

About 50 mutations in the 5ARD2 gene have been identified in people with 5-alpha reductase deficiency. Most of these mutations change single protein building blocks (amino acids) in steroid 5-alpha reductase 2. Some of these genetic changes render the enzyme completely inactive. Other mutations reduce but do not eliminate the enzyme's function.

As a result of 5ARD2 mutations, the body cannot effectively convert testosterone to DHT in reproductive tissues (Niu et al, 2011). A shortage of DHT disrupts the formation of external genitalia before birth. People with 5-alpha reductase deficiency are genetically male, with one X and one Y chromosome in each cell, but they may be born with external genitalia that look predominantly female, or that are not clearly male or clearly female (sometimes called ambiguous genitalia). Other affected infants have external genitalia that appear predominantly male, but they often have an unusually small penis (micropenis) and the urethra opening on the underside of the penis (hypospadias).

During puberty, the testes produce more testosterone. Researchers believe that people with 5-alpha reductase deficiency develop secondary male sex characteristics in response to higher levels of this hormone. Some affected people also retain a small amount of 5-alpha reductase 2 activity, which may produce DHT and contribute to the development of secondary sex characteristics during puberty.

Certain normal variations (polymorphisms) in the 5ARD2 gene may be associated with prostate cancer. Two of these polymorphisms have been studied extensively. The most common variation replaces the amino acid valine with the amino acid leucine at position 89 in steroid 5-alpha reductase 2 (written as Val89Leu or V89L). The other variation replaces the amino acid alanine with the amino acid threonine at position 49 in the enzyme (written as Ala49Thr or A49T). Some studies (Niu et al, 2011) have suggested that these variations are associated with an increased risk of developing prostate cancer or having a more aggressive form of the disease. Other studies, however, have not shown these associations. It remains unclear what role 5ARD2 polymorphisms play in prostate cancer risk.

Some gene mutations are acquired during a person's lifetime and are present only in certain cells. These changes, which are called somatic mutations, are not inherited. Studies have shown that somatic 5ARD2 mutations in prostate cancer cells may be associated with the progression of prostate cancer. These mutations may increase the activity of steroid 5-alpha reductase 2, which would raise the levels of DHT in prostate tissue.

The mechanism of androgen action varies in different tissues, but in the majority of androgen target tissues either testosterone or 5 alpha-dihydrotestosterone (DHT) binds to a specific androgen receptor to form a complex that can regulate gene expression. Testosterone is

metabolized to DHT by the enzyme 5 alpha-reductase. The autosomal recessive genetic disorder of 5 alpha-reductase deficiency has clearly shown that the requirement for DHT formation varies with different tissues. In this syndrome genetic males contain normal male internal structures including testes, but exhibit ambiguous or female external genitalia at birth; at puberty they undergo partial virilization which includes development of a male gender identity even if brought up as females. Their development suggests that testosterone itself is able to stimulate psychosexual behaviour, development of the embryonic wolffian duct, muscle development, voice deepening, spermatogenesis, and axillary and pubic hair growth; DHT seems to be essential for prostate development and growth, the development of the external genitalia and male patterns of facial and body hair growth or male-pattern baldness. How different hormones operate to regulate genes via the same receptor is currently unknown, but appears to involve cell-specific factors. The 5-alpha-reductase enzyme has proved difficult to isolate biochemically, but recently at least two human isoenzymes have been identified using molecular biological methods. All the various 5 alpha-reductase-deficient kindreds have been shown to have mutations in 5 alpha-reductase 2, the predominant form in the prostate. The biological role of 5 alpha-reductase 1 has not yet been ascertained, but at present it cannot be ruled out that some of the actions ascribed to testosterone are indeed in cells producing DHT via this enzyme. The activity of 5 alpha-reductase is also implicated in benign prostatic hypertrophy, hirsutism and possibly male-pattern baldness (Vassiliadi and Barber , 2009).; recent evidence discounts the role of 5 alpha reductase 2 in sebaceous glands and acne. Specific inhibitors of both enzymes are now available and finasteride, a 5 alpha-reductase 2 inhibitor, has been used successfully in clinical trials of benign prostatic hypertrophy. Knowledge of 5 alpha-reductase is expanding dramatically

at the moment with the application of molecular biological methods. The advent of antibodies to the isoenzymes should herald further understanding of their biological and clinical roles.

2.49 Risks, safety, danger, side effects of 5 alpha reductase Inhibitors

Reduced libido and impotence can be a concern with the use of finasteride and dutasteride. The 5alpha-reductase inhibitors, finasteride and dutasteride, are associated with erectile dysfunction (ED), ejaculatory dysfunction and decreased libido. Prostate enlargement is a common problem in middle-aged and elderly men (Berry et al, 1984). First-line medical therapy includes alpha 1 blockers and 5alpha-reductase inhibitors (5ARIs), such as finasteride and dutasteride. 5ARI use has been associated with adverse sexual outcomes. These effects occur early in therapy. A proposed mechanism for sexual dysfunction involves decreased nitric oxide synthase activity due to decreased dihydrotestosterone. Though theories have been proposed, there is more needs to know about the exact mechanisms behind 5ARI-related sexual dysfunction. Many men report lower vitality, lower mood, and easier abdominal weight gain. Many herbal aphrodisiacs can reverse some of the decline in libido.

Clinical trials with 5ARI report reveal decreased circulating dihydrotestosterone (DHT) resulting in diminished sexual desire and/or orgasm (Gur et al, 2013). The presence of adverse sexual effects is associated with decreased self-esteem, quality of life and ability to maintain an intimate relationship. Inhibition of 5ARI additionally influences progesterone and deoxycorticosterone levels and may alter psychological functions, including increased depression, melancholy and loss of general well-being.

2.50 Vitamin D

Vitamin D refers to a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc. In humans, the most important compounds in this group are Vitamin D_3 and Vitamin D_2. Vitamin D is naturally present in ery fre foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, food, and supplements is biologically inert and must undergo two hydroxylation in the body for activation. The first occurs in the liver and converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as calcidiol. The second occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol.

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts. Without sufficient vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D also helps protect older adults from osteoporosis.

Vitamin D has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation (Holick et al 2006). Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many cells have vitamin D receptors, and some convert 25(OH) D to $1,25\ (OH)_2D$.

Serum concentration of 25(OH) D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half-life of 15 days. Serum25 (OH) D functions as a biomarker of exposure, but it is not clear to what extent 25(OH)D levels also serve as a biomarker of effect (i.e., relating to health status or outcomes) . Serum 25(OH)D levels do not indicate the amount of vitamin D stored in body tissues.

In contrast to 25(OH) D, circulating 1,25 $(OH)_2D$ is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate (Jones, 2008). Levels of 1,25 $(OH)_2D$ do not typically decrease until vitamin D deficiency is severe (Holick, 2007).

Recommended Dietary Allowances (RDAs) of Vitamin D is 800IU for adults

2.51 Sources of Vitamin D

Very few foods in nature contain vitamin D. The flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils are among the best sources (United State Department of Agriculture, 2011). Small amounts of vitamin D are found in beef liver, cheese, and egg yolks. Vitamin D in these foods is primarily in the form of vitamin D_3 and its metabolite 25(OH)D_3 (Mattila et al ,1994). Some mushrooms provide vitamin D_2 in variable amounts.

Fortified foods provide most of the vitamin D in the American diet (Calvo et al, 2004). For example, almost all of the U.S. milk supply is voluntarily fortified with 100 IU/cup. (In Canada, milk is fortified by law with 35–40 IU/100 mL, as in margarine at ≥530 IU/100 g.) In the 1930s, a milk fortification program was implemented in the United States to combat rickets, then a major public health problem. Other dairy products made from milk, such as cheese and ice

cream, are generally not fortified. Ready-to-eat breakfast cereals often contain added vitamin D, as do some brands of orange juice, yogurt, margarine and other food products.

Sun Exposure: Most people meet at least some of their vitamin D needs through exposure to sunlight. Ultraviolet (UV) B radiation with a wavelength of 290–320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to previtamin D_3, which in turn becomes vitamin D_3. Season, time of day, length of day, cloud cover, smog, skin melanin content, and sunscreen are among the factors that affect UV radiation exposure and vitamin D synthesis. Perhaps surprisingly, geographic latitude does not consistently predict average serum 25(OH) D levels in a population. Ample opportunities exist to form vitamin D (and store it in the liver and fat) from exposure to sunlight during the spring, summer, and fall months even in the far north latitudes.

Complete cloud cover reduces UV energy by 50%; shade (including that produced by severe pollution) reduces it by 60% (Wharton and Bishop 2003). UVB radiation does not penetrate glass, so exposure to sunshine indoors through a window does not produce vitamin D. Sunscreens with a sun protection factor (SPF) of 8 or more appear to block vitamin D-producing UV rays, although in practice people generally do not apply sufficient amounts, cover all sun-exposed skin, or reapply sunscreen regularly. Therefore, skin likely synthesizes some vitamin D even when it is protected by sunscreen as typically applied.

The factors that affect UV radiation exposure and research to date on the amount of sun exposure needed to maintain adequate vitamin D levels make it difficult to provide general guidelines. It has been suggested by some vitamin D researchers, for example, that approximately 5–30 minutes of sun exposure between 10 am and 3 pm at least twice a week to the face, arms, legs, or

back without sunscreen usually lead to sufficient vitamin D synthesis and that the moderate use of commercial tanning beds that emit 2%–6% UVB radiation is also effective (Holick, 2002). Individuals with limited sun exposure need to include good sources of vitamin D in their diet or take a supplement to achieve recommended levels of intake.

Despite the importance of the sun for vitamin D synthesis, it is prudent to limit exposure of skin to sunlight and UV radiations (International Agency for Research on Cancer, 2006). Ultravoilet radiation is a carcinogen responsible for most of the estimated 1.5 million skin cancers and the 8,000 deaths due to metastatic melanoma that occur annually in the United States. Lifetime cumulative UV damage to skin is also largely responsible for some age-associated dryness and other cosmetic changes. The American Academy of Dermatology advises that photoprotective measures be taken, including the use of sunscreen, whenever one is exposed to the sun. Assessment of vitamin D requirements cannot address the level of sun exposure because of these public health concerns about skin cancer, and there are no studies to determine whether UVB-induced synthesis of vitamin D can occur without increased risk of skin cancer.

2.52 Vitamin D Deficiency

Nutrient deficiencies are usually the result of dietary inadequacy, impaired absorption and use, increased requirement, or increased excretion. A vitamin D deficiency can occur when usual intake is lower than recommended levels over time, exposure to sunlight is limited, the kidneys cannot convert 25(OH)D to its active form, or absorption of vitamin D from the digestive tract is inadequate. Vitamin D-deficient diets are associated with milk allergy, lactose intolerance, ovo-vegetarianism, and veganism. Rickets and osteomalacia are the classical vitamin D deficiency diseases. In children, vitamin D deficiency causes rickets, a disease characterized by a failure of

bone tissue to properly mineralize, resulting in soft bones and skeletal deformities (Wharton and Bishop, 2003). In adults, vitamin D deficiency can lead to osteomalacia, resulting in weak bones. Symptoms of bone pain and muscle weakness can indicate inadequate vitamin D levels, but such symptoms can be subtle and go undetected in the initial stages.

2.53 Groups at Risk of Vitamin D Inadequacy

Obtaining sufficient vitamin D from natural food sources alone is difficult. For many people, consuming vitamin D-fortified foods and, arguably, being exposed to some sunlight are essential for maintaining a healthy vitamin D status. In some groups, dietary supplements might be required to meet the daily need for vitamin D.

2.54 Breastfed Infants

Vitamin D requirements cannot ordinarily be met by human milk alone (Picciano, 2001), which provides <25 IU/L to 78 IU/L (Wagner and Greer, 2008) . (The vitamin D content of human milk is related to the mother's vitamin D status, so mothers who supplement with high doses of vitamin D may have correspondingly high levels of this nutrient in their milk . A review of reports of nutritional rickets found that a majority of cases occurred among young, breastfed African Americans (Weisberg, 2004). Survey of Canadian pediatricians found the incidence of rickets in their patients to be 2.9 per 100,000; almost all those with rickets had been breast fed (Ward et al 2007).

2.55 Older Adults

Older adults are at increased risk of developing vitamin D insufficiency in part because, as they age, skin cannot synthesize vitamin D as efficiently, they are likely to spend more time indoors,

and they may have inadequate intakes of the vitamin . As many as half of older adults in the United States with hip fractures could have serum 25(OH) D levels <30 nmol/L (<12 ng/mL).

2.56 People with Limited Sun Exposure

Homebound individuals, women who wear long robes and head coverings for religious reasons, and people with occupations that limit sun exposure are unlikely to obtain adequate vitamin D from sunlight (Webb et al 1998). Because the extent and frequency of use of sunscreen are unknown, the significance of the role that sunscreen may play in reducing vitamin D synthesis is unclear. Ingesting RDA levels of vitamin D from foods and/or supplements will provide these individuals with adequate amounts of this nutrient.

2.57 People with Dark Skin

Greater amounts of the pigment melanin in the epidermal layer result in darker skin and reduce the skin's ability to produce vitamin D from sunlight. Various reports consistently show lower serum 25(OH) D levels in persons identified as black compared with those identified as white. It is not clear that lower levels of 25(OH) D for persons with dark skin have significant health consequences. Those of African American ancestry, for example, have reduced rates of fracture and osteoporosis compared with Caucasians. Ingesting RDA levels of vitamin D from foods and/or supplements will provide these individuals with adequate amounts of this nutrient.

2.58 Vitamin D and Cancer

Laboratory and animal evidence as well as epidemiologic data suggest that vitamin D status could affect cancer risk. Strong biological and mechanistic bases indicate that vitamin D plays a role in the prevention of colon, prostate, and breast cancers.

Vitamin D supplements have been widely marketed for their claimed anticancer properties (Byers, 2010) Associations have been shown in observational studies between low vitamin D levels and the risk of development of cancer (Ma et al, 2011, Feldman et al, 2014) but it is unclear if taking vitamin D affects the risk of cancer with the evidence being "inconsistent, inconclusive as to causality, and insufficient to inform nutritional requirements"(Chung et al, 2011).

2014 review found that supplements had no significant effect on cancer risk (Bolland et al, 2014) Another review suggested that vitamin D3 may slightly decrease the risk of death from cancer (one fewer death in 150 people over 5 years), but concerns with the quality of the data were noted.

Insufficient evidence exists to recommend vitamin D supplements for people with cancer, although some evidence suggests hypovitaminosis D may be associated with a worse outcome for some cancers and that higher 25-hydroxy vitamin D levels at the time of diagnosis are associated with better outcomes.

2.59 Selenium

Selenium is a trace element that is naturally present in many foods, added to others, and available as a dietary supplement. Selenium, which is nutritionally essential for humans, is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Sunde, 2006, Sunde, 2012).

Selenium exists in two forms: inorganic (selenate and selenite) and organic (selenomethionine and selenocysteine). Both forms can be good dietary sources of selenium (Terry and Diamond 2012). Soils contain inorganic selenites and selenates that plants accumulate and convert to organic forms, mostly selenocysteine and selenomethionine and their methylated derivatives.

Most selenium is in the form of selenomethionine in animal and human tissues, where it can be incorporated nonspecifically with the amino acid methionine in body proteins. Skeletal muscle is the major site of selenium storage, accounting for approximately 28% to 46% of the total selenium pool (Terry and Diamond, 2012). Both selenocysteine and selenite are reduced to generate hydrogen selenide, which in turn is converted to selenophosphate for selenoprotein biosynthesis (Devis 2012).

The most commonly used measures of selenium status are plasma and serum selenium concentrations. Concentrations of seleniun in blood and urine reflect recent selenium intake. Analyses of hair or nail selenium content can be used to monitor longer-term intakes over months or years. The quantitative analysis of one or more selenoproteins (such as glutathione peroxidase and selenoprotein P) is also used as a functional measure of selenium status ((Terry and Diamond, 2012). The Plasma or serum selenium concentrations of 8 micrograms (µg)/dL or higher in healthy people typically meet needs for selenoprotein synthesis.

2.60 Sources of Selenium

Seafoods and organ meats are the richest food sources of selenium (Sunde, 2012) . Other sources include muscle meats, cereals and other grains, and dairy products. The amount of selenium in drinking water is not nutritionally significant in most geographic regions. The major food

sources of selenium in the diet are breads, grains, meat, poultry, fish, and eggs (Chun et al, 2010).

The amount of selenium in a given type of plant-based food depends on the amount of selenium in the soil and several other factors, such as soil pH, amount of organic matter in the soil, and whether the selenium is in a form that is amenable to plant uptake (Rayman, 2012). As a result, selenium concentrations in plant-based foods vary widely by geographic location (Sunde, 2012). For example, according to the U.S. Department of Agriculture Food Composition Database, Brazil nuts have 544µg selenium/ounce, but values from other analyses vary widely.

The selenium content of soil affects the amounts of selenium in the plants that animals eat, so the quantities of selenium in animal products also vary (Sunde, 2010). However, selenium concentration in soil has a smaller effect on selenium levels in animal products than in plant-based foods because animals maintain predictable tissue concentrations of selenium through homeostatic mechanisms

Selenium is available in multivitamin/multimineral supplements and as a stand-alone supplement, often in the forms of selenomethionine or of selenium-enriched yeast (grown in a high-selenium medium) or as sodium selenite or sodium selenate. The human body absorbs more than 90% of selenomethionine but only about 50% of selenium from selenite.

Few studies have compared the relative absorption and bioavailability of different forms of selenium. In one investigation, 10 groups of selenium-replete subjects were randomly assigned to receive a placebo or either 200 or 600 µg/day selenium as selenomethionine, sodium selenite, or high-selenium yeast (in which an estimated 75% of selenium was in the form of selenomethionine) for 16 weeks (Burk, et al 2006). Selenium bioavailability, based on urinary

excretion, was greatest for selenomethionine and lowest for selenite. However, supplementation with any of these forms only affected plasma selenium levels and not glutathione peroxidase activity or selenoprotein P concentration, confirming that study participants were selenium replete before they began taking selenium supplements.

The Recommended Dietary Allowance (RDA) which is the average daily level of intake of selenium sufficient to meet the nutrient requirements of nearly all healthy individuals is 55µg for adults, 60µg and 70µg for pregnant and lactating mothers respectively (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes, 2000)

2.61 Selenium Intakes and Status

Most people consume adequate amounts of selenium in their diet. According to an analysis of data from the 2009–2010 National Health and Nutrition Examination Survey, the average daily selenium intake in Americans aged 2 years and older from foods is 108.5µg and from both foods and supplements is 120.8µg . Adult men have higher daily intakes (134µg from foods and 151µg from foods and supplements) than adult women (93 µg from foods and 108 µg from foods and supplements). In the United States, 18% to 19% of adults and children use a dietary supplement containing selenium (Bailey et al, 2011).

Men have slightly higher serum selenium levels than women, and whites have higher levels than African Americans (Xun et al, 2011). Selenium concentration is higher in the thyroid gland than in any other organ in the body, and, like iodine, selenium has important functions in thyroid hormone synthesis and metabolism.

Selenium intakes and serum concentrations in the United States and Canada vary somewhat by region because of differences in the amounts of selenium in soil and in local foods consumed (Kafia and Ganji, 2003). For example, concentrations are higher in residents of the Midwestern and Western United States than in the South and Northeast. The extensive transport of food typically allows people living in low-selenium areas to obtain sufficient amounts of selenium.

Selenium deficiency is also associated with male infertility and might play a role in Kashin-Beck disease, a type of osteoarthritis that occurs in certain low-selenium areas of China, Tibet, and Siberia. Selenium deficiency could exacerbate iodine deficiency, potentially increasing the risk of cretinism in infants (Sunde, 2010).

2.62 Groups at Risk of Selenium Inadequacy

The following groups are among those most likely to have inadequate intakes of selenium.

People living in selenium-deficient regions.

Selenium intakes in most part of the world, even in low-selenium regions, are well above the RDA (Niskar et al, 2003). However, people in some other countries whose diet consists primarily of vegetables grown in low-selenium areas are at risk of deficiency. The lowest selenium intakes in the world are in certain parts of China, where large proportions of the population have a primarily vegetarian diet and soil selenium levels are very low. Average selenium intakes are also low in some European countries, especially among populations consuming vegan diets.

(Sunde, 2010). Although intakes in New Zealand were low in the past, they rose after the country increased its importation of high-selenium wheat (Rayman, 2012). Selenium levels are

significantly lower in patients undergoing long-term hemodialysis than in healthy individuals. Hemodialysis removes some selenium from the blood (Tonelli et al 2009). In addition, hemodialysis patients are at risk of low dietary selenium intakes due to anorexia resulting from uremia and dietary restrictions. Although selenium supplementation increases blood levels in hemodialysis patients, more evidence is needed to determine whether supplements have beneficial clinical effects in these individuals. Selenium levels are often low in people living with HIV, possibly because of inadequate intakes (especially in developing countries), excessive losses due to diarrhea, and malabsorption (Stone et al, 2010). Serum selenium concentrations decline with age. Marginal or deficient selenium concentrations might be associated with age-related declines in brain function, possibly due to decreases in selenium's antioxidant activity (Rees et al 2013).

Sequel to the effects on DNA repair, apoptosis, and the endocrine and immune systems as well as other mechanisms, including its antioxidant properties, selenium might play a role in the prevention of cancer (Allen et al, 2008, Rayman et al 2012). Epidemiological studies have suggested an inverse association between selenium status and the risk of colorectal, prostate, lung, bladder, skin, esophageal, and gastric cancers. In a Cochrane review of selenium and cancer prevention studies, compared with the lowest category of selenium intake, the highest intake category had a 31% lower cancer risk and 45% lower cancer mortality risk as well as a 33% lower risk of bladder cancer and, in men, 22% lower risk of prostate cancer. The authors found no association between selenium intake and risk of breast cancer. A meta-analysis of 20 epidemiologic studies showed a potential inverse association between toenail, serum, and plasma selenium levels and prostate cancer risk (Brinkman et al, 2006).

