THE CYTOKINE PROFILE AND PROSTATE SPECIFIC ANTIGEN LEVELS IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL

LIZA KIENDE MWIRIGI (BSc.)
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A Research Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Infectious Diseases - Immunology) in the School of Medicine, Kenyatta University

AUGUST 2015
DECLARATION

This thesis is my original work and has not been presented for a degree or award in any other University or for any other award.

Signature:................................................................. Date:.................................

Liza Kiende Mwirigi (P150/21492/2012)
Department of Medical Laboratory Science

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Signature:................................................................. Date:.................................

Dr. Margaret Muturi (PhD)
Department of Medical Laboratory Sciences
Kenyatta University

Signature:................................................................. Date:.................................

Dr. Minda P. Okemwa
Department of Human Pathology
University of Nairobi
DEDICATION

I