Randomized controlled trials of selenium supplementation for cancer prevention have yielded conflicting results. The authors of a Cochrane review concluded, based on nine randomized clinical trials, that selenium might help prevent gastrointestinal cancers but noted that these results need to be confirmed in more appropriately designed randomized clinical trials. A secondary analysis of the double-blind, randomized, controlled Nutritional Prevention of Cancer Trial in 1,312 U.S. adults with a history of basal cell or squamous cell carcinomas of the skin found that 200 µg/day selenium as high-selenium baker's yeast for 6 years was associated with a 52% to 65% lower risk of prostate cancer. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) (Klein et al, 2011), a randomized, controlled trial in 35,533 men aged 50 years or older from the United States, Canada, and Puerto Rico, was discontinued after 5.5 years when analyses showed no association between supplementation with 200 µg/day selenium with or without 400 international units (IU)/day vitamin E and prostate cancer risk (Duffield-Lilico et al, 2003). An additional 1.5 years of follow-up data on participants after they stopped taking the study supplements confirmed the lack of a significant association between selenium supplementation and prostate cancer risk (Lippman et al, 2009).

Selenoproteins help prevent the oxidative modification of lipids, reducing inflammation and preventing platelets from aggregating (Rayman, 2012). For these reasons, experts have suggested that selenium supplements could reduce the risk of cardiovascular disease or deaths associated with cardiovascular disease.

2.63 Magnesium

Magnesium, an abundant mineral in the body, is naturally present in many foods, added to other food products, available as a dietary supplement, and present in some medicines (such as

antacids and laxatives). Magnesium in general is essential for the survival of our cells but takes on further importance in the age of toxicity where our bodies are being bombarded on a daily basis with heavy metals. Without sufficient magnesium, the body accumulates toxins and acid residues, degenerates rapidly, and ages prematurely. Glutathione requires magnesium for its synthesis. Glutathione synthetase requires y-glutamyl cysteine, glycine, ATP, and magnesium ions to form glutathione. In magnesium deficiency, the enzyme y-glutamyl transpeptidase is lowered. Lower levels magnesium is associated with dramatic increases in free radical generation as well as glutathione depletion and this is vital since glutathione is one of the few antioxidant molecules known to neutralize mercury (Zhang et al, 2003). Without the cleaning and chelating work of glutathione (magnesium), cells begin to decay as cellular filth and heavy metals accumulate; excellent environments to attract deadly infection/cancer. Magnesium has an effect on a variety of cell membranes through a process involving calcium channels and ion transport mechanisms. It is responsible for the maintenance of the trans-membrane gradients of sodium and potassium. Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (Rude et al, 2010:Rude, 2012). Magnesium is required for energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione. Magnesium also plays a role in the active transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm (Rude, 2012).

An adult body contains approximately 25 g magnesium, with 50% to 60% present in the bones and most of the rest in soft tissues. Less than 1% of total magnesium is in blood serum, and these

levels are kept under tight control. Normal serum magnesium concentrations range between 0.75 and 0.95 millimoles (mmol)/L (Elin, 2010). Hypomagnesemia is defined as a serum magnesium level less than 0.75 mmol/L. Magnesium homeostasis is largely controlled by the kidney, which typically excretes about 120 mg magnesium into the urine each day (Rude, 2010). Urinary excretion is reduced when magnesium status is low.

Assessing magnesium status is difficult because most magnesium is inside cells or in bone. The most commonly used and readily available method for assessing magnesium status is measurement of serum magnesium concentration, even though serum levels have little correlation with total body magnesium levels or concentrations in specific tissues. Other methods for assessing magnesium status include measuring magnesium concentrations in erythrocytes, saliva, and urine; measuring ionized magnesium concentrations in blood, plasma, or serum; and conducting a magnesium-loading (or "tolerance") test. No single method is considered satisfactory. Some experts consider the tolerance test (in which urinary magnesium is measured after parenteral infusion of a dose of magnesium) to be the best method to assess magnesium status in adults. To comprehensively evaluate magnesium status, both laboratory tests and a clinical assessment are required.

2.64 Sources of Magnesium

Magnesium is widely distributed in plant and animal foods and in beverages. Green leafy vegetables, such as spinach, legumes, nuts, seeds, and whole grains and fruits such as banana, apple are good sources. In general, foods containing dietary fiber provide magnesium. Magnesium is also added to some breakfast cereals and other fortified foods. Some types of food processing, such as refining grains in ways that remove the nutrient-rich germ and bran, lower

magnesium content substantially. Tap, mineral, and bottled waters can also be sources of magnesium, but the amount of magnesium in water varies by source and brand (ranging from 1 mg/L to more than 120 mg/L) (Azoulay, 2001).

The Recommended Dietary Allowance of magnesium in adult is 320mg and 420mg for females and males respectively.

2.65 Magnesium Deficiency

Symptomatic magnesium deficiency due to low dietary intake in otherwise-healthy people is uncommon because the kidneys limit urinary excretion of this mineral. However, habitually low intakes or excessive losses of magnesium due to certain health conditions, chronic alcoholism, and/or the use of certain medications can lead to magnesium deficiency. Early signs of magnesium deficiency include loss of appetite, nausea, vomiting, fatigue, and weakness. As magnesium deficiency worsens, numbness, tingling, muscle contractions and cramps, seizures, personality changes, abnormal heart rhythms, and coronary spasms can occur. Magnesium supplements are available in a variety of forms, including magnesium oxide, citrate, and chloride (Rude, 2012) The Supplement Facts panel on a dietary supplement label declares the amount of elemental magnesium in the product, not the weight of the entire magnesium-containing compound. Absorption of magnesium from different kinds of magnesium supplements varies. Forms of magnesium that dissolve well in liquid are more completely absorbed in the gut than less soluble forms (Fine et al, 1991). Small studies have found that magnesium in the aspartate, citrate, lactate, and chloride forms is absorbed more completely and is more bioavailable than magnesium oxide and magnesium sulfate (Muhlbauer et al, 1991). One study found that very high doses of zinc from supplements (142 mg/day) can interfere with magnesium absorption and

disrupt the magnesium balance in the body (Lindberg, 1990). Magnesium deficiency seems to be carcinogenic, and in case of solid tumors, a high level of supplemented magnesium inhibits carcinogenesis. Both carcinogenesis and magnesium deficiency increase the plasma membrane permeability and fluidity.

2.66 Vitamin E

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. "Vitamin E" is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities (Traber, 2006).

Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity. Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements.

Serum concentrations of vitamin E (alpha-tocopherol) depend on the liver, which takes up the nutrient after the various forms are absorbed from the small intestine. The liver preferentially resecretes only alpha-tocopherol via the hepatic alpha-tocopherol transfer protein (Traber, 2006); the liver metabolizes and excretes the other vitamin E forms (Traber, 2007). As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of alpha-tocopherol and have been the subjects of less research (Sen et al, 2006, Dietrich et al, 2006)

Vitamin E is an antioxidant which protects cells from the damaging effects of free radicals, which are molecules that contain an unshared electron. Free radicals damage cells and might contribute to the development of cardiovascular disease and cancer (Verhagen et al, 2006). Unshared electrons are highly energetic and react rapidly with oxygen to form reactive oxygen

species (ROS). The body forms ROS endogenously when it converts food to energy, and antioxidants might protect cells from the damaging effects of ROS. The body is also exposed to free radicals from environmental exposures, such as cigarette smoke, air pollution, and ultraviolet radiation from the sun. ROS are part of signaling mechanisms among cells.

Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation (Buettner, 1993). Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might help prevent or delay the chronic diseases associated with free radicals.

In addition to its activities as an antioxidant, vitamin E is involved in immune function and, as shown primarily by in vitro studies of cells, cell signaling, regulation of gene expression, and other metabolic processes. Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes. Vitamin-E–replete endothelial cells lining the interior surface of blood vessels are better able to resist blood-cell components adhering to this surface. Vitamin E also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation.

2.67 Sources of Vitamin E

Numerous foods provide vitamin E. Nuts, seeds, and vegetable oils are among the best sources of alpha-tocopherol, and significant amounts are available in green leafy vegetables and fortified

cereals (USDA, 2011). Most vitamin E in American diets is in the form of gamma-tocopherol from soybean, canola, corn, and other vegetable oils and food products.

Palm oil is exceptionally high in tocotrienols. Other good dietary sources of vitamin E are virtually all nuts and seeds, particularly almonds and walnuts, and, olive, and coconut oil.

Recommended dietary allowance of Vitamin E for adult is 24.1IU and about 28.4IU for pregnant and lactating mothers.

2.68 Vitamin E and Health

Many claims have been made about vitamin E's potential to promote health and prevent and treat disease. The mechanisms by which vitamin E might provide this protection include its function as an antioxidant and its roles in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement.

Antioxidant nutrients like vitamin E protect cell constituents from the damaging effects of free radicals that, if unchecked, might contribute to cancer development (USDA, 2011). Vitamin E might also block the formation of carcinogenic nitrosamines formed in the stomach from nitrites in foods and protect against cancer by enhancing immune function (Weitberg and Corvese, 1997). Unfortunately, human trials and surveys that have attempted to associate vitamin E intake with cancer incidence have found that vitamin E is not beneficial in most cases.

Several studies have examined whether vitamin E intake and/or supplemental vitamin E affects the risk of developing prostate cancer. A prospective cohort study of >29,000 men found no association between dietary or supplemental vitamin E intake and prostate cancer risk (Kirsh et al, 2006). However, among current smokers and men who had quit, vitamin E intakes of more

than 400 IU/day were associated with a statistically significant 71% reduction in the risk of advanced prostate cancer. In a clinical trial involving 29,133 male smokers, men randomly assigned to take daily supplements of 50 IU synthetic vitamin E for 5–8 years had 32% fewer prostate cancers compared to subjects who did not take the supplements (.Heinonen et al, 1998). Based in part on the promising results of this study, a large randomized clinical trial, called the SELECT trial, began in 2001 to determine whether 7–12 years of daily supplementation with synthetic vitamin E (400 IU, as dl-alpha-tocopheryl acetate), with or without selenium (200 µg, as L-selenomethionine), reduced the number of new prostate cancers in 35,533 healthy men, aged 50 and older. The trial was discontinued in October 2008 when an analysis found that the supplements, taken alone or together for about 5.5 years, did not prevent prostate cancer. Results from an additional 1.5 years of follow-up from this trial (during which the subjects no longer received vitamin E or selenium), showed that the men who had taken the vitamin E had a 17 percent increased risk of prostate cancer compared to men only taking placebos, a statistically significant difference (Klein et al, 2011). The risk of developing prostate cancer was also slightly increased in subjects taking vitamin E plus selenium or selenium alone, but the differences were not statistically significant. No differences were found among groups in the incidence of lung or colorectal cancers or all cancers combined.

However, results from the recently published, large SELECT trial show that vitamin E supplements (400 IU/day) may harm adult men in the general population by increasing their risk of prostate cancer. Follow-up studies are assessing whether the cancer risk was associated with baseline blood levels of vitamin E and selenium prior to supplementation as well as whether changes in one or more genes might increase a man’s risk of developing prostate cancer while taking vitamin E.

2.69 Vitamin C

Vitamin C is probably best known as an antioxidant. Antioxidants help prevent excessive activity on the part of free radical molecules. (Free radicals are forms of molecules that tend to be very reactive, and too many free radicals in the wrong place at the wrong time can do damage to our cells and tissue.) Vitamin C and other antioxidants help prevent that damage. Damage to the lens of the eye, damage to molecules circulating around in our bloodstream, and damage to genetic material (DNA) in our cells are all examples of damage that have been shown to be prevented under certain circumstances by vitamin C. One interesting application of vitamin C as an antioxidant is its ability to transform iron into a state that is better absorbed in the intestine, including vitamin C-rich foods in recipes with the best iron sources can potentially be a way to enhance iron absorption. Vitamin C has the ability to protect against lipid peroxidase (LPO) by acting as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the vitamin E redox cycle.

Vitamin C is required to produce collagen, a protein that plays a critical role in the structure of the body. Collagen is the framework for our skin and our bones, and without it, we would quite literally fall apart. This is exactly what we see with severe vitamin C deficiency, or scurvy. People who have this condition lose teeth, bleed easily, and lose the strength of their bones. Luckily, it doesn't take much vitamin C to prevent this problem. As we've known for more than two centuries, a single lime per day would usually be enough. (However, as described earlier, we have dozens and dozens of great food choices that will give us as much vitamin C as a single lime!)

Brain Health: Vitamin C is necessary to make certain neurotransmitters. These neurotransmitters are the signals that carry thoughts, feelings, and commands around our brains and throughout our nervous system.

In particular, we need vitamin C to produce serotonin, a hormone that plays a critical role in wide variety of body systems, including the nervous system, endocrine system, immune system, and digestive system. Many of our moods, daily bodily rhythms (including sleep-wake cycles), and experiences of stress and pain have serotonin included as a factor in their occurrence. Some of the most commonly used prescription medications for depression (SSRIs, or Selective Serotonin Reupdate Inhibitors) also target this hormone. Meanwhile it has been suggested that dietary intake of vitamin C may not automatically improve the quality of any experiences described above, but it is recommend that one should include vitamin C-rich foods on a daily basis as part of ones overall well-being.

2.70 Food Sources of Vitamin C

Our best food sources of vitamin C have a single thing in common: they are all plant foods. Even though many animals make vitamin C in their bodies, only plants make it to the degree that they provide a rich source of the nutrient when eaten. The citrus fruits (orange, grapefruit, lime, and lemon) are excellent sources of vitamin C.

Many non-citrus fruits are highly rated sources, as well. Papaya, strawberries, pineapple, kiwifruit, cantaloupe, and raspberries are also excellent vitamin C sources. Cranberries, blueberries, and watermelon are examples of very good sources, while apples, pears, and bananas are in the good category. One would expect almost any fresh fruit to be a good, very good, or excellent source of dietary vitamin C. Many green leafy vegetables contain vitamin C.

Very good sources of vitamin C in the vegetable group include summer and winter squash, green beans, and carrots.

2.71 Relationship with other Nutrients

Vitamin C can increase the absorption of iron (especially the iron found in plant foods) and may help lower the risk of dietary iron deficiency. Antioxidants in foods tend to work together in important and synergistic ways to provide protection against free radical damage. The most well-known of these connections is that between vitamin E and vitamin C. Specifically, vitamin C helps to protect vitamin E in people, such as smokers, who have chronic overproduction of free radicals (Bruno et all, 2005). This synergistic protection is but one of many potential explanations for why the health benefits of plant-based diets cannot be replicated by nutrient supplements.

2.72 Zinc

Zinc is an essential trace element that plays an important role in many body processes.

It is suggested that dietary zinc supplementation protects against oxidative damage, reduces cancer risk (Ho, 2004) and has therapeutic potentials against prostate cancer (Franklin & Costello, 2007). Epidemiologic studies, however, provided contradictory findings on the effectiveness of zinc in prevention against prostate cancer. While there are studies that showed zinc reduces the risk of developing prostate cancer (Key et al., 1997; Kristal et al., (1999), others showed that advanced prostate cancer is associated with high intake of zinc and potentiate the development of benign prostate hyperplasia (BPH) and progression towards cancer (Moyad, 2004; Gallus et al, 2007; Lawson et al, 2007). Various mechanisms have been proposed to explain how high zinc regulates prostate health and indirectly, how its

absence or low concentration could have contributed to the occurrence of prostate cancer. Restoration of high zinc to cancerous prostate tissues has been shown to inhibit prostate cancer cells proliferation (Feng et al., 2000) and invasion . In contrast, there are studies which showed that zinc under non-physiological conditions could promote cancer cell growth and invasion. Because of the diverse roles of zinc in cell signaling, the exact pathways and genes affected by the absence or presence of high zinc concentrations in prostate cancer cells remain unraveled.

2.73 Zinc and Human Health

Zinc is an essential trace element, critical for diverse biological functions in the human body. Its importance in humans was not discovered until 1961 where zinc deficiency was found to be the cause of growth retardation and hypogonadism in Iranian and Egyptian patients (Prasad et al., 2014). Zinc in the body plays an important role in normal testicular development, spermatogenesis, and sperm motility (Madding et al, 1986). Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa (Lin et al, 2000). Sperm motility is significantly influenced by zinc. Zinc copes up with excessive amount of superoxide anions, thus adequate amount of zinc in seminal plasma exerts protective effect by virtue of this antioxidant activity (Gavella and Lipovac, 1998). Zinc appears to protect sperm from bacteria and chromosomal damage (O'Connor, 2000)

Meat, poultry, oyster, dairy products, legumes and cereals are food rich in zinc. However, phytates, which are present in some cereals and legumes can reduce the bioavailability of zinc by inhibiting absorption of zinc in the intestines . The recommended dietary allowance (RDA) for daily intake of zinc in 97- 98% healthy individuals is 8 mg/d for women, 11 mg/d for men, 11-12

mg/d for pregnant and lactating women. Children from 1-3 years old, 4-8 years and 9-13 years old require 3, 5 and 8 mg/d of zinc, respectively (Food and Nutritional Board of Medicine, 2010a). The tolerable upper intake level (UL), defined as the maximal daily intake unlikely to cause adverse health effects is 40 mg/d for adults. For children of 1-3, 4-8 and 9-13 years old, ULs are 7 mg/d, 12 and 23 mg/d, respectively, (Food and Nutritional Board of Medicine, 2010b).

Deficiency of zinc is associated with significant health problems. Early manifestations of zinc deficiency include decreased immunity resulting in increased susceptibility to infections such as diarrhea, common cold, acute lower respiratory infection and malaria (Christopher et al, 2005)

Zinc-deficient individuals also display dermatitis, delayed wound healing and alopecia. Prolonged deficiency leads to retardation of growth, genital development, hypogonadism and impaired neuropsychological functions (Prasad, 2008). Zinc deficiency in pregnancy retards fetal growth and postnatal development, causes neural tube defects and premature birth but the use of zinc supplementation in women still requires further studies.

Zinc supplements are found in the forms of zinc gluconate, zinc sulfate, and zinc acetate, where the percentage of elemental zinc varies (Haase et al., 2008). Studies have shown that supplementation with zinc corrects growth and gonadal development, improves immune functions and hastens recovery from diarrhea, common cold, acute lower respiratory infection and malaria (Fischer Walker & Black, 2004). It is also used to treat genetic disorders such as acrodermatitis enteropathica (Maverakis et al., 2007) and Wilson’s disease (Brewer, 2001). Zinc excess can induce copper deficiency and it is used in Wilson's disease to interfere with uptake of copper and subsequently reduce excessive copper accumulation.

While zinc supplementation may be beneficial in certain conditions excessive zinc intake can pose serious health risks. Acute zinc toxicity causes gastrointestinal-related symptoms such nausea, vomiting, tenesmus (a feeling that one needs to pass stool, even though your bowels are already empty) and diarrhea (Brown et al., 1964). Chronic zinc toxicity can lead to copper deficiency and its related hematological and neurological manifestations (Maret & Sandstead, 2006). Chronic high intake of zinc can also cause abnormalities of genitourinary functions (Johnson et al., 2007).

2.74 Zinc Homeostasis and Transport

Total zinc in human body is about 2-3 g for a 70 kg adult (Wastney et al., 1986). Ninety percent is incorporated in the muscle, most of which are poorly exchangeable and tightly bound to high molecular weight ligands such as Metalloenzymes, metalloproteins, nucleoproteins and nucleic acids. The remaining 10% of zinc is readily exchangeable and loosely bound to amino acid and citrate (Outten & O'Halloran, 2001).

This pool of zinc is found in plasma, liver and bone and is metabolically active, rapidly exchanged and sensitive to changes in the bioavailability of zinc in the diet. The prostate gland, pancreas, adrenal gland, certain areas of the brain, inner ear and eye, skin, nails, hair, red and white blood cells are known to accumulate high concentrations of zinc.

The concentrations of zinc are strictly regulated in vivo because dysregulation of zinc homeostasis can result in pathogenic consequences. Approximately 30-40% of intracellular zinc is localized in the nucleus, 50% in the cytosol and cytosolic organelles and the remainder is associated with cell membranes. Zinc transporters play important roles in maintaining zinc homeostasis, as zinc ions are hydrophilic and do not cross cell membranes

by passive diffusion (Cousins & McMahon, 2000).

2.75 Functions of Zinc

2.76 Structural and Catalytic Functions

Zinc has structural, catalytic and regulatory functions. It is a structural element found in many enzymes essential for DNA synthesis, transcription, aminoacyl-tRNA synthesis and ribosomal functions. It is found in zinc finger motifs of more than two thousand of transcription factors where these motifs provide a platform for interaction with DNA or other proteins. Zinc is also found in LIM domains in proteins important for cytoskeletal organization, organ development and oncogenesis (Kadrmas & Beckerle, 2004). Zinc regulates cell proliferation and differentiation by modulating nucleic acid metabolisms and protein synthesis. It also controls cell growth by activating, transporting and modulating growth hormone, insulin-like growth factor-1, prolactin, testosterone and other steroid hormones. Zinc, unlike the highly reactive copper and iron, does not participate in redox reactions (Berg & Shi, 1996). The incorporation of zinc into proteins instead of these highly reactive elements helps prevent the generation of free radicals (Ho, 2004). Zinc, hence, has anti-oxidant activities. It maintains proper folding, stability and activity of zinc-dependent enzymes by protecting these enzymes from free radicals attacks (Coleman, 1992). Some of the zinc-dependent enzymes include superoxide dismutase, carbonic anhydrase, alkaline phosphatase, glutamic dehydrogenase, nucleotidase, carboxypeptidase A, retinal dehydrogenase and angiotensin-converting enzymes.

Intracellular zinc also protects several compounds from oxidative damage and these include citrate in the prostate gland (Omu et al., 1998) and insulin in the secretory granules of the islet beta cells. In addition, zinc exerts its anti-oxidant abilities indirectly by maintaining an adequate level of the free radical scavengers such as metallothioneins (MTs). It also stabilizes

cell membrane structure such as that of the red blood cells and sperm cells (Omu et al., 1998). Zinc-bound melanin granules of the skin, choroids, iris, retina, photoreceptors of the eye also provide protection against oxidative damage and apoptosis (Borovansky, 1994).

2.77 Zinc and Immunity

Zinc is critical for immune function and is involved in many aspects of the immune system. Deficiency of zinc causes dysfunction of cells involved in innate 'immunity such as macrophages, neutrophils and natural killer (NK) cells and affects phagocytosis, intracellular killing, cytokine production, complement activity and delayed type hypersensitivity. Zinc is also important for gene regulation in T lymphocytes. It activates pituitary growth hormone and thymulin, the thymic hormone that stimulates division, differentiation and maturation of T-cells, lymphocyte proliferation and cytotoxic activity of natural killer cells (Baum et al., 2000). It also modulates the function of T-helper cells by regulating lymphokines, interleukin-2 (IL-2), interferon-gamma (IFN-) and tumour necrosis factor-alpha (TNF-) in response to invasion of pathogens (Baum et al., (1999) It is also known that zinc helps in tissue repair and wound healing by stimulating keratinocyte proliferation and migration to the injured area and scavenges proinflammatory cytokines-produced nitric oxide to prevent tissue damage.

Zinc regulates the transcription of genes that is important for cell proliferation and differentiation by modulating transcription factors such as metal response element-binding transcription factor-1, MTF-1 (Langmade et al., 2000): Egr-1 (Adamson et al., 2003); AP-1 and NF- • B (Herbein et al., 2006) Jun and ATF-2. Among these, MTF-1 to date is the best-characterized zinc-activated transcription factors. MTF-1 responds acutely to changes in intracellular zinc concentrations by binding with zinc and translocates to nucleus. It then activates genes transcription by binding to

promoters of other known zinc-responsive genes such as metallothioneins (MTs) and zinc transporter, ZnT-1 that regulate intracellular zinc concentrations (Langmade et al., 2000). Other zinc finger transcription factors such as Egr-1, AP-1 and NF- • B are important for the regulation of genes that control cells proliferation. Deregulation of these transcription factors contributes to cancer cells proliferation, metastasis and angiogenesis. How zinc affects the regulation of these transcription factors and their outcomes, however, varies. It is shown in a number of studies, that zinc at different concentrations activate or suppress the activities of NF- B in different cell lines resulting in different cellular responses (Kim et al., 2007).

2.78 Association of Zinc with Prostate Cancer

The role of zinc in malignant diseases is still unclear but abnormal levels of zinc in serum of cancer patients and malignant tissues are widely reported. In prostate cancer, decreased zinc levels are consistently observed in malignant tissue samples from different populations and at various stages of malignancy. Analysis of malignant prostate tissues showed a 60-70% reduction of zinc levels in comparison to those of the normal peripheral zone tissues. The plasma zinc level between patients with malignancy is also significantly lower than normal patients (Goel & Sankhwar, 2006).

2.79 Epidemiologic Studies

It is reported that dietary zinc supplementation protects against oxidative damage, reduces cancer risk (Ho, 2004) and is beneficial against prostate tumorigenesis (Franklin & Costello, 2007). Findings from several epidemiologic studies, however, attain no consensus on the effectiveness of zinc against prostate cancer, partly because of differences in experimental design, amount of zinc administered and methods in determining plasma/serum zinc status (Haase et al., 2008).

Several studies showed that there are either no beneficiary effects of zinc or there are no potential adverse effects of dietary zinc on prostate cancer risk. In contrast, there are reports which concluded that zinc reduces prostate cancer risk (Kristal et al, 1999 :Epstein et al, 2011). Gonzalez et al. (2009) reported that dietary zinc was not associated with prostate cancer and 10-year average intake of supplemental zinc does not reduce the overall risk of prostate cancer. However, they found that risk of advanced prostate cancer decreases with greater intake of supplemental zinc in their Vitamins And Lifestyle (VITAL) cohort.

2.80 Antioxidants

Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A. and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress is damage to cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation. Oxidative stress seems to play a significant role in many human diseases, including cancers. Antioxidants in pharmacology have been extensively studied, particularly as treatments for stroke and neurodegenerative diseases. For these reasons, oxidative stress can be considered to be both the cause and the consequence of some diseases.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested

that excess supplementation with certain putative antioxidants may be harmful. (Jha et al, 1995, Bjelakovic et al, 2007, Baillie et al 2009). Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

2.81 History of Antioxidants

As part of their adaptation from marine life, terrestrial plants began producing non-marine antioxidants such as ascorbic acid (Vitamin C), polyphenols and tocopherols. The evolution of angiosperm plants between 50 and 200 million years ago resulted in the development of many antioxidant pigments - particularly during the Jurassic period - as chemical defenses against reactive oxygen species that are byproducts of photosynthesis (Benzie, 2003). Originally, the term antioxidant specifically referred to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th centuries, extensive study concentrated on the use of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines (Mattill, 1947).

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A. C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Jacob, 1996, Knight, 1998). The possible mechanisms of action of antioxidants were first explored when it was recognized that a

substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005)

A paradox in metabolism is that, while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Vertuani, 2004) In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Davies, 1995). However, reactive oxygen species also have useful cellular functions, such as redox signaling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, (2006)

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HCIO), and free radicals such as the hydroxyl radical (OH^-) and the superoxide anion (O_2). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules (Valko et al 2007). This species is produced from hydrogen peroxide in metal- catalyzed redox reactions such as the Fenton reaction (Stohs, and Bagchi, 1995). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms (Nakabeppu et al, 2006, Valko

et al, 2007). Damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992).

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (Raha, and Robinson, 2000). In this process, the superoxide anion is produced as a by-nroduct of several steps in the electron transport chain (Lenaz, 2001). Particularly important is the reduction of coenzvme Q in complex III, since a highly reactive free radical is formed as an intermediate. This unstable intermediate can lead to electron "leakage", when electrons jump directly to oxygen and form the superoxide anion, instead of moving through the normal series of well-controlled reactions of the electron transport chain (Finkel, 2000). Peroxide is also produced from the oxidation of reduced flavoproteins. However, although these enzymes can produce oxidants, the relative importance of the electron transfer chain to other processes that generate peroxide is unclear (Imlay, 2003, Seaver and Imlay, 2004). In plants, algae, and cyanobacteria, reactive oxygen species are also produced during photosynthesis (Demmig and Adams 2002). Particularly under conditions of high light intensity (Krieger-Liszkay, 2004). This effect is partly offset by the involvement of carotenoids in photo-inhibition, and in algae and cyanobacteria, by large amount of iodide and selenium, which involves these antioxidants reacting with over-reduced forms of the photosynthetic reaction centres to prevent the production of reactive oxygen species.

2.82 Classification of Antioxidants

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell

membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors (Miller and Britigan, 1997).

The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another (Sies, 1993: Chaudiere and Ferrari,1999) The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes.

2.83 Natural Antioxidants

Naturally occurring antioxidants of high or low molecular weight can differ in their composition, in their physical and chemical properties, in their mechanism and in theirsite of action. They can

be divided into the following categories: (i) Enzymes: Enzyme such as superoxide dismutase (SOD), catalase and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds. Superoxide dismutase which was discovered in late 60s, catalyses the transformation of the superoxide radical into hydrogen peroxide, which can then be transformed by enzyme catalase into water and molecular oxygen. While superoxide anion in itself is not particularly reactive, it can reduce transition metal ions, such as iron and gets converted to most reactive radicals -the hydroxyl radical. Thus, elimination of superoxide radical can attenuate the formation of hydroxyl radical. Glutathione peroxidase (GPx) reduces lipid peroxides (ROOH), formed by the oxidation of polyunsaturated fatty acids (PUFA), to a stable, non toxic molecule - hydroxyl fatty acid (ROH). Together with phosholipase, GPx can also convert phospholipids hydro peroxide (PL-OOH) into phospholipids hydroxide (PL-OH) (Ursini et al., 1987).

(ii) Low molecular weight antioxidants: These are subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly through free radical scavenging property. Lipids soluble antioxidants: These antioxidants tend to accumulate in lipid plasma lipoprotein (eg. LDL); upon supplementation. This group of antioxidants are supposed to act as highly efficient scavengers, such as against lipid peroxylradical, which are formed within the lipoprotein as a consequence of free radical chain reaction of lipid peroxidation.

2.84 Mode of Action of Antioxidants

In general, the antioxidants act by the following routes·

I. Chain breaking reaction eg. α-tocopherol, which act in lipid phase to trap free radicals.

II. By reducing concentration of reactive oxygen species eg. Glutathione.

III. By scavenging initiating radicals e.g superoxide dismutase which act in the lipid phase to trap superoxide free radicals.

IV. By chelating transition metal catalyst: a group of compound which act by sequestration of transition metals that are well established proxidants. In this way transferring, lactoferrin and ferritin function to keep iron induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrants

2.85 Antioxidants and Cancer

Epidemiological evidence (Hennekens, 1994) consistently reveals that a diet low in antioxidants or a low level of antioxidants in the blood can increase cancer risk. Smoking as well as chronic inflammation - two of the principal causes of cancer - have a strong free radical component in their action mechanisms. Oxidative damage has recently been related to various clinical conditions as having a critical role and these conditions include malignant diseases. Reactive oxygen species (ROS) can cause DNA oxidation and damage to proteins, damage to tumor suppressor genes and an increase in proto-oncogenic expression. Cancer shows a pro-oxidative change in the redox state. ROS are potential carcinogens due to the fact that they facilitate mutagenesis in addition to tumor promotion and progression. Even normal cells show an increased proliferation and expression of growth related genes if they are exposed to hydrogen peroxide or superoxide. The majority of ROS-induced mutations seem to envelop guanine,

causing Guanine-Tyrosine transversions. If this is related to critical genes such as oncogenes or tumor suppressor genes it can result in cancer initiation or progression. Chronic prostatic hyperplasia is diagnosed in the majority of men around the age of 40 years. But the late appearance of PCa suggests that a multiplestep process is involved in carcinogenesis and the most reasonable candidates for the endogenous formation of genotoxins in late stages of life are accumulated ROS (Waris and Ahsan, 2006). To protect against ROS toxic effects and to modulate their physiological effects, cells have developed an intrinsic antioxidant defense system. The antioxidant enzymatic system is very complex and is composed of small molecules with great antioxidant weight (vitamins E, C, A,) primary antioxidant enzymes (manganese, copper, zinc superoxide dismutase, catalase, glutathione peroxidase) and secondary antioxidant enzymes (glutathione reductase, glucose-6-phosphate dehydrogenase). DNA repairing proteins and enzymes are considered to be part of the antioxidant system just as metal kidnapping proteins are important in antioxidant cell state modulation. Nitric oxide modulates ROS levels partly by its reaction with the superoxide anion and finally the proteins involved in cellular stress response are also important in oxidative damage modulation. Each component of the antioxidant system is specifically located in subcellular compartments. Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of the superoxide radical to hydrogen peroxide and oxygen which in turn can be eliminated by catalase or glutathione peroxidase. The role of Mn-SOD in carcinogenesis is still uncertain. Superoxide dismutase induction in tumors may be due to stress and genotoxicity induced by oxidant and inflammation exposure. This enzyme has also been observed to be implicated in the resistance of tumor cells to cytotoxic drugs and radiation. Many studies have suggested that the Mn-SOD gene could be a tumor suppressor gene and that

malignant tumors have lower Mn-SOD activity. Nevertheless, many types of tumor cells have been found to contain high levels of Mn-SOD compared with their non-malignant counterpart.

2.86 Pro-oxidant Activities

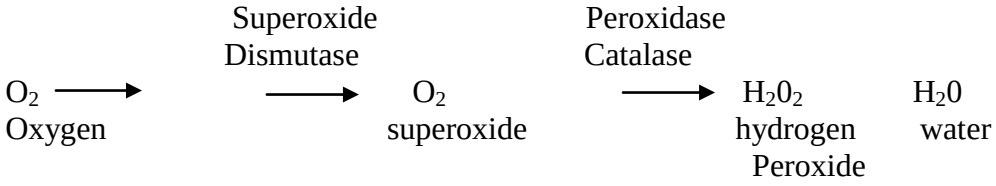
Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide (Duarte and Lunec 2005). However, it will also reduce metal ions that generate free radicals through the Fenton reaction (Carr and Frei, 1999)

$2\ Fe^{3+}$ + Ascorbate —> $2\ Fe^{2+}$ + Dehydroascorbate

$2\ Fe^{2+} + 2\ H_20_2$ -> $2\ Fe^{3+}$ + 2 OH- + 2 OH“

The relative importance of the antioxidant and pro-oxidant activities of antioxidants are an area of current research, but vitamin C, which exerts its effects as a vitamin by oxidizing polypeptides, appears to have a mostly antioxidant action in the human body (Valko et al, 2005) However, less data is available for other dietary antioxidants, such as vitamin E, (Schneider, 2005) or the polyphenols (Halliwell, 2008, Ristow and Zarse, 2010) Likewise, the pathogenesis of diseases involving hyperuricemia likely involve uric acid's direct and indirect pro-oxidant properties. That is, paradoxically, agents which are normally considered antioxidants can act as conditional pro-oxidants and actually increase oxidative stress. Besides ascorbate, medically important conditional pro-oxidants include uric acid and sulfhydryl amino acids such as homocysteine. Typically, this involves some transition-series metal such as copper or iron as catalyst.

Enzyme Systems



Enzymatic pathway for detoxification of reactive oxygen species.

2.87 Overview of the Antioxidants

As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another. Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms (Aruoma, 1994)

2.88 Superoxide Dismutase

Each cell of the body has a personalized system for removing toxic matter. The key cleansing tool used for this process is the cell's network of enzymes and enzyme systems. These enzymes work to break down toxic materials and flush them out of the cell. When there are more toxins than a cell's enzymes can handle, or when there aren't enough enzymes in the first place, cells are at risk to become cancer cells.

One enzyme in particular is a major power player in a cell's detox system. Its name: Superoxide dismutase (SOD). Its mission: Scavenge for and dismantle one of the body's most deadly free radical toxins, superoxide. Superoxide (the enemy) is a reactive particle that bounces around a cell. It damages everything it comes in contact with. This might seem surprising, but the superoxide toxin is actually produced by cells themselves, as a byproduct of their metabolic process to produce energy.

Through a series of enzymatic reactions, cells strip away electrons in order to create energy. During the process, electrons attach to oxygen molecules and thereby create the toxic chemical, superoxide. This process of creating energy creates a handful of other toxic radicals too, like hydrogen peroxide and hydroxyl radicals.

When these toxic radicals are produced, the body's natural supply of cell enzymes like SOD acts to shield the cell and quickly eliminate the toxins before they harm the cells. This is probably recognized as the usual antioxidant-free radical reaction. In addition to breaking down toxins created by cell metabolism, enzymes also tackle toxins that enter the body by way of air pollution, smoking, or other forms of ingestion. Each enzyme serves a specific function by attacking an assigned free radical. Superoxide dismutase, for instance, functions solely to attack superoxide and break it up into hydrogen peroxide and oxygen. Hydrogen peroxide itself is a toxic radical, so another type of enzyme, catalase, is then tasked with decomposing the substance into water and oxygen.

Superoxide Dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. Superoxide dismutase is found in

both the dermis and the epidermis, and is key to the production of healthy fibroblasts (skin-building cells). Superoxide dismutase acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes.

Researchers are currently studying the potential of superoxide dismutase as an anti-aging treatment, since it is now known that SOD levels drop while free radical levels increase as one ages. Superoxide Dismutase helps the body use zinc, copper, and manganese. There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cells' cytoplasm, and Mn SOD protects their mitochondria from free radical damage.

2.89 Oxidative Stress in Disease

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease (Christen, 2000; Nunomura et al, 2006), Parkinson's disease (Wood-Kaczmar et al, 2006) the pathologies caused by diabetes (Giugliano et al 1996, Davì et al, 2005) rheumatoid arthritis (Hitchon and El-Gabalawy, 2004) and neurodegeneration in motor neuron diseases (Cookson and Shaw, 1999). In many of these cases, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage; One case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. Here, low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally cardiovascular disease (Aviram, 2000, Van Gaa et al, 2006)

Oxidative damage in DNA can cause cancer. Several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase etc.

protect DNA from oxidative stress. It has been proposed that polymorphisms in these enzymes are associated with DNA damage and subsequently the individual's risk of cancer susceptibility (Khan et al, 2010)

A low calorie diet extends median and maximum lifespan in many animals. This effect may involve a reduction in oxidative stress (López-Lluch et al, 2006). While there is some evidence to support the role of oxidative stress in aging in model organisms such as Drosophila melanosaster and Caenorhabditis eleeans (Larsen, 1993, Helfand and Rogina 2003), the evidence in mammals is less clear (Sohal et al, 2002: Rattan, 2006). Indeed, a 2009 review of experiments in mice concluded that almost all manipulations of antioxidant systems had no effect on aging (Pérez et al, 2009). Diets high in fruit and vegetables, which are high in antioxidants, promote health and reduce the effects of aging; antioxidant vitamin supplementation has no detectable effect on the aging process, so the effects of fruit and vegetables may be unrelated to their antioxidant contents (Ward, 1998, Thomas, 2004) One reason for this might be the fact that consuming antioxidant molecules such as polyphenols and vitamin E will produce changes in other parts of metabolism, and it may be these other effects that are the real reason these compounds are important in human nutrition (Aggarwal and Shishodia, 2006).

2.90 Potential Health Effects

Organ Function

The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids, the target of lipid peroxidation. Consequently, antioxidants are commonly used as medications to treat various forms of brain injury. Here, superoxide

dismutase mimetics, sodium thiopental and propolol are used to treat reperfusion injury and traumatic brain injury, while the experimental drugs disufenton sodium and ebselen are being applied in the treatment of stroke. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, and as a way to prevent noise-induced hearing loss. Targeted antioxidants may lead to better medicinal effects. Mitochondria-targeted ubiquinone, for example, may prevent damage to the liver caused by excessive alcohol.

People who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases, and there is evidence that some types of vegetables, and fruits in general, may lower risk against some cancers. Since fruits and vegetables happen to be good sources of nutrients and phytochemicals, this suggested that antioxidant compounds might lower risk against several diseases. This idea has been tested in a limited manner in clinical trials and does not seem to be true, as antioxidant supplements have no clear effect on the risk of chronic diseases such as cancer and heart disease. This suggests that these health benefits come from other substances in fruits and vegetables (possibly dietary fiber) or come from a complex mix of compounds. For example, the antioxidant effect of flavonoid-rich foods seems to be due to fructose-induced increases in the synthesis of the antioxidant uric acid and not to dietary antioxidants per se.

It is thought that oxidation of low density lipoprotein in the blood contributes to heart disease, and initial observational studies found that people taking Vitamin E supplements had a lower risk of developing heart disease. Consequently, at least seven large clinical trials were conducted to test the effects of antioxidant supplement with Vitamin E, in doses ranging from 50 to 600 mg per day. None of these trials found a statistically significant effect

of Vitamin E on overall number of deaths or on deaths due to heart disease. Further studies have also been negative. It is not clear if the doses used in these trials or in most dietary supplements are capable of producing any significant decrease in oxidative stress. Overall, despite the clear role of oxidative stress in cardiovascular disease, controlled studies using antioxidant vitamins have observed no reduction in either the risk of developing heart disease, or the rate of progression of existing disease.

Several trials have investigated supplements with high doses of antioxidants, the study tested the effect of supplementation with doses comparable to those in a healthy diet. Over 12,500 French men and women took either low-dose antioxidants (120 mg of ascorbic acid, 30 mg of vitamin E, 6 mg of beta carotene, 100 µg of selenium, and 20 mg of zinc) or placebo pills for an average of 7.5 years. The study concluded that low-dose antioxidant supplementation lowered total cancer incidence and all-cause mortality in men but not in women. Supplementation may be effective in men only because of their lower baseline status of certain antioxidants, especially beta carotene.

Although some levels of antioxidant vitamins and minerals in the diet are required for good health, there is considerable doubt as to whether antioxidant supplements are beneficial or harmful; and if they are actually beneficial, which antioxidant(s) are needed and in what amount. Indeed, some authors argue that the hypothesis that antioxidants could prevent chronic diseases has now been disproved and that the idea was misguided from the beginning. Rather, dietary polyphenols may have non-antioxidant roles in minute concentrations that affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity or gene regulation.

For overall life expectancy, it has even been suggested that moderate levels of oxidative stress may increase lifespan in the worm Caenorhabditis elegans, by inducing a protective

response to increased levels of reactive oxygen species. The suggestion that increased life expectancy comes from increased oxidative stress conflicts with results seen in the yeast Saccharomyces cerevisiae and the situation in mammals is even less clear. Nevertheless antioxidant supplements do not appear to increase life expectancy in humans.

2.91 Physical Exercise

During exercise, oxygen consumption can increase by a factor of more than 10. This leads to a large increase in the production of oxidants and results in damage that contributes to muscular fatigue during and after exercise. The inflammatory response that occurs after strenuous exercise is also associated with oxidative stress, especially in the 24 hours after an exercise session. The immune system response to the damage done by exercise peaks 2 to 7 days after exercise, which is the period during which most of the adaptation that leads to greater fitness occurs. During this process, free radicals are produced by neutrophils to remove damaged tissue. As a result, excessive antioxidant levels may inhibit recovery and adaptation mechanisms. Antioxidant supplements may also prevent any of the health gains that normally come from exercise, such as increased insulin sensitivity.

The evidence for benefits from antioxidant supplementation in vigorous exercise is mixed. There is strong evidence that one of the adaptations resulting from exercise is a strengthening of the body's antioxidant defenses, particularly the glutathione system, to regulate the increased oxidative stress. This effect may be to some extent protective against diseases which are associated with oxidative stress, which would provide a partial explanation for the lower incidence of major diseases and better health of those who undertake regular exercise.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Subject Recruitment

A total of one hundred and twenty patients, aged between 50 years and above (50-99years) were recruited for this study. They were divided into two groups. Sixty patients had prostate cancer (group A), sixty had benign prostate hyperplasia (group B) and sixty apparently heathy volenteers of the same age served as control subjects (group C). The patients with prostate cancer were futher reclassified as follows: 1. using the grade of the tumour, 2. using the Gleason score and 3. metastasis and non metastasis. The patients were attending Urology clinic in Nnamdi Azikiwe University Teaching Hospital, Nnewi (NAUTH) and 60 control subjects of the same age. All subjects were properly medically examined by the consultant Urologist. A structured questionnaire was administered to all participants which helped furnish us with the information concerning their age, sex, other biodata.

3.2 Inclusion and Exclusion Criteria

It was only patients suffering from prostrate disorders as confirmed by the Consultant Urologist who were recruited to this study. Patients with prostrate disorders on androgen deprivation therapy were excluded in the study.

3.3 Calculation of Sample Size (Naing et al, 2006).

The formula is given as:

$$N = \frac{Z^2 pq}{d^2}$$

Where q = 1-p, N = Minimum sample siz, Z = Confidence interval of 95% equivalent to confidence coefficient of 1.96, P = prevalence rate in percentage (2%), Desired level of precision or significance = 0.05

$$N = \frac{1.96^2 \times p\,(1-p)}{0.05^2}$$

$$N = \frac{3.84 \times 0.02\,(1-0.02)}{0.05^2}$$

$$N = \frac{0.165\,(0.957)}{0.0025}$$

$$N = \frac{0.0768\,(0.98)}{0.0025}$$

$$N = 31$$

3.3 Sample Collection

About 10 mls of blood sample was collected from the patients and controls through venepuncture into plane test tubes and was allowed to clot at room temperature. Serum was obtained after centrifugation of the blood samples at 4000rpm and stored in aliquots at -20^0C until used for the estimation of biochemical parameters.

3.4 Quality Control Measures

Quality control sera/sample were analyzed along with the test samples in each batch. Standard deviation and coefficient of variation were calculated.

3.5 Biochemical Analyses

The laboratory analyses were carried out in the Chemical Pathology Department laboratory, Nnamdi Azikiwe University Teaching Hospital and Springboard Research laboratory Awka,

Anambra state. Prostate specific antigen, Free prostate specific antigen, Sarcosine, engrailed-2 gen proteins (EN2), 5-alpha reductase, total antioxidant capacity, Superoxide dismutase (SOD) and creatinine were determined in the Department of chemical pathology laboratory, Nnamdi Azikiwe University Hospital, Nnewi. Magnesium (Mg) and Zinc (Zn) levels were determined in the Biotechnology Unit of Nnamdi Azikiwe University, Awka, while the micronutrients selenium (Se), Vitamins C, D and E were determined in Springboard laboratory Awka.

3.6 Ethical Approval

Research design was submitted to the Ethical Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH) for ethical clearance and approval which was obtained for this study. The copy of the approval is attached in appendix (NAUTH/CS/66/VOL4/009)

3.7 Informed Consent

The consent of all the participants were duely obtained through the form that was administered to all the participants. Every information from the subjects was treated with strict confidence. No monetary inducement was demanded from or paid to the participants. The informed consent form is attached.

3.8 Assay Methods

3.9 Method of Assay for Prostate Specific Antigen (PSA) (Stowel et al, 1991) (Cat No BC-1019)

3.10 Principle of the Test

The PSA ELISA method is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay system utilizes a goat anti-PSA antibody directed against PSA for solid phase Immobilization (on the micro titer wells). A monoclonal anti-PSA antibody

conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized goat antibody at room temperature for 1 hour. The wells are washed to remove any unbound or excess antigen. The monoclonal anti-PSA-HRP conjugate is then added and allowed to react with the immobilized antigen for 1 hour at room temperature. The wells are washed with water to remove unbound-labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes resulting to the formation of a blue colour. The colour development is stopped with the addition of stop solution changing the colour to yellow. The concentration of PSA is directly proportional to the color intensity of the test sample which is measured spectrophotometrically at 450nm.

3.11 Assay Procedure

All reagents were brought to room temperature before use.

The desired numbers of coated wells were determined in the holder and 50 µl of standards, samples and controls were dispensed into the appropriate wells, then 50µl of zero buffer was added to the wells and thoroughly mixed for 30 seconds and this was incubated at room temperature for 1 hour. After the incubation the mixture was emptied into a waste container and the microplate rinsed 5 times with distilled water. Afterwards the wells was well drained with an absorbent paper to remove all residual water droplets. 100µl of enzyme conjugate was added to each well and gently mixed. The plate was incubated at room temperature for 1 hour after which the the incubation mixture was emptied into a waste container and the microtiter was washed 5 times.The absorbent paper was used to remove the residual water droplets and 100µl of TMB reagent was added to each well and gently mixed and incubated at room temperature for 20

minutes. Finally 100µl of stop solution was added to each well and the plate read with plate reader at 450nm.

3.12 Assay Method for Free PSA (Catalona, 1995) (Cat. No. BC-1021)

3.13 Principle of the Test

The f-PSA ELISA method is a solid phase two site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and a goat anti-PSA antibody labelled with horseradish peroxidase is used as a tracer. The f-PSA molecules present in the sample are sandwiched between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is the assayed colourimetrically using a microplate reader at 450nm.

3.14 Assay Procedure Free PSA

All reagents were brought to room temperature before use.

The desired numbers of coated wells were determined in the holder and 50 µl of standards, samples and controls were dispensed into the appropriate wells, then 100µl of zero buffer was added to the wells, gently mixed for 30 seconds and this was incubated at room temperature for 1 hour. After the incubation the mixture was emptied into a waste container and the micro titer wells rinsed for 5 times with distilled water. Afterwards the wells were well drained with an absorbent paper to remove all residual water droplets. 200µl of enzyme conjugate was added to each well and gently mixed. The plate was incubated at room temperature for 1 hour after which the incubation mixture was emptied into a waste container and the microtiter wells were washed 5 times. The absorbent paper was also used to remove the residual water droplets and 100µl of

TMB reagent was added to each well and gently mixed and incubated at room temperature for 20 minutes. Finally 100µl of stop solution was added to each well and the plate was read with plate reader at 450nm.

3.15 Determination of Engrailed Homeobox Protein 2 (EN2) (life science Inc, China)(sE98982Hu

3.16 Principle of the Test

The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to EN2. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for EN2. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain EN2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of EN2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

3.17 Reagent Preparation

The kit components were brought to room temperature (18-25oC) before use. Standard was reconstituted with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature and mixed gently (no foams). The concentration of the standard in the stock solution is 20ng/mL.

Seven tubes were prepared containing 0.5mL Standard Diluent which was used to produce a double dilution series according to the concentration shown below. Each tube was mixed thoroughly before the next transfer. After Setting up 7 points of diluted standard such as

20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, and the last (8^{th}) tubes with Standard Diluent is the blank as 0ng/mL.

Assay Diluent A and Assay Diluent B were diluted with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B.

Detection Reagent A and Detection Reagent B were briefly centrifuged before use and diluted to the working concentration with working Assay Diluent A or B, respectively (1:100)

Wash Solution: 20ml of wash solution concentrate was diluted with 580mL of distilled water to prepare 600 mL of Wash Solution.TMB substrate: Ready to use. The needed volume of the solution was aspirated with sterilized tips.

Note:

1. Making serial dilution in the wells directly is not recommended.
2. Standard was prepared within 15 minutes before assay
3. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once
4. Contaminated water or container for reagent preparation will influence the detection result.

3.18 Assay Procedure for Engrailed 2 gene

The wells for diluted standard (7 wells), blank and sample was determined, and 100µL each of dilutions of standard (see Reagent Preparation), blank and samples into the appropriate wells. The wells were covered with the plate sealer and incubated for 2 hours at 37oC.The liquid of each well was carefully removed, (not washed). Then 100µL of detection reagent A working solution was added to each well and incubated for 1 hour at 37oC after covering it with the Plate sealer. The solution was aspirated and the wells washed with 350µL of wash solution (3 times)

using a wash bottle or multi-channel pipette, and the remaining liquid from all wells was completely removed by snapping the plate onto absorbent paper. Then 100µL of detection Reagent B working solution was added to each well and incubated for 30 minutes at 37oC after covering it with the plate sealer. The aspiration/wash process was repeated as described above 5 times, after which 90µL of Substrate Solution was added to each well and covered with a new plate sealer and incubated for 15 - 25 minutes at 37oC. The liquid turned blue by the addition of substrate solution. Finally 50µL of stop solution was added to each well and the liquid mixed properly by tapping the side of the plate. Then the absorbance/concentration was measured immediately using micro plate reader at 450nm.

3.19 Assay Method for 5-Alpha Reductase (5ARD2) (life science Inc, China)(sE81285Hu)

3.20 Principle of the Test

The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to $5ARD_2$. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for $5ARD_2$. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain $5ARD_2$, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured Spectrophotometrically at wavelength of 450nm. The concentration of $5ARD_2$ in the samples is then determined by comparing the O.D. of the samples to the standard curve.

3.21 Reagent Preparation for 5-Alpha Reductase

The kit components were brought to room temperature (18-25oC) before use. Standard was reconstituted with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature and mixed gently (without foaming). The concentration of the standard in the stock solution is 20ng/mL.

Seven tubes were prepared containing 0.5mL Standard Diluent which was used to produce a double dilution series according to the concentration shown below. Each tube was mixed thoroughly before the next transfer. After Setting up 7 points of diluted standard such as 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, and the last (8^{th}) tube with Standard Diluent is the blank as 0ng/mL.

Assay Diluent A and Assay Diluent B were diluted with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B.

Detection Reagent A and Detection Reagent B were briefly centrifuged before use and diluted to the working concentration with working Assay Diluent A or B, respectively (1:100)

Wash Solution: 20ml of wash solution concentrate was diluted with 580mL of distilled water to prepare 600 mL of Wash Solution. TMB substrate: Ready to use. The needed volume of the solution was aspirated with sterilized tips.

3.22 Assay Procedure for 5-alpha reductase

The wells for diluted standard (7 wells), blank, sample and controls were determined, and 100µL each of dilutions of standard (see reagent preparation), blank and samples into the appropriate wells. The wells were covered with the plate sealer and incubated for 2 hours at 37oC.The liquid of each well was carefully removed, without washing. Then 100µL of detection reagent A

working solution was added to each well and incubated for 1 hour at 37oC after covering it with the Plate sealer. The solution was aspirated and the wells washed with 350µL of wash solution (3 times) using a wash bottle or multi-channel pipette, and the remaining liquid from all wells was completely removed by snapping the plate onto absorbent paper. Then 100µL of detection Reagent B working solution was added to each well and incubated for 30 minutes at 37oC after covering it with the plate sealer. The aspiration/wash process was repeated as described above 5 times, after which 90µL of Substrate Solution was added to each well and covered with a new plate sealer and incubated for 15 - 25 minutes at 37oC. The liquid turned blue by the addition of substrate solution. Finally 50µL of stop solution was added to each well which turned the liquid yellow by the addition of Stop solution and the liquid mixed properly by tapping the side of the plate. Then the absorbance/concentration was measured immediately using micro plate reader at 450nm.

3.23 Total Antioxidant Capacity (Biovision USA) (K274-100)

3.24 Principle of the Test

The TAC assay kit (Biovision) measures either the combination of both small molecule antioxidants and proteins or small molecule alone in the presence of proprietary protein mask. The Cu^{2+} ion is reduced to Cu^{+} by both small molecules and proteins. The reduced Cu^{+} ion is chelated with a diluent (colourimetric probe) to produce a deep blue colour which was read at 570nm.

Kit Contents:

Cu2+ Reagent	0.2 ml	K274-100-1
Assay Diluent	10 ml	K274-100-2
Protein Mask	10 ml	K274-100-3
Trolox Standard	(1 μmol)	K274-100-4

3.25 Reconstitution of Reagents:

Cu^{2+} Reagent, Assay Diluent, Protein Mask: Ready to use as supplied and were kept at room temperature as stipulated on the vial. Trolox Standard lyophilized was dissolved in 20 μl of pure dimethyl sulfoxide (DMSO) by vertxing, then 980 μl of distilled water was added and mixed well, generating a 1 mmol solution. Following reconstitution, aliquots were stored frozen. The reconstituted standard is stable for 4 months when stored frozen.

3.26 Reagent Preparation for Total Antioxidant Capacity (TAC)

Trolox standard curve: The standard curve was prepared by adding 0, 4, 8, 12, 16, 20 μl of the Trolox standard to individual wells and then the total volume was adjusted to 100 μl with distilled H2O to give 0, 4, 8, 12, 16, 20 nmol of Trolox standard.

Preparation of working solutions: One part Cu^{2+}reagent was diluted with 49 parts 0f assay diluent. Enough working solution was diluted for the number of assays. Each well requires 100 μl of Cu^{2+} working solution.

3.27 Assay Procedure

20μl of samples, standard and controls were added to the wells and then 100 μl Cu^{2+} working solution was added to all standard, sample and control wells. The plate was covered and

incubated at room temperature for 1.5 hours. The absorbance read at 570 nm using the plate reader. The standard curve was plotted which was used to extrapolate the

Concentration of the tests.

3.28 Test Method for Superoxide Dismutase (SOD) (Biovision kit USA)(K335-100)

3.29 Principle of the Test

The sensitive SOD assay kit utilizes WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by a colorimetric method at 450 nm.

Kit Contents:

WST Solution	1 ml	Red	K335-100-1
SOD Enzyme Solution	20µl	Green	K335-100-2
SOD Assay Buffer	20 ml		K335-100-3
SOD Dilution Buffer	10 ml		K335-100-4

3.30 Reagent Preparation and Storage Conditions

WST Working Solution: 1 ml of WST solution was diluted with 19 ml of assay buffer solution. The diluted solution is stable for up to 2 months at 4°C. Enzyme Working Solution was Centrifuged for 5 seconds and mixed well by pipetting (The step is necessary, as the enzyme has two layers and must be mixed well before dilution). Then 15µl of enzyme working solution was diluted with 2.5 ml of dilution buffer. The diluted enzyme solution is stable for up to 3 weeks at 4°C

3.31 Superoxide Dismutase Assay Procedure

20µl of Sample and blank 2 were added to the appropriate wells and 20µl of distilled water was added to each blank 1 and blank 3 wells. Then 200µl of the WST working solution was added to each well after which 20µl of dilution buffer were added to each Blank 2 and Blank 3 well. Finally 20µl of enzyme working solution was added to each sample and Blank 1 well, the wells were mixed thoroughly and incubated plates at 37°C for 20 minutes. The absorbance was read at 450 nm using a microplate reader.

3.32 Test Method for Sarcosine (Biovision kit USA)(K636-100)

Sarcosine was measured using colourimetric method.

3.33 Principle of the Test

The assay Kit (Biovision) provides an accurate, convenient measure of sarcosine in variety biological samples. In the assay, sarcosine is specifically oxidized to generate a product that converts a colorless probe to a product with intense red color which is measured colourimetrically at 570 nm.

Kit Contents:

Sarcosine assay buffer	25ml		K-636-1
Sarcosine Probe in DMSO)	Lyophilized		K-636-2
Sarcosine Enzyme mix	Lyophilized	Green	K-636-3
Sarcosine Standard (10 µmol)	Lyophilized	Yellow	K-636-4

3.34 Reagent Preparation and Storage Conditions

Sarcosine assay buffer**:** This is ready to use as supplied. It was stored at 4°C or frozen. as indiceted on the vial. Sarcosine probe was briefly warmed at 37°C for 1-2 min to dissolve, mixed well and Stored frozen. This reagent is stable for at least 2 months. Enzyme mix was **r**econstituted with 220 µl of sarcosine assay buffer, aliquoted and stored until needed. Freezing/thawing was strictly avoided as was instructed. Sarcosine standard was reconstituted with 100 µl of distilled water and stored frozen, stable for 2 months.

3.35 Assay Procedure

The Standard was prepared by mixing 10 µl of the reconstituted sarcosine standard with 990 µl of assay buffer to generate the standard working solution. Then 0, 2, 4, 6, 8, 10 µl of the working standard solution were added to 6 consecutive wells to bring the volume to 50 µl each well with assay buffer. Enough reaction Mix was prepared for the standards and samples as follows: For each assay, 46µl assay buffer, 2µl enzyme and 2µl of probe were added together and mixed well. Then 50 µl of the reaction mix was added to each standard, sample and control wells and mixed thoroughly and incubated at 37°C for 1 hr. The plate was read with a plate reader at 570 nm,

3.36 Test Method for determination of Vitamins C, D and E (Wenzl, 2006)

Instrument: Buck 930 gas chromatograph equipped with an on – column automatic injector, Mass spectroscopy, HP 88 capillary column (100m x .25um film thickness,) CA, USA.

3.37 Principle of analyses

The principle of measuring vitamins C, D and E using gas chromatography involves separation of components of the sample under test due to partition in between

gaseous mobile phase and stationary liquid phase. Gas chromatography runs on the principle of partition chromatography for separation of components. In terms of stationary and mobile phases it is categorized under gas-liquid type of chromatography i.e stationary phase is a liquid layer supported over a stationary phase while the mobile phase is an inert and stable gas. Hence the perfect name as Gas Liquid chromatography (GLC). The gas is set to flow at a constant rate from the cylinder on to the liquid layer impregnated on solid support in a column. The sample is injected into the injection point and is carried by the mobile gas into the column. Inside the column, the components get separated by differential partition in between the mobile phase gas and stationary phase liquid. The component that partitioned into gas comes out of the column first and is detected by detector. The one partitioned into liquid phase comes out later and is also detected through a detector that produces an electrical signal which is translated to concentration.

3.38 Sample Preparation for Vitamin Determination Using Gas Chromatography

200ul of plasma working solution were prepared in 20ml ethanol. To determine the concentration of vitamin in human plasma, an aliquot of the plasma sample was deproteinized with ethanol. Vitamin C, D and E was extracted with a mixture of 6ml hexane and 2ml dichloromethane. Gas chromatography separation was performed using a Hp 5 captllary column. Helium was used as a carrier gas at a flow rate of 2ml/min.

Fixed Setting: Generally the operator must adjust gas flows to the coloumns, the inlets, the detectors, and the split ratio. In addition, the injector and detector temperatures must be set. The detectors are generally held at the high end of the oven temperature range to minimize the risk of analyte precipitation. All of these parameters should have been set to the correct values, but double check all the parameters to ensure that the values are correct.

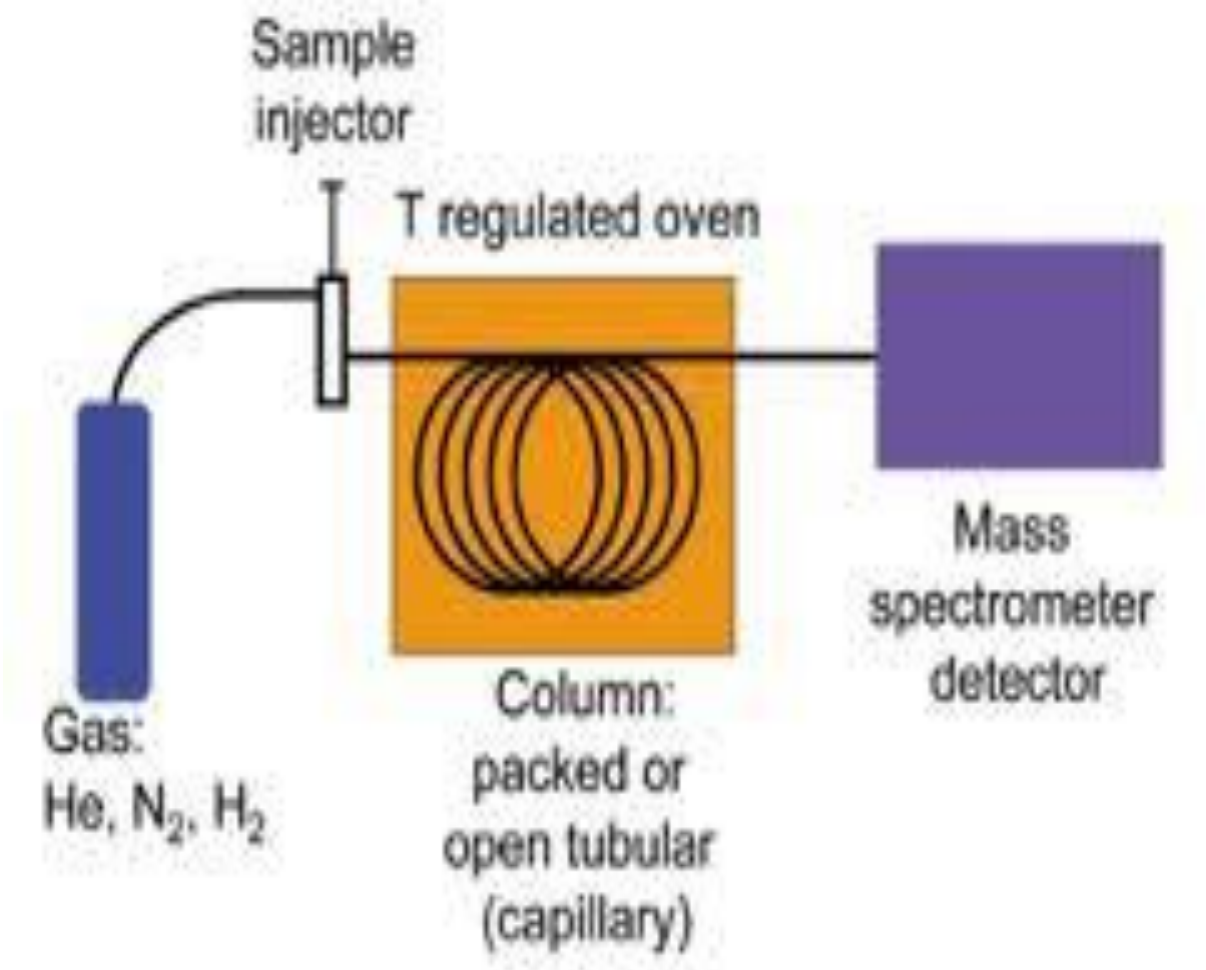


Fig 3.1 Gas Chromatography/mass spectroscopy

Alon and Amirav, 2006

3.39 Assay Procedure for Magnesium (Mg) (Abarca et al, 2001), Zinc (Zn) (Smith et al, 1997) and Selenium (Se)(Tsalev et al, 2001)

3.40 Working Principle of the Atomic absorption spectrophotometer.

The principle is based on the sample being aspirated into the flame. The element is atomized and when AASs light beam is directed through into the monochromator and unto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelengths, a lamp source composed of that element is used making the method relatively free from spectral or radiational interfereces. The amount of energy of the characteristic wavelength in the flame is proportional to the concentration of the element.

Material

FS 240 Varian Atomic absorption spectrophotometer. (USA).

3.41 Reagent Preparation

The Mg, Zn, Se working standards were prepared from the stock (1000ppm)

100ppm of Mg, Zn and Se were first prepared in 100ml of distilled water from the stock using the formula below:

$C_1V_1 = C_2V_2$

Where

C_1 = concentration of the working solution.

V_1 = Volume of distilled water to prepare the working solution.

C_2 = concentration of Mg, Zn and Se Stock (1000ppm)

V_2 = volume of stock that is diluted.

$\frac{100ppm \times 100ml}{1000ppm} = V_2$

V_2 = 10ml of stock solution.

Thereafter, 10ppm 0f Mg, Zn and se were prepared from 100ppm working solution by adding 10ml of working solution to 90ml of distilled water.

3.41 Sample Preparation

The sample was prepared using 1:4 dilution which was used for the analysis.

3.42 Assay Procedure

The machine was switched on and allowed to run for 2 minutes. The standard / sample were aspirated into the oxidizing acetylene-nitrous oxide flame. The concentration was read from the system.

3.43 Method of Assay for Creatinine (Vratislav, 2008)

3.44 Principle of the Test

Craetinine in alkaline solution reacts with picric acid to form a brownish-yellow. The colour intensity is directly proportional to the creatinine concentration measured at 510nm

3.45 Reagent Supplied and the Preparation

The working solution was prepared by mixing equal volumes of R1a and R1b. this solution is stable when stored for 3 days at 15 to 25^{oc}

3.46 Procedure

0.5ml of the working solution was added to the test tubes prepared for the tests. Then 0.1 ml of the standard was added to the tubes and mixed very well and read using the chemistry analyzer.

This process also calibrates the machine. The above procedure was repeated for all the samples and control to get their direct concentration. After running 10 to 15 samples, the standard was also run again to recalibrate the machine.

CHAPTER FOUR

4.0 RESULTS

The results obtained from the study are represented in the tables and figures below. The age, height, weight, body mass index and the blood pressure of test groups and control are shown in table 4.1. The mean value of age of control was 70±9.0 years while that of the study group with benign prostate (BPH) and prostate cancer were 73±9.0 and 70±9.0 years respectively. There was no significant difference in the mean age of the three groups studied. The mean values of the height of the test group were 1.67±0.55m and 1.66±0.56m for BPH and Pca respectively while that of the control was 1.67±0.07m. There was also no significant difference in the mean values of the height. The mean value of weight for the control was 73.47±10.3Kg while that of the BPH and Pca were 75.63±11.4 Kg and 80.07±11.3 Kg respectively. The analysis of variance showed that there is significant difference in the mean weight of the three groups. The mean values of body mass index for BPH and Pca were 26.59±3.9 and 29.43±4.5Kg/m^2 respectively while the value for control was 26.10±2.7Kg/m^2. There was also significant difference between Pca and control. The mean values of systolic and diastolic blood pressure were 133.6±15.8 mmHg and 84.6±7.4mmHg respectively for control while those of the test groups were 133.0±13.1 and 85.7±8.4 mmHg systolic and diastolic for BPH, 132.8±15.3 mmHg and 81.8±8.5 mmHg systolic and diastolic for Pca. There were no significant difference in the mean value of both diastolic and systolic blood pressure.

Table 4.1: Mean (±SD), p-value, F-value of age, height, body weight and blood pressure measurements in the all groups.

Group	Age (Yr)	Height (m)	Weight (Kg)	BMI (Kg/m^2)	DBP (mmHg)	SBP (mmHg)
Pca (A)	70±9.0	1.66±0.56	80.07±11.3	29.43±4.5	81.8±8.5	132.8±15.3
BPH (B)	73±9.0	1.67±0.55	75.63±11.4	26.59±3.9	85.7±8.4	133.0±13.1
CONT (C)	70±9.0	1.67±0.07	73.43±10.3	26.10±2.7	84.6±7.4	133.6±15.8
F-Value	1.61	0.491	5.522	14.381	3.67	0.053
P-value	0.202	0.613	0.005*	0.0001*	0.027	0.948
POST HOC						
A vs B	0.150	0.396	0.030*	0.0001*	0.010*	0.948
A vs C	0.839	0.388	0.0001*	0.0001*	0.058	0.758
B vs C	0.101	0.989	0.286	0.469	0.476	0.806

N = 60 for each group

BPH = benign prostate hyperplasia

Pca = Prostate cancer

CONT = Control

$P<0.05$

* Shows significant value

The values of total prostate specific antigen, free prostate specific antigen, percentage free prostate specific antigen and creatinine of prostate cancer and BPH and the control group are in table 4.2. There was a statistical difference in the mean values of total prostate specific antigen (F = 92.26), FPSA (F = 40.0), %FPSA (F = 19.6) and creatinine (F = 12.14) among three groups studied. The mean values of TPSA, FPSA, %FPSA were found to be 5.24±4.97, 0.84±0.98, 17.01±14.78 for controls respectively, while, the values of TPSA, FPSA and %FPSA were 42.80±49.15, 2.99±5.21, 7.51±4.66 respectively for BPH. The values for Pca were 104±49.05, 9.41±7.83 and 8.20±4.65 for TPSA, FPSA and %FPSA respectively. There was a significant difference in the mean value of both TPSA, FPSA between control and BPH, between control and Pca. A significant difference was also observed in the mean values of TPSA and FPSA between BPH and Pca. The plasma concentration of creatinine was found to be 82.18±77.78 µmol/L for controls which was sinificantly lower than that of the test groups with the values of 125.10±66.4 µmol/L and 152.11±89.06µmol/l for BPH and Pca respectively.

Table 4.2: levels 0f TPSA, FPSA, %FPSA and creatinine in Prostate cancer, BPH and controls.

Group	TPSA (ng/ml)	FPSA (ng/m)	%FPSA	Cr (µmol/L)
Pca (A)	104±49.05	9.41±7.83	8.20±4.65	152.11±89.06
BPH (B)	42.80±49.15	2.99±5.21	7.51±4.66	125.10±66.43
CONT (C)	5.24±4.97	0.84±0.98	17.01±14.78	82.18±77.78
F-value	92.652	40.00	19.253	12.149
P-value	0.0001*	0.0001*	0.0001*	0.0001*
POST HOC				
A vs B	0.0001*	0.0001*	0.685	0.062
A vs C	0.0001*	0.0001*	0.0001*	0.0001*
B vs C	0.0001*	0.033*	0.0001*	0.003*

N= 60 for each group

BPH = benign prostate hyperplasia

Pca = Prostate cancer

CONT = Control

P<0.05

* Shows significant value

The mean concentrations of sarcosine, total antioxidant capacity, engrailed -2-gene protein, and 5-alpha reductase as contained in table 4.3 showed that the mean value of sarcosine was 3.0±0.52 nmol/l for control which was significantly lower than that of Pca group with the value of 5.36±1.6nmol/l ($P<0.050$). The value of sarcosine for BPH was 3.09±0.34nmol/l, there was no significant difference between the control group and BPH. The concentration of sarcosine in BPH was also significantly lower ($P<0.05$) than the Pca group. The mean level of TAC was found to be 15.23±7.3 nmol/l for the control group which was significantly higher ($P<0.05$) than the test groups with the values of 7.40±2.60nmol/l and 7.2±2.2nmol/l respectively for BPH and Pca. In contrast,s no significant difference was observed between BPH and Pca. The values of EN 2 was found to be 5.51±2.33ng/ml for controls and 5.63±2.92, 6.50±3.86ng/ml for BPH and Pca respectively.There was an increase in the mean value of EN2 in the Pca group which was not significantly difference when compared with the control ($P>0.05$). The value of 5-alpha reductase was 3.7±1.02ng/ml in control group which significantly lower ($P<0.05$) than in the test groups with mean values of 6.55±1.9 for BPH and 7.10±7.31ng/ml for Pca respectively. In cotrast there was no significant difference in the mean value of 5-alpha reductase of subjects with BPH and Pca.

Table 4.3: Concentration of Sarcosine, TAC, EN2 and 5-ARD in prostate cancer, BPH and contols.

Group	Sarcosine (nmol/l)	TAC (nmol/ml)	EN2 (ng/ml)	5-ARD (ng/ml)
Pca (A)	5.36±1.60	7.2±2.2	6.50±3.86	7.10±1.31
BPH (B)	3.09±0.34	7.40±2.60	5.63±2.92	6.55±1.90
CONT (C)	3.0±0.52	15.23±7.3	5.51±2.33	3.7±1.02
F-value	96.117	57.705	1.738	5.656
P-value	0.0001*	0.0001*	0.179	0.004*
POST HOC				
A vs B	0.0001*	0.885	0.136	0.608
A vs C	0.0001*	0.0001*	0.09	0.002*
B vs C	0.267	0.0001*	0.834	0.010*

N= 60 for each group

BPH = benign prostate hyperplasia

Pca = Prostate cancer

CONT = Control

$P<0.05$

* Shows significant value.

Table 4.4 below shows the mean concentrations of seleniun, zinc, and magnesium. The serum level of selenium was 38.63±18.45mg/dl for control group which was significantly higher than test groups with mean values of 27.53±9.49 and 20.81±8.80 mg/dl for BPH and Pca respectively. The mean values of zinc were found to be significantly higher in controls (58.17±27.81 mg/dl) than the test groups which were 20.01±13.28 and 18.99±18.29mg/dl for BPH and Pca respectively. The mean values of magnesium were 5.17±1.06, 3.18±0.22 and 1.89±1.79mg/dl for controls, BPH and Pca respectively. The mean value was significantly higher in controls than both BPH and Pca. Meanwhile the mean value was also significantly lower in Pca than BPH

Table 4.4: Levels of selenium, zinc and magnesium in subjects with Prostate cancer, BPH and controls

Group	Se (mg/dl)	Zn (mg/dl)	Mg (mg/dl)
Pca (A)	20.81±8.80	18.99±18.29	1.89±1.79
BPH (B)	27.53±9.49	20.01±13.28	3.18±0.22
CONT (C)	38.63±18.45	48.17±27.81	5.17±1.06
F-value	6.792	42.182	25.77
P-value	0.001*	0.0001*	0.0001*
POST HOC			
A vs B	0.071	0.834	0.004*
A vs C	0.0001*	0.0001*	0.001*
B vs C	0.024*	0.0001*	0.002*

N= 60 for each group

BPH = benign prostate hyperplasia

Pca = Prostate cancer

CONT = Control

$P<0.05$

* Shows significant value

The levels of vitamins C, D, E and SOD are shown in table 4.5. The mean concentration of vitamin C was 6.94±4.5mg/dl for control while the mean concentrations were 5.84±6.65 and 5.18±8.93 mg/dl for BPH and prostate cancer subjects respectively. There was no significant ($p > 0.05$) different between the three groups studied. The concentration of vitamin D for control was 23.33±19.75mg/dl which was significantly higher than the mean concentration of the BPH and prostate cancer patients which were 10.91±13.49 and 8.82±14.66mg/dl. The concentration of vitamin E was found to be 10.71±0.44 for control which was also significantly ($p<0.05$) higher than that of the BPH and prostate cancer with the values 6.17g/dl±7.87 and 6.07g/dl±0.48 respectively. The activity of SOD was 1.41±0.63 IU/l for Prostate cancer subjects which was significantly ($p<0.05$) lower than tha of BPH and control which were 2.18±1.1 and 2.11±0.96 respectivrly. There was no significant different between BPH and control.

Table 4.5: The mean concentration of Vitamin C, D , E and SOD

Group	Vit C (Mg/dl)	Vit D (mg/dl)	Vit E (mg/dl)	SOD (IU/l)
Pca (A)	5.18±8.93	8.82±14.66	6.07±0.48	1.41±0.63
BPH (B)	5.84±6.65	10.91±13.49	6.17±7.87	2.11±0.96
CONT (C)	6.94±4.51	23.33±19.75	10.71±0.44	2.18±1.1
F-value	0.383	6.110	1.680	12.05
P-value	0.683	0.003*	0.038*	0.0001*
POST HOC				
A vs B	0.386	0.642	0.972	0.001*
A vs C	0.232	0.001*	0.048*	0.0001*
B vs C	0.600	0.006*	0.031*	0. 694

N= 60 for each group

BPH = benign prostate hyperplasia

Pca = Prostate cancer

CONT = Control

P<0.05

* Shows significant value

Table 4.6: Correlations of parameters in BPH patients.

Parameters	**r-value**	**P-value**
FPSA Vs %FPSA	0.36	0.007
FPSA Vs 5ARD	0.42	0.001
FPSA Vs Zn	0.44	0.001
TAC Vs Vit D	0.33	0.01
5ARD Vs Se	0.30	0.024
Age Vs TPSA	0.40	0.001
Hgt Vs Wgt	0.47	0.001
5ARD Vs FPSA	0.42	0.001
Vit. D Vs % PSA	0.27	0.03
Vit. D Vs TAC	0.32	0.011

Table 4.7: Correlations of parameters in prostate cancer patients.

Parameters	**r-value**	**P-value**
BMI Vs Wgt	0.81	0.0001
TPSA Vs BMI	0.28	0.029
%FPSA Vs Vit D	0.27	0.034
TAC Vs Vit D	0.32	0.011
5ARD Vs Se	-0.293	0.024
Se Vs Zn	0.27	0.034
Vit E Vs Mg	0.44	0.0001
TPSA Vs 5ARD	0.29	0.025
Se Vs SOD	0.34	0.006
TAC Vs BMI	-0.35	0.005
TAC Vs Se	0.50	0.0001
Vit D Vs Vit E	0.50	0.0001

Tumor markers are substances that are produced by cancer or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. They are biological molecules found in blood, other body fluids, or tissues that can be objectively measured and evaluated as a sign of a normal/abnormal biological process and a pathogenic condition/disease. Most tumor markers are made by normal cells as well as by cancer cells; however they are produced at much higher levels in cancerous conditions. These substances can be found in the blood, urine, tumor tissue, or other tissues or body fluids of some patients with cancer. Because tumor markers can be used to assess the response of a tumor to treatment and for prognosis, researchers have hoped that they might also be useful in screening tests that aim to detect cancer early, before there are any symptoms. For a screening test to be useful, it should have very high sensitivity (ability to correctly identify people who have the disease) and specificity (ability to correctly identify people who do not have the disease). If a test is highly sensitive, it will identify most people with the disease, it will result in very few false-negative results. If a test is highly specific, only a small number of people will test positive for the disease that they do not have, in other words, it will result in very few false-positive results. The sensitivity and specificity were calculated using the formulae bellow.

The formula to calculate the sensitivity and specificity (Bradley 2005) are as follows:

$$\text{Sensitivity} = \frac{\text{No. of patients (test group) with high (true positive) levels of parameter}}{\text{Total no. of patients used in the study}} \times \frac{100}{1}$$

$$\text{Specificity} = \frac{\text{No. of control without high (true negative) levels of parameter}}{\text{Total no. of controls used in the study.}} \times \frac{100}{1}$$

Total no of Prostate cancer patients = 60

Total no of control subjects = 60

Subtituting the values in the equation for example sarcosine:

Sensitivity of sarcosine = $\frac{45}{60} \times 100 = 75\%$, Specificity of sarcosine = $\frac{43}{60} \times 100 = 71\%$

Where 45 is the number of cancer group that are true positive (for sensitivity)

43 is the number of control group that are true negative (for specificity)

Sensitivity of TAC = $\frac{19}{60} \times 100 = 31\%$, Specificity of TAC = $\frac{37}{60} \times 100 = 61\%$

Sensitivity of 5ARD = $\frac{35}{60} \times 100 = 58\%$, Specificity of 5ARD = $\frac{41}{60} \times 100 = 68\%$

Sensitivity of SOD = $\frac{40}{60} \times 100 = 68\%$, Specificity of SOD = $\frac{33}{60} \times 100 = 55\%$

Sensitivity of EN2 = $\frac{31}{60} \times 100 = 51\%$, Specificity of EN2 = $\frac{25}{60} \times 100 = 41\%$

Sensitivity of TPSA = $\frac{23}{60} \times 100 = 38\%$, Specificity of TPSA $\frac{27}{60} \times 100 = 45\%$

Table 4.8: The Sensitivity and Specificity of the markers (Sarcosine, TAC, 5ARD, EN2 and SOD).

Parameters	No. of positive		No. of negatives		Sensitivity (%)	Specificity (%)
	Control	Test	Control	Test		
Sarcosine	17	45	43	15	75	71
TAC	23	19	37	41	31	61
5ARD	19	35	41	25	58	68
SOD	27	40	33	20	68	55
EN2	35	31	25	29	51	41
TPSA	33	23	27	37	38	45

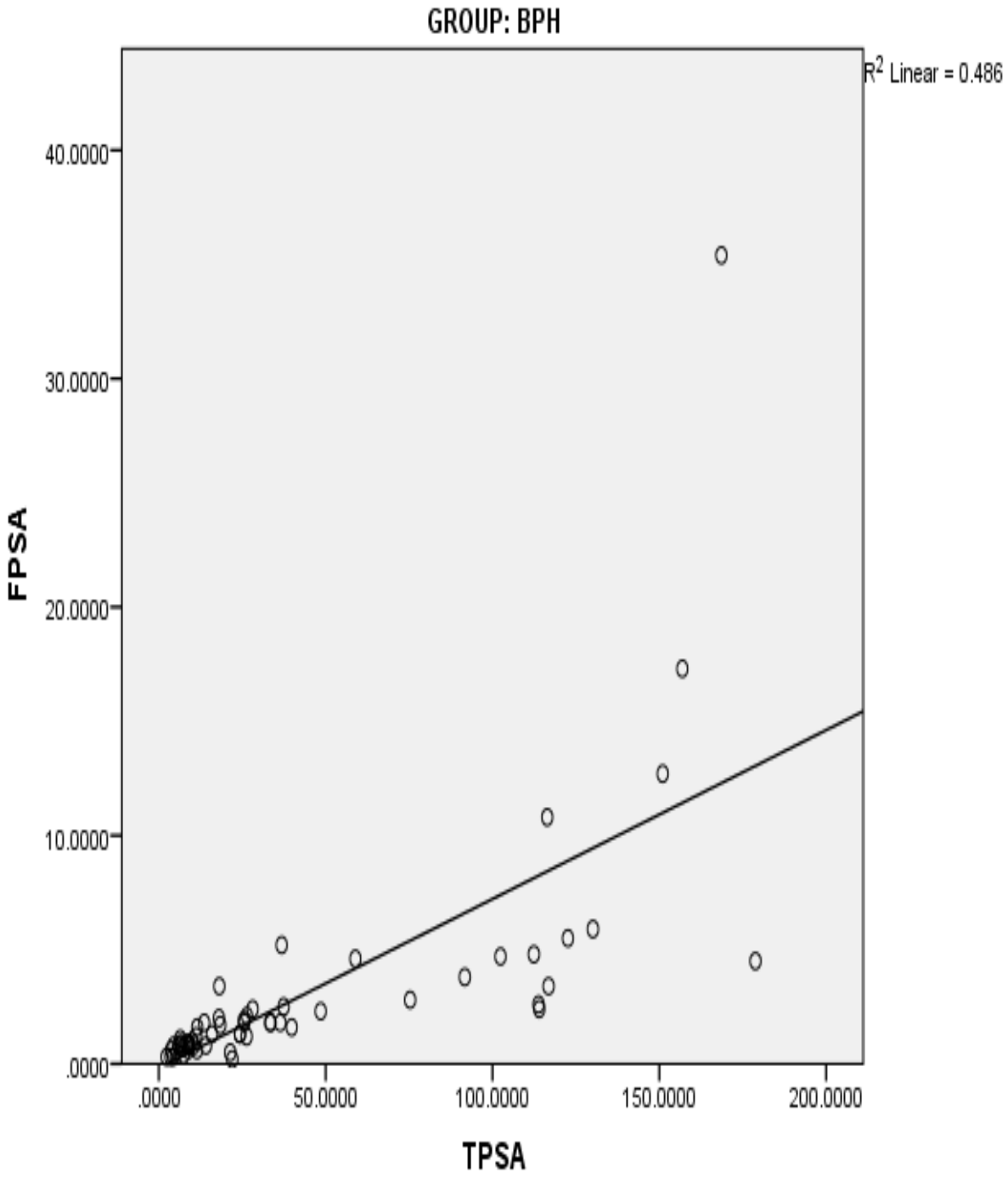


Fig 4.1 Correlation between FPSA and TPSA in BPH subjects

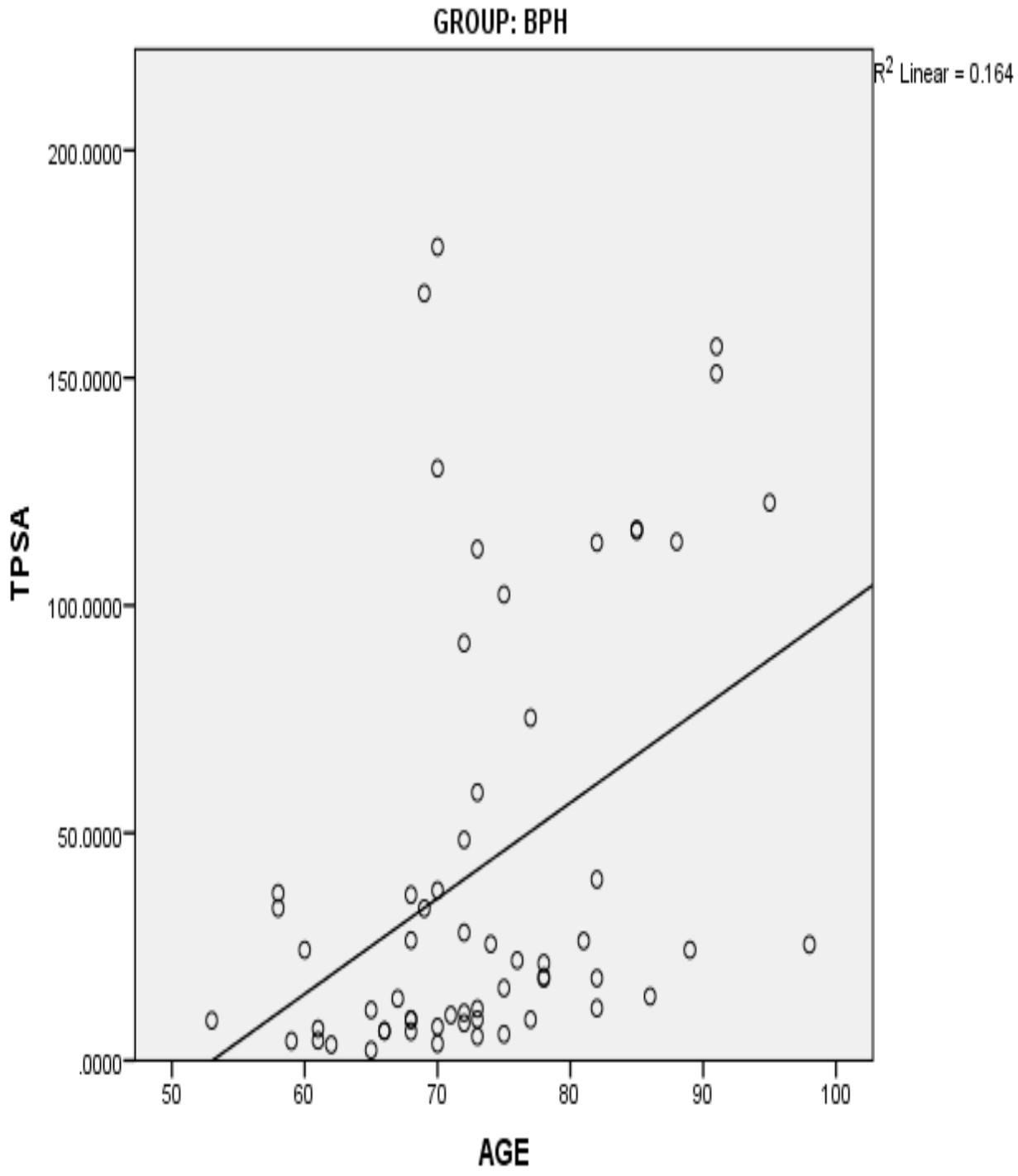


Fig 4.2: Correlation between TPSA and Age

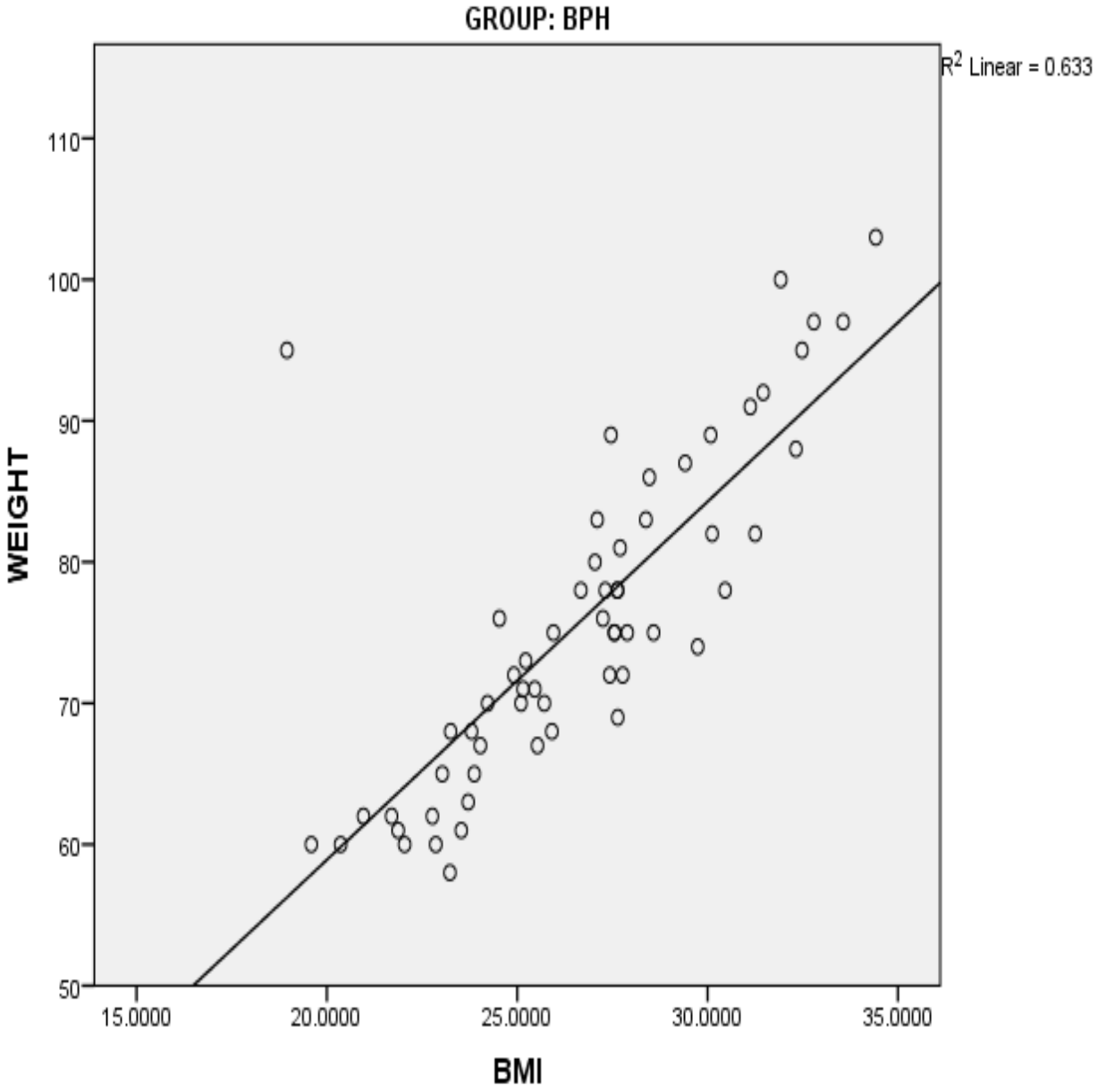


Fig 4.3: Correlation betweenWeight and BMI

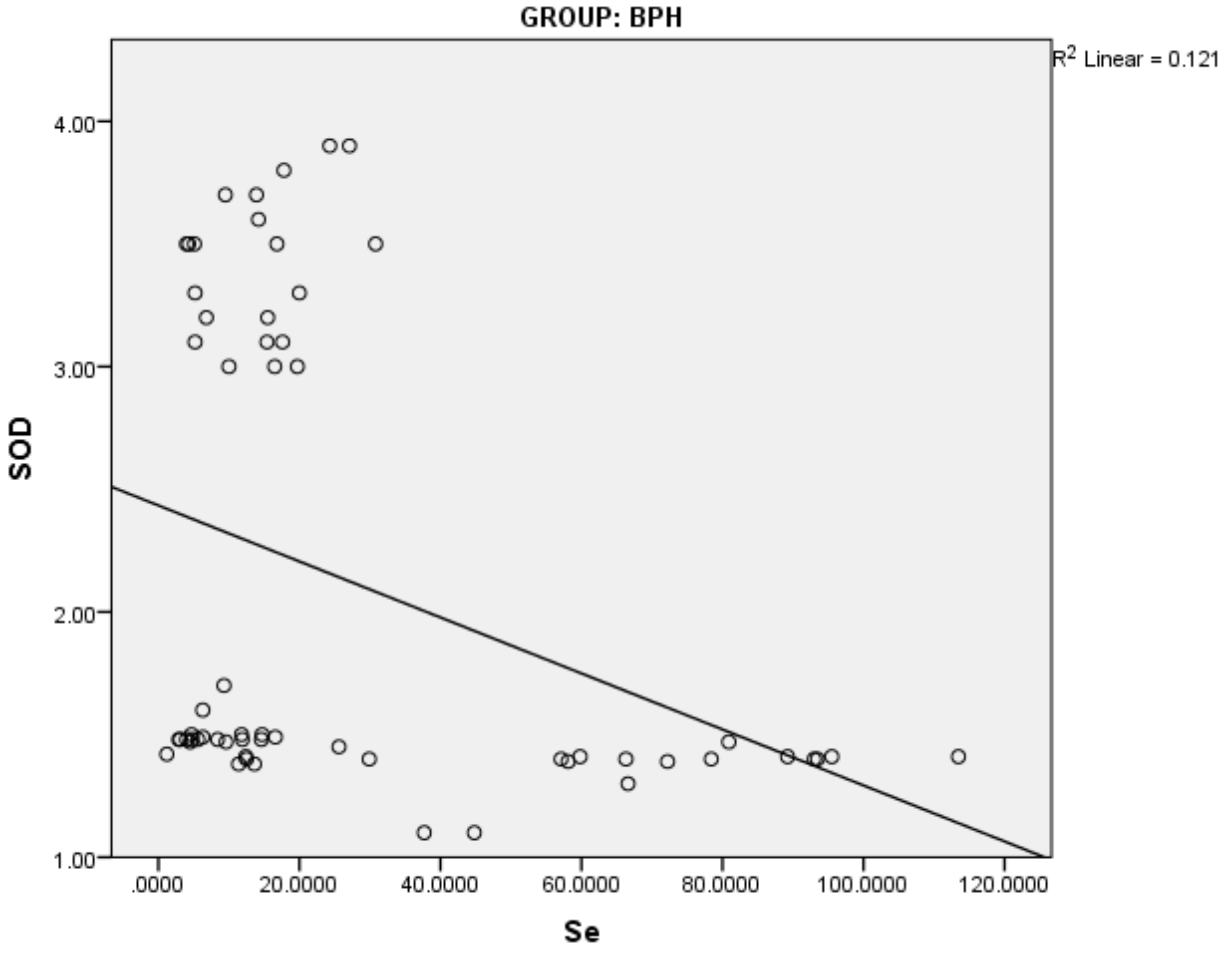


Fig 4.4: Correlation between SOD and Se

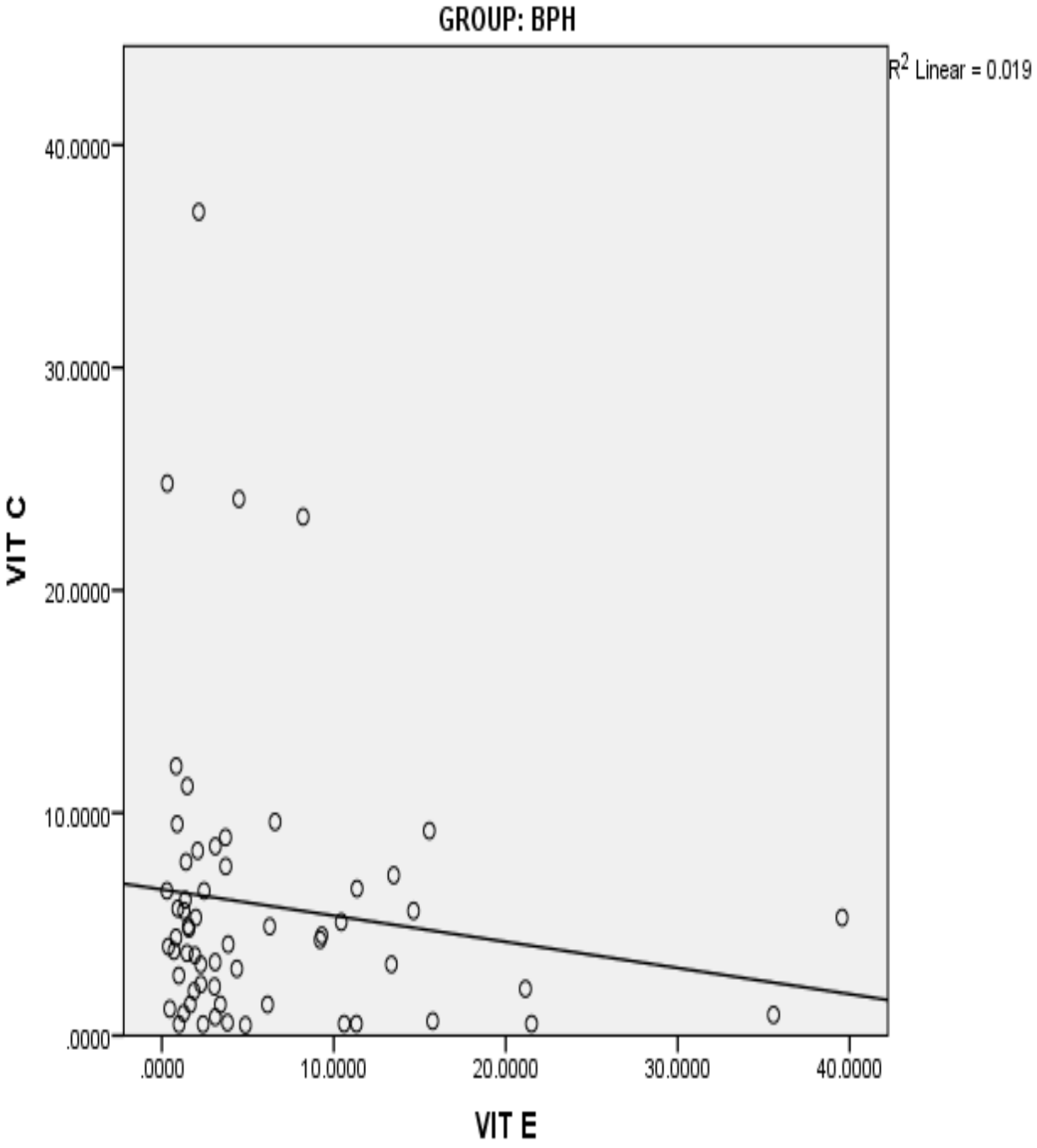


Fig 4.5: Correlation between Vit C and Vit E

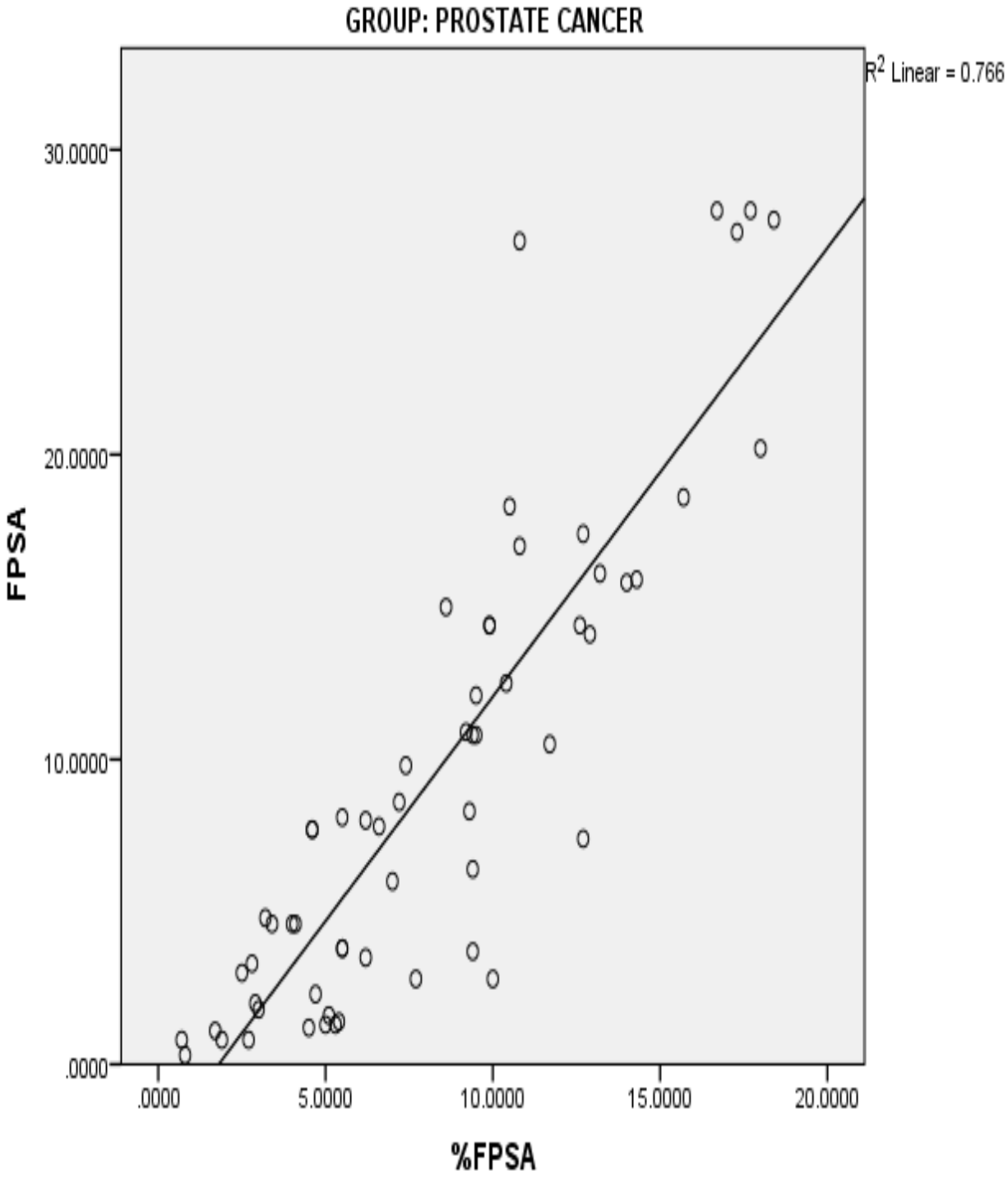


Fig4.6: Correlation between FPSA and %FPSA.

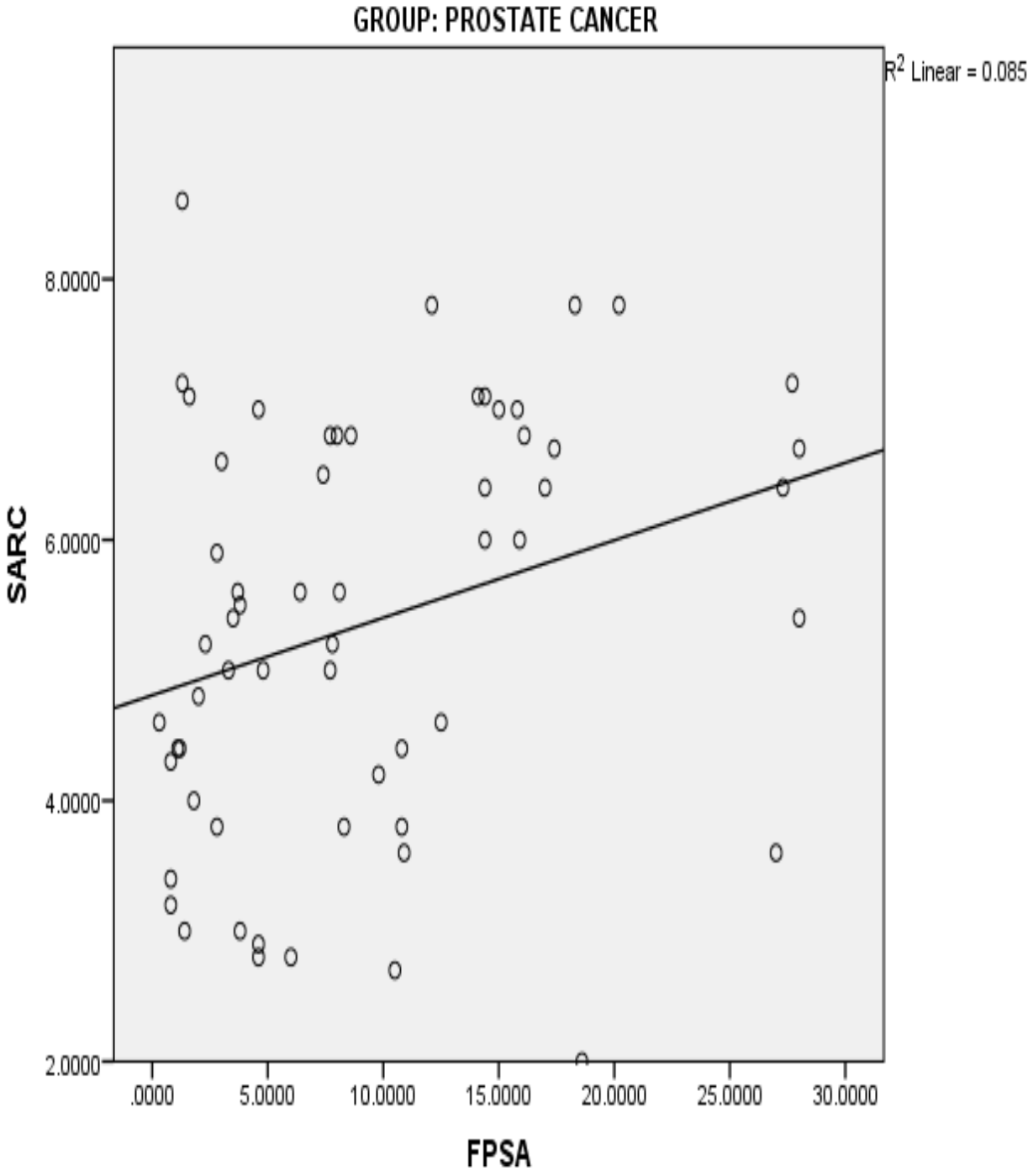


Fig4.7: Correlation between SARC and FPSA.

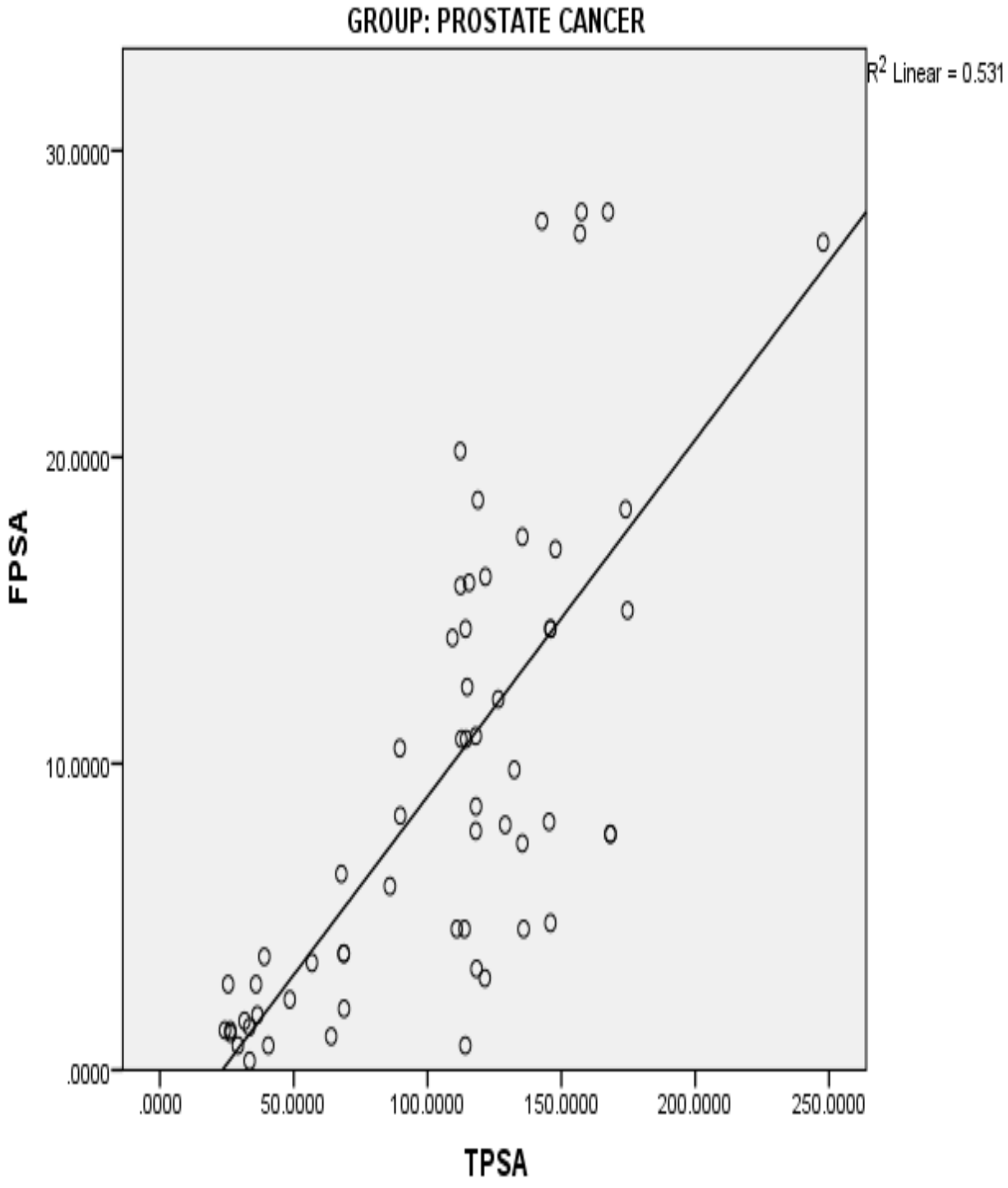


Fig4.8: Correlation between FPSA and TPSA.

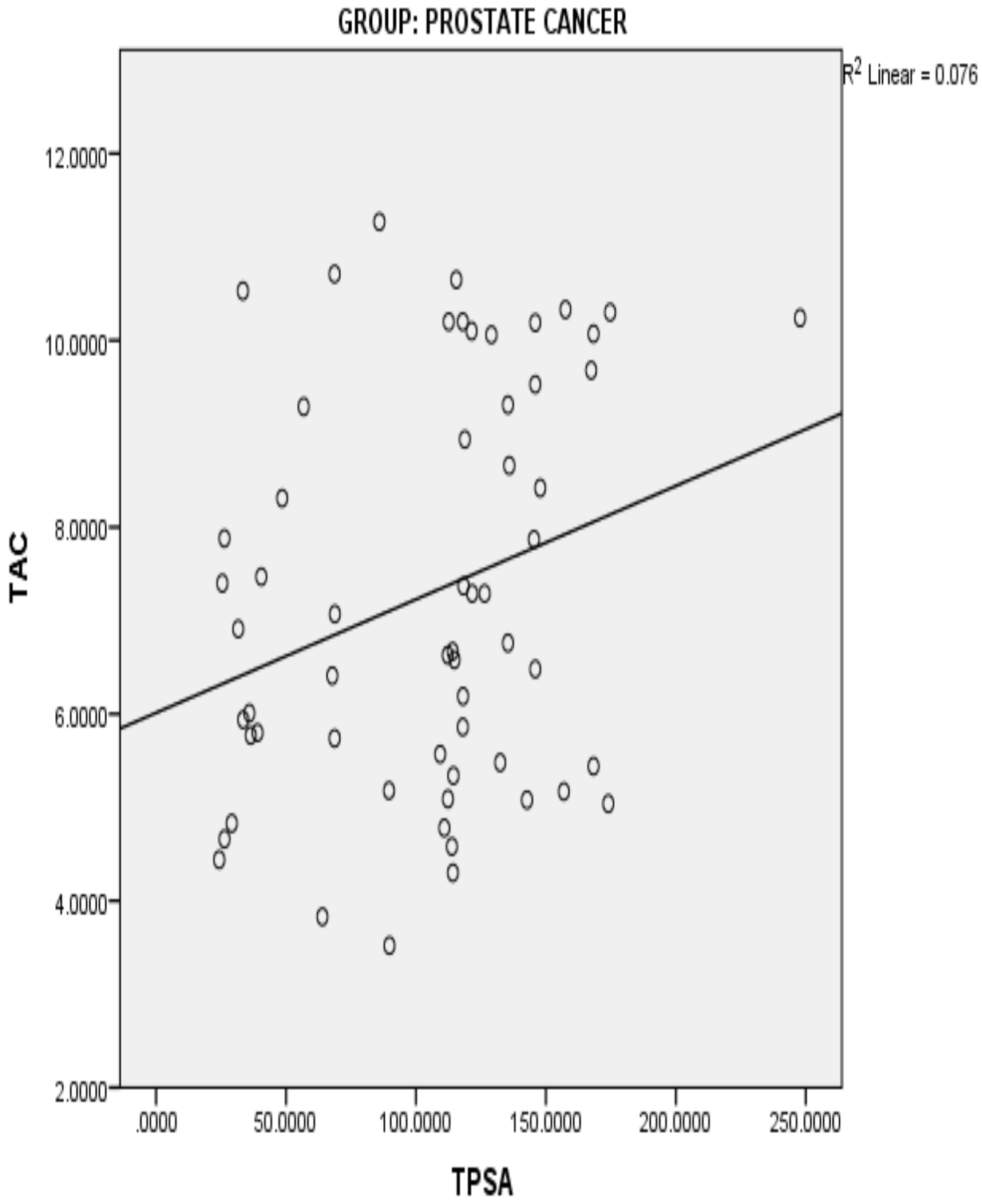


Fig 4.9: Correlation between TAC and TPSA.

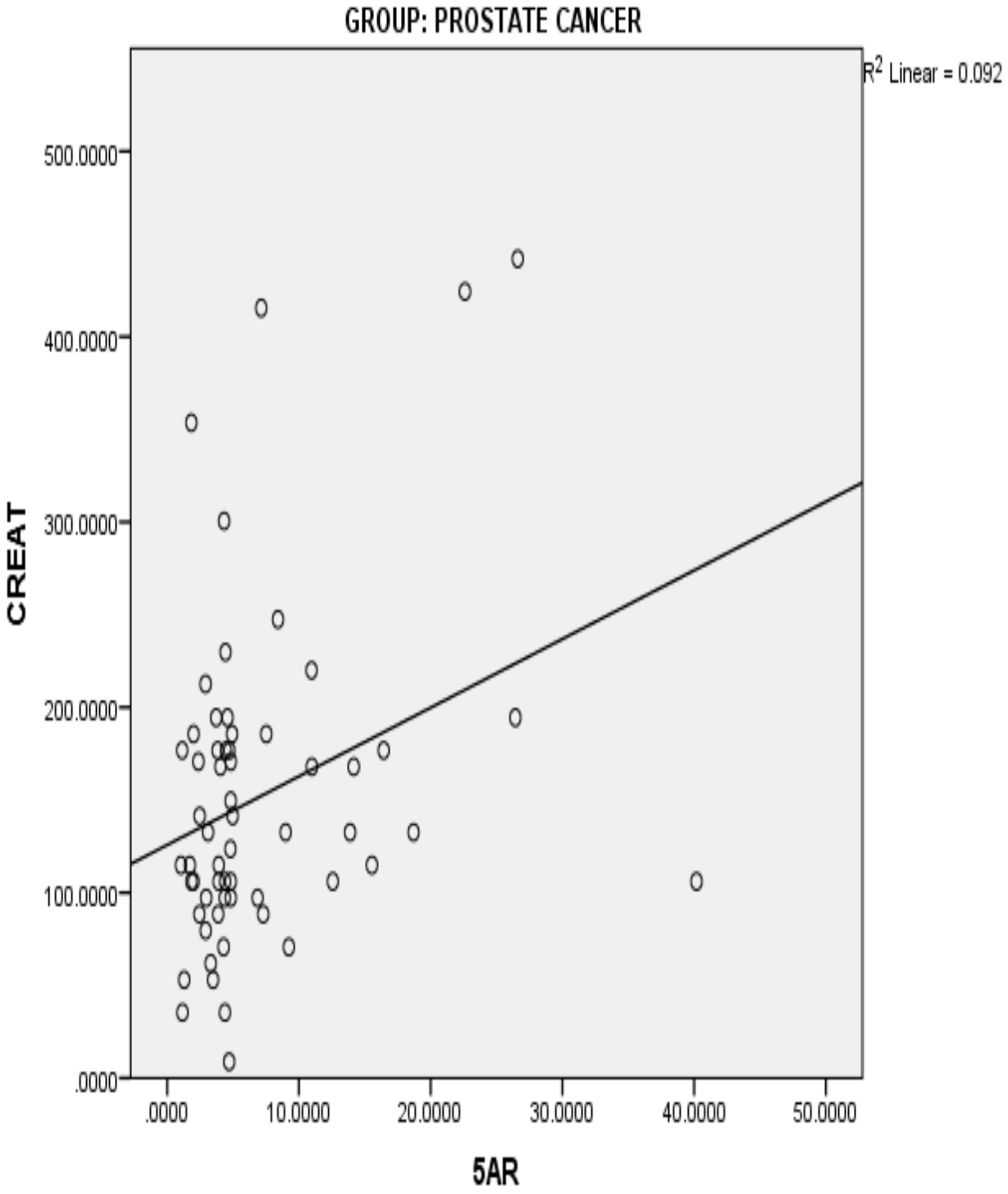


Fig 4.10: Correlation between CREAT and 5AR.

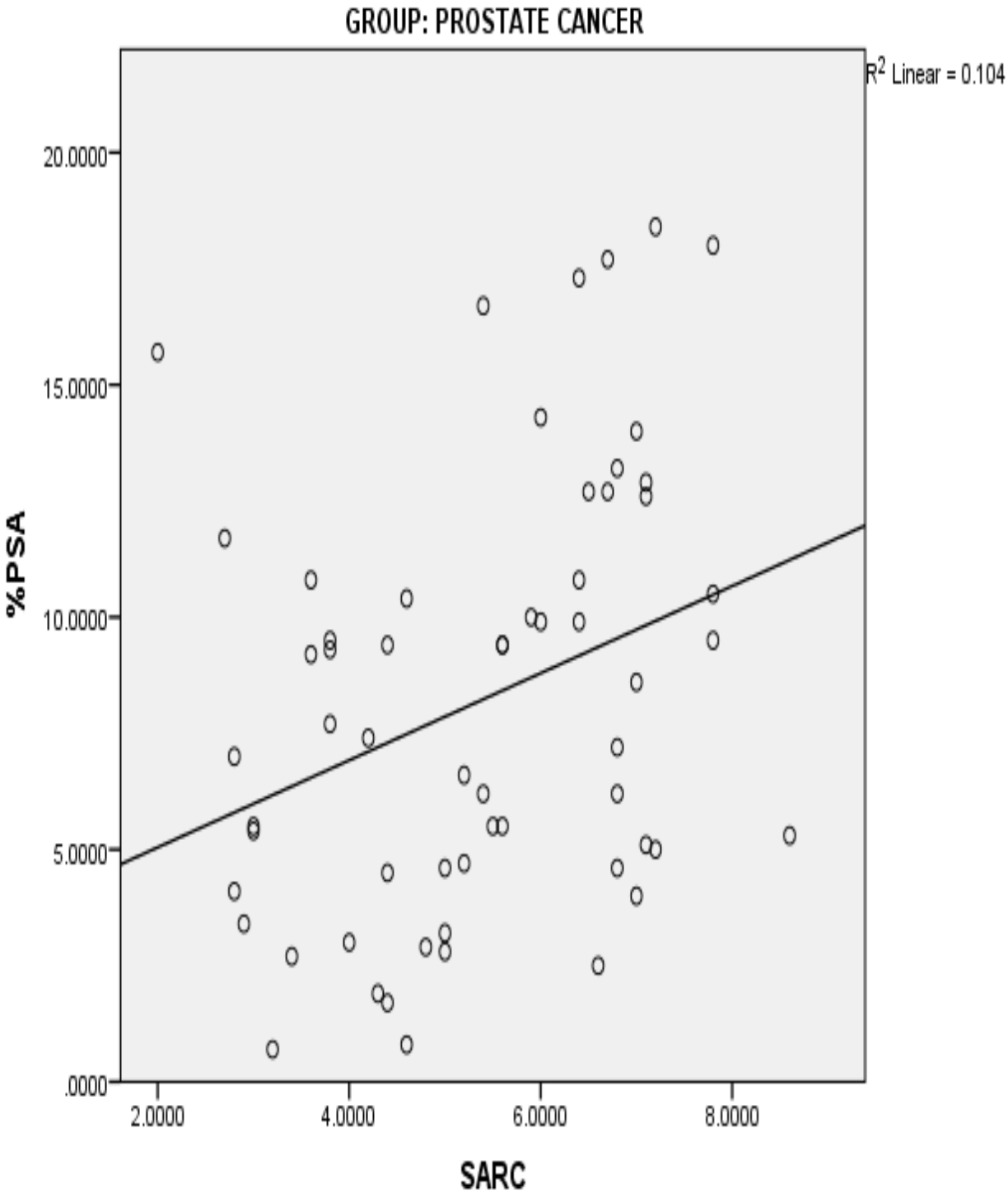


Fig4.11: Correlation between %FPSA and SARC.

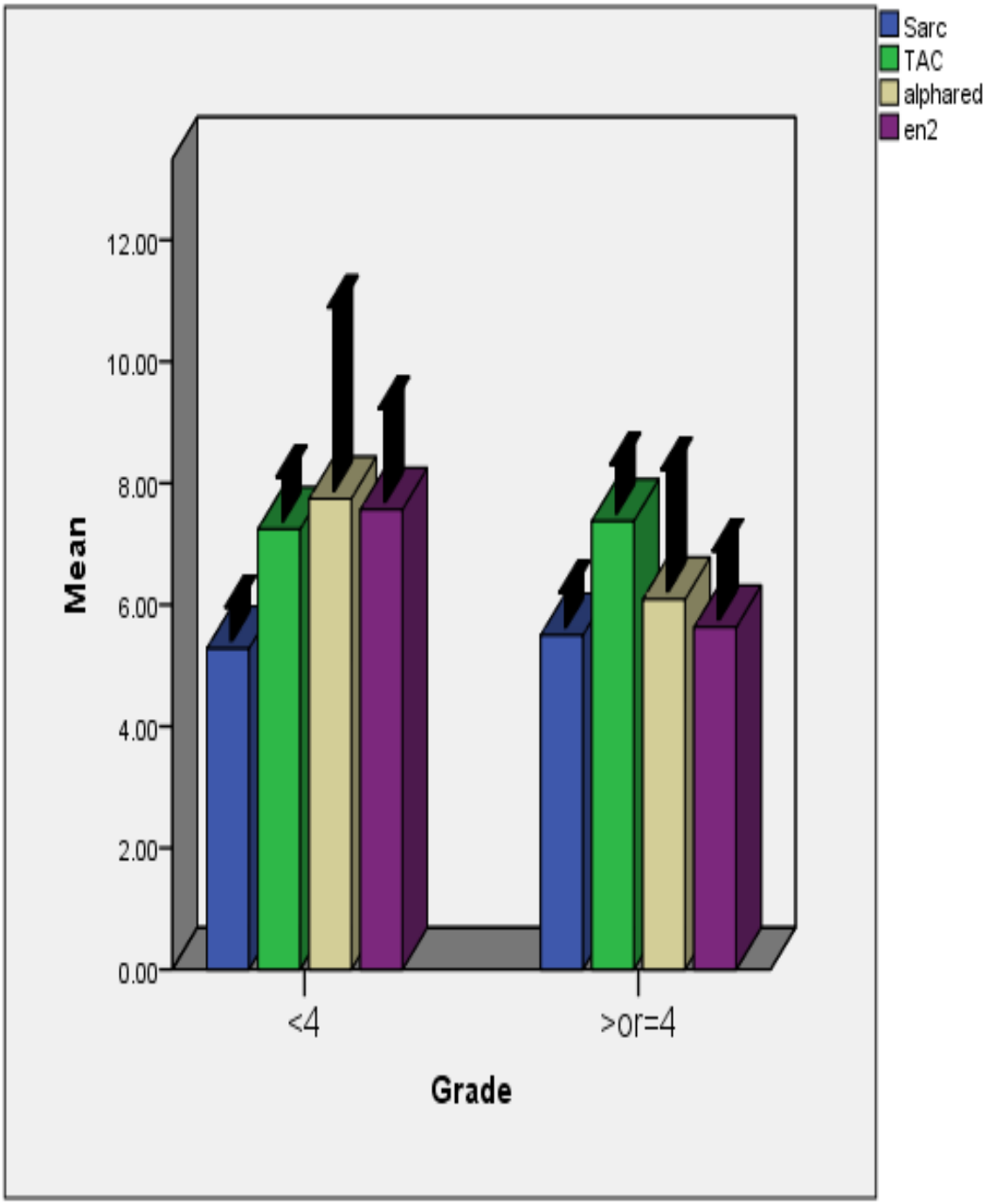


Fig 4.12: Mean concentration of Sarc, TAC, 5 ARD and EN2 of the prostate subject when they were classified using the grade of the tumor

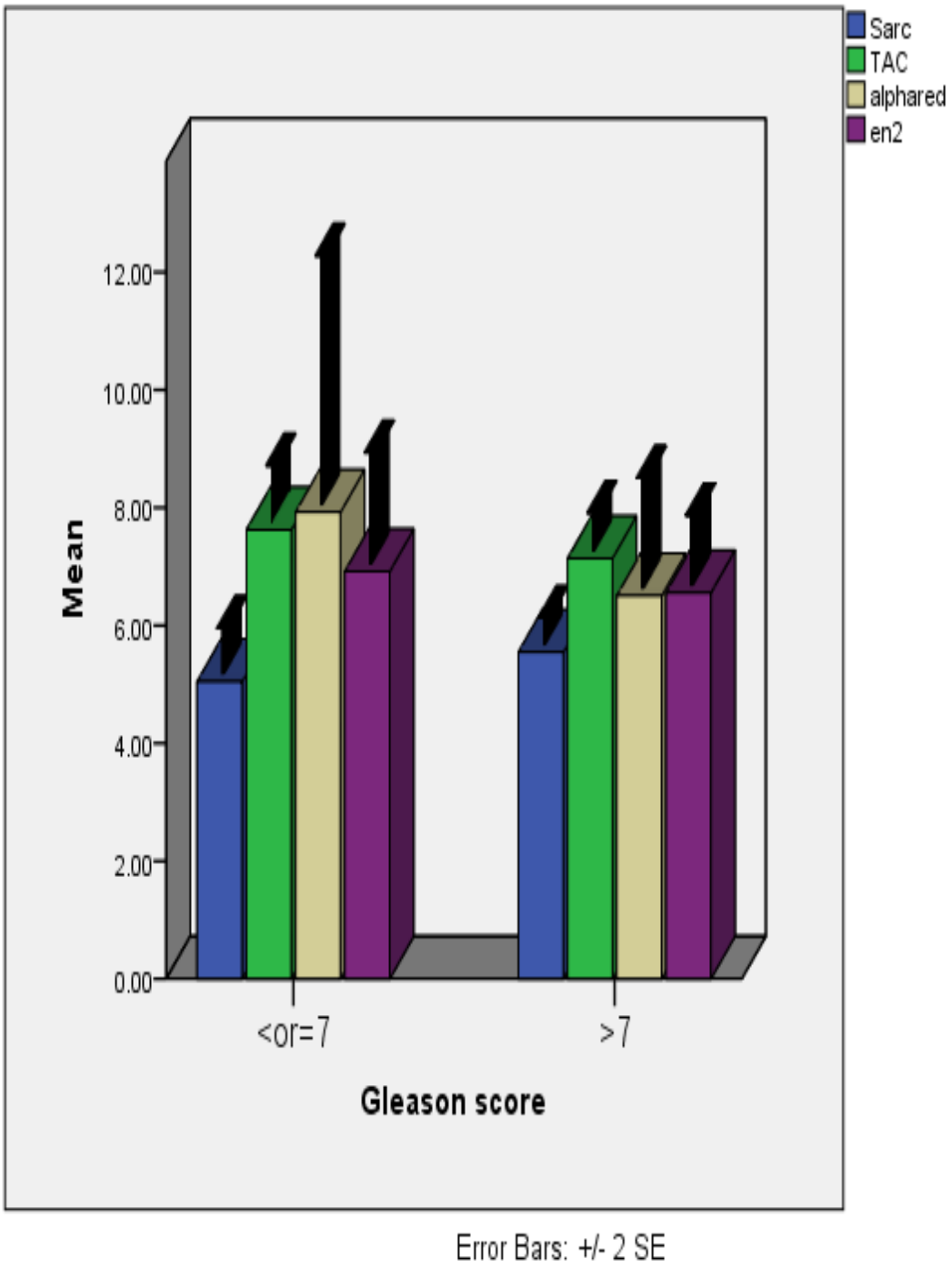


Fig 4.13: Mean concentration of Sarc, TAC, 5 ARD and EN2 of the prostate subject using their Gleason score

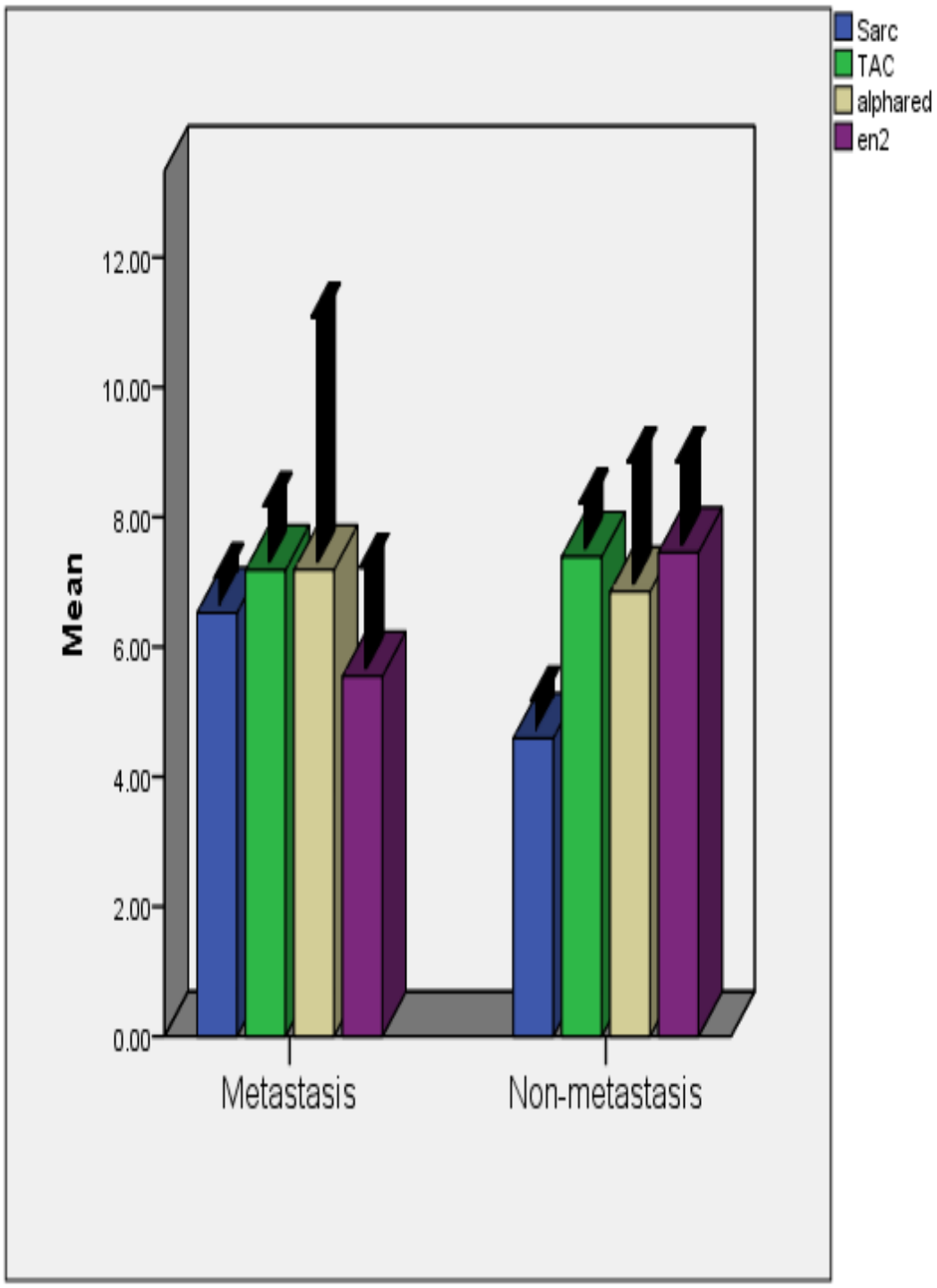


Fig 4.14: Mean concentration of Sarc, TAC, 5 ARD and EN2 of the prostate subject based on classification of metastasis and non metastasis

CHAPTER 5

5.0 DISCUSSION

In this present study, the level of total prostate specific antigen (PSA) was found to be significantly elevated in prostate cancer and benign prostate hyperplasia partistipants when compared with controls. There is wildly held view that men with total PSA level greater than 10.0ng/ml are at increased risk of developing prostate cancer. Previous reports from the American cancer Society (ACS, 2014) showed that there is more than a 67% chance of risk of having Pca with elevated PSA, the mecahanism being that the PSA is produced at much higher levels in cancerous conditions. The ACS report showed that PSA levels between 4.0ng/ml and 10ng/ml may indicate prostate cancer in about 25% of the studied population, while there is a 75% chance of having BPH or prostatitis. The effect of age on PSA is well established, elevation in the levels of PSA are more common in the elderly (Babaian et al, 1992)

The levels of free prostate specific antigen were significantly higher in both Pca and BPH than controls, the level being significantly higher in Pca than BPH. The elevation of free PSA seen in this study is probably due to increased secretion by the tumour itself or the normal body tissues in response to cancer. It is only in the grey zone (total PSA of between 4.0ng/ml and 10ng/ml) that the free PSA is most useful or valuable in diagnosis. When men in the grey zone have decreased levels of free PSA, they have higher probability of prostate cancer, but when they have elevated level of free PSA, the risk is diminished. The ratio of free to total PSA may aid in deciding whether or not a prostate biopsy should be performed. There was also significant difference in the mean value of percentage free PSA for the three groups, the value being significantly higher in control than the patients groups. There was no significant difference in BPH and Pca. Some evidence suggests that lower levels of percentage free PSA may be

associated with more aggressive cancer. Evidence also suggest that in men with PSA levels between 4.0 to 10.0ng/ml, performing a prostate biopsy when the percentage free PSA is 24 or below would detect more than 90% of Prostate cancer. This would reduce the number of unnecessary biopsy by 20% (John Hopkins health alert, 2014).

The mean value of creatinine was found to be significantly higher in patients with Pca than control, also the value was significantly increased in BPH than controls, thus there were significant differences in the three groups. Urinary retention is a common presentation in BPH and Pca thus urinary retention affects creatinine values. The synthesis of creatine, which is converted to creatinine in muscle cells, requires a methyl group from S-adenosylmethionine, which in turn is converted via adenosylhomocysteine to homocysteine. It is therefore conceivable that creatinine could serve as a marker for homocysteine status. Studies among prostate cancer patients have shown that serum creatinine is associated with more advanced disease (Chiong et al, 2005) and with decreased survival (Fossa et al, 1992), although this relationship is not supported by some studies (Merseburger et al, 2001).

The significant raised sarcosine was due to hypomethylation (decreased methylation) of DNA, which triggers increase synthesis of glycine –N- methyl transferase. Due to properties of GNMT, its excessive production causes a cleavage of glycine to sarcosine through increased utilization of SAM and this causes the elevation of sarcosine in blood and urine. This makes sarcosine interesting in the field of non-invasive cancer biomarkers. This finding is in agreement with the finding of Sreekumar et al (2009) who reported that sarcosine was greatly increased during cancer progression. However another study revealed that sarcosine was not detected in the urine of cancer patients (Jentzmik et al 2011). The discrepancy may be due to the sample used for

analysis. Sreekumar et al, (2009) used serum while Jentzmik used urine sample. Hence for any substance to appear in urine, it must have exceeded its reabsorptve capacity. Meanwhile other studies reported significant elevation of sarcosine levels in prostate cancer patients (Giuseppe et al 2012, Khan et al 2013). Hence these studies found that sacosine may be potentially useful for the diagnosis and prognosis of prostate cancer. The value of sarcosine showed no significant difference in the patients with BPH and the controls. This finding appears to be the major advantage of sarcosine over prostate specific antigen as a diagnostic tool for prostate cancer.

Proliferating cells exhibit considerably different metabolic requirements from most normal differentiated cells. In order to support their high rates of proliferation, cancer cells consume additional nutrients and divert those nutrients into macromolecular synthetic pathways, metabolic pathways must therefore be rewired in such a way as to balance biosynthetic process with sufficient ATP production to support cell growth and survival (Griffin et al, 2004). Prostatic fluid from patient with Pca have characteristic lower citrate and higher spermin levels as a consequence of the metabolic shift associated with the acquisition of malignant phenotype. The citrate is converted by enzymes that are androgen regulated to acetyl COA which is a precursor for lipid biosynthesis and cholesterogenesis. The increase in lipid biosynthesis is essential for cell proliferation, membrane formation and signal transduction (Etinger, 2004). In prostate cancer, a metabolic profile characterized by an increased amino acid metabolism and methylation have been reported (Swanson et al 2006; Mohit et al 2012). This increased methylation process involves S- adenosylmethionine (SAM) dependent methyl transferase (GNMT) which catalyzes the transfer of a methyl group from SAM to amino acid group of glycine producing sarcosine (N- methyl glycine) and S-adosinyl homocystein (SAH). Amjad et al (2011) reported that GNMT which produce sarcosine plays a functional role in prostate cancer

progression hence they stated that GNMT protein levels are strongly associated with aggressiveness. They also demonstrated that stable knockdown of the enzyme GNMT inhibits cell proliferation, induces apoptosis and attenuates cell invasion. The studies of Khan et al (2013) was in agreement with above findings, they also reported that the expression of sarcosine biosynthesis enzyme, glycine N-methyltransferase was elevated in prostate cancer, while sarcosine dehydrogenase (SARDH) and pipecolic acid oxidase (PIPOX) which metabolize sarcosine to glycine were reduced in prostate cancer. Consistent with this GNMT promotes the oncogenic potential of the prostate cells by facilitating sarcosine production while SARDH and PIPOX reduce the oncogenic potential of the prostate cells by metabolizing sarcosine. The sarcosine pathway has also been shown to be regulated by androgen receptor and gene fusion (TMPRSS2-ETS) in Pca cell lines (Sreekumar, 2009).

Biomarkers signifying the presence of any cancer may be defined on the basis of gene products uniquely expressed or overexpressed in tissues, serum or urine in cancer compared to non-cancer. A number of genes are involved in the early embryonic development and are subsequently re-expressed in cancer. This study assessed engrail 2 gene protein also known as homeobox protein. The mean levels of this protein in serum were higher in prostate cancer but the increase was not statistically significant. However many studies have reported significantly increased levels of engrail 2 gene proteins in urine of prostate cancer patients. (Morgan et al, 2011, Brimelow, 2011). The result of this study suggests that the use of this biomarker in diagnosis should be evaluated further.

There was a significant decrease in the mean levels of vitamin E in prostate cancer patients. This finding is in agreement with that of Ozman (2006) who also reported significantly lower levels

of vitamin E in prostate cancer patients. However, there was no significant difference in the mean value of vitamin E between the BPH and the controls. Meanwhile the Selenium and Vitamin E Cancer Prevention Trial (SELECT) (Klein et al 2011) study did not establish any significant difference in the mean value of vitamin E between the cancer patients and controls. Another by study Venkalowaran (2002) argued that SELET was not able to establish significant difference because SELECT did not use the d-gamma tocopherol which is the natural form of vitamin E. Vitamin E is a well-known antioxidant promoted as a preventive agent for several health problems. It has been the subject of many studies for the prevention and amelioration of prostate disorders and in combination with selenium is probably the most valuable vitamin for prostate health. Considerable evidence exists for a synergistic relationship between vitamin E and selenium. Studies have shown that vitamin E and selenium are protective against both prostate and breast disorders. This synergistic relationship is particularly strong in regard to switching on the full effects of the body's apoptotic mechanisms (natural cell death) machinery against cancerous growths. Vitamin E in particular generally inhibits cell proliferation and enhances apoptosis in both breast and prostate cancer cells (Zu, 2003). This alteration of growth and natural cell death of dividing cells by vitamin E and selenium is significantly more effective in breast and prostate cancers (Gunawardena, 2000). The mean value of selenium was also found to be significantly lower in cancer patient than controls. Selenium is an important dietary trace element and also an antioxidant. It has been found that selenium increase immunity in a way that is protective against both breast and prostate disorders. It seems selenium offers a mediating effect by actually killing cancer cells at different stages (Jiang 2002), thus selenium appears to target both non clinical and clinical diseases. Selenium mediates cancer cell development by

preventing the cells from developing and by causing developing cancer cells to self-destruct (Li, 2004).

Vitamin D was also found to be significantly lower in patients with prostate cancer and BPH than controls. Researches have shown that vitamin D may work to help slow prostate cancer progression by increasing the death of cancerous cells. Studies (Tretli et al 2009) have also shown that men with the lowest levels of vitamin D had almost double the chance of dying from prostate cancer or having it spread to other body parts, compared to men with the highest levels of vitamin D. Cells in the prostate are able to take the inactive form of vitamin D and activate it. Some of the cancerous cells in the prostate lose this ability, but they still have receptors for vitamin D, which could mean that supplementation may help to slow the growth of cancerous cells. These Vitamin D receptors in prostate tissue when bound to vitamin D, a signal is generated which may cause the cancerous cells to die, stop growing or stop spreading to other parts of the body. The anti-proliferative effect of vitamin D in prostate cells is mediated through the vitamin D receptors (VDR), which is a member of the steroid/nuclear receptor super family. In target cells, VDR binds vitamin D with high affinity and specifically interacts with retinoid X receptor (RXR). This heterodimeric complex contains two characteristic zincfinger motifs that binds to specific DNA sequence motif called vitamin D response element (VDRE) in the promoter region of vitamin D regulated genes and they regulate the rate of RNA polymerase II mediated transcription of these genes which then regulate the anti-proliferative effect (Freedman, 1999) . Studies have reported also that more aggressive and advanced tumors are linked to lower levels of vitamin D (Der et al, 2014). Studies of cancer cells and tumor in mice have reported the effect of vitamin D which slows or prevents the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth, stimulating cell death

(apoptosis) and reducing tumor blood vessel formation (angiogenesis) (Moore et al, 2005, Deeb et al, 2007). Data from this study showed that there were no significant difference in the value of vitamin C in the patient with prostate cancer, BPH and controls. The result of vitamin C in other studies seems inconclusive.

This study also demonstrated significantly lower levels of zinc in prostate cancer patients than controls. Zinc is an essential trace element that plays an important role in many body processes. Prostate glands containing cancer generally have lower levels of zinc (Goel & Sankhwar, 2006) than healthy gland. There are many processes in the body involved in the repair of DNA that require zinc to function properly, and since the prostate has the highest concentration of zinc than any other organ in the body, it is reasonable to assume that zinc deficiency would affect the prostate significantly. This is partially mediated by the action of hormone testosterone. Feng (2000) showed that one effect of zinc accumulation is to inhibit abnormal growth of the prostate, primarily by increasing the rate of normal programmed cell death (apoptosis). The prostate gland accumulates and secretes extraordinary high levels of citrate. This process is dependent on ample supply of zinc in the prostate. In an unhealthy prostate the ability to accumulate both zinc and citrate is altered, and hence with prostate cancer the ability to accumulate zinc and produce citrate is impaired. Reduced zinc and poor citrates metabolism in prostatic cells is characteristic of men with prostate cancer. Studies have shown that zinc exerts both growth and metabolic effects on prostate cells and contributes to the development of prostate malignancy by altering citrate metabolism (Costello et al, 2004). It has also been reported that zinc inhibits invitro cultured human prostate cell lines and rat ventral prostate epithelium cells proliferation by inducing mitochondrial apoptogenesis by releasing cytochrome c which then activates subsequent caspase mediated apoptotic cascading events leading to apoptosis. The prostate gland

comprises of peripheral (70%), the central (about 25%) and transition (5%) zones. In regard to zinc relationship and the development of malignancy, the peripheral zone is the major component. The central gland normally contains much lower zinc levels than in the peripheral zone, therefore, the normal glandular secretory epithelial cells of the peripheral but not central gland, are zinc accumulating cells. Zinc transporter, ZIP1 is important in the uptake and accumulation of zinc by the prostate cells. Upregulation of ZIP1 in prostate cells increases zinc accumulation, which inhibits cell growth and increases net citrate production.

In this study, the activities of 5-alpha reductase were found to be significantly higher in both prostate cancer and BPH patients than controls. The prostate gland depends on androgen stimulation for its development and growth. Testosterone which is the most common androgen in the prostate gland is converted to dihydrotestosterone (DHT) by this enzyme 5-alpa reductase in the prostatic stromal and basal cells. Dihydrotestosterone is primarily responsible for prostate development and the pathogenesis of benign prostatic hyperplasia and prostate cancer (Zhou et al, 2009). Inhibition of the enzyme 5-alpha reductase reduces the size of the prostate. This reduction in glandular tissue is achieved by the induction of apoptosis which is histologically manifested by ductal atrophy. Inhibition also diminishes the number of blood vessels in the prostate because of the reduction in vascular derived endothelial growth factor. The mechanism of androgen action varies in different tissues but in the majority of androgen target tissue either testosterone or 5-alpha reductase with DHT binds to a specific androgens receptor to form a complex that regulates gene expression (Heinlein and Chang, 2002)

Total antioxidant capacity was found to be significantly lower in both prostate and BPH than controls. The measure of antioxidant capacity considers the combined action of all the

antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than simple sum of measurable antioxidants. Determining plasma total antioxidant capacity may help to identify conditions affecting oxidative status. Reactive oxygen species signaling may play an important role in the development and the progression of prostate cancer. Increased reactive oxygen species, otherwise known as oxidative stress is associated with several pathological conditions including inflammation and infection. Reactive oxygen species are products of normal cellular metabolism which play vital roles in stimulation of signaling pathways in response to changing intra and extracellular environmental conditions. Elevated levels of cellular reactive oxygen species significantly contribute to the initiation and progress of cancer and the degree of ROS generation correlates with aggressive phenotype of the cancer. Chronic elevation of ROS over time is known to induce somatic mutations and neoplastic transformation. Hydroxyl radicals, peroxides and superoxide are ROS that are generated during every day metabolic process in normal cell. Reactive oxygen species generation has also been traditionally associated with tissue injury or DNA damage which are general manifestation of pathological conditions associated with infection and DNA mutations and cellular proliferation (Naka et al, 2008). Processes associated with proliferation and apoptosis may be a result of the activation of signaling pathways in response to intracellular changes in ROS levels (Sauer et al, 2001). Excessive production of ROS or inadequacy in a normal cell's antioxidants defense system can cause the cell to experience oxidative stress and thus when antioxidant defenses are weakend, body cells and tissues becomes more prone to develop dysfunctions. Thus the adequate maintenance of antioxidant levels is essential to prevent or even manage a great number of disease conditions. The potential role of ROS in the regulation of cellular process controlling malignant transformation holds a lot of promise in understanding the etiology and progression of

cancer in general and prostate cancer in particular, as this may open doors for the development of therapies for cancer prevention and treatment. In the case of the prostate, besides acting as a DNA damaging agent, moderately elevated levels of ROS may act as secondary messengers and can control various signaling pathways which are essential for the maintenance of oncogenic phenotype by virtue of activating many transcription factors in prostate cancer.

The activity of super oxide dismutase was found to be significantly lower in prostate cancer compared with BPH and controls. At the same time there was no significant difference in this enzyme between BPH and controls. Super oxide dismutase acts as both an antioxidant and anti-inflammatory enzyme in the body (Vouldoukis et al, 2004) neutralizing the free radicals that lead to wrinkles and precancerous cell changes. This enzyme breaks down superoxide particles into harmless substances, a process called dismutation.

Magnesium was found to be significantly lower in patients with prostate cancer than controls. There was no significant difference between prostate cancer and BPH. This finding is in agreement with Dai et al (2011) who also reported significant lower levels of magnesium in patients with prostate cancer. Magnesium plays an essential role in deoxyribonucleic acid repair, cell differentiation, apoptosis and angiogenesis (Iseri and French, 1984). Deficiency of magnesium has been linked to inflammatory response and oxidative stress (Weglicki and Phillips, 1992). Given the broad range of biological functions dependent on magnesium, it is highly plausible that magnesium deficiency may affect multiple pathways towards tumorgenesis.

Examining the biomarker of interest based on the grouping of the patients with prostate cancer, it was only sarcosine that showed statistically significant difference in those patients with

metastasis. Aditionally the mean value of sarcosine and total antioxidant capacity were slightly increased in those patients with high grade of ≥4 but the increase was not statistically significant.

Furthermore, the calculation of sensitivity and specificity using Bradley (2005) formula showed that sarcosine exhibited higher sensitivity and specificity than all other parameters. Superoxide dismutase and 5-alpha reductase had moderately high sensitivity and specicifity, while total specific antigen exhibited low sensitivity and specificity in this study. This is in aggreement with the statement of problem of this study that PSA exhibits low specificity among other key limitations.

5.1 Conclusion

The ultimate goal in clinical practice is to avoid unnecessary biopsy, to improve patient care in terms of prostate cancer burden, assessment and diagnosis at early stage, reducing invasive diagnostic procedures as well as helping to evaluate treatment response. This study has shown that sarcosine level was significantly higher in prostate cancer than in the patients with BPH and controls. Additionally the mean value of sarcosine in patients with BPH was not statistically different from the controls. This finding suggests that sarcosine has some degree of specificity for prostate cancer which is an advantage over prostate specific antigen. In addition, the level of sarcosine was significantly higher in those patients with metastasis than in those with no metastasis. This finding appears to show that sarcosine is a potential biomarker (useful for diagnosis and prognosis) for prostate cancer. This study also showed that 5-alpha reductase and creatinine were significantly higher in both BPH and prostate cancer than the control. Total antioxidant capacity, superoxide dismutase, selenium, zinc, magnesium, vitamin E and D were all significantly lower in patients with prostate cancer than controls.

5.2 Recommendations.

1. This study showed significant increase in the level of sarcosine, and also sarcosine exhibited high sensitivity and specificity, it is therefore recommended that sarcosine be part of the routine test of the patients with prostate disorders.
2. This study have demonstrated that some antioxidant vitamin and minerals were significantly decreased in prostate disorders such as benigh prostate hyperplasia and prostrate cancer, therefore supplementation of these antioxidant vitamin and minerals could help the pateints.
3. Other potential markers such as pro- PSA and HOXB13 gen should be further investigated for the diagnosis of prostate cancer.

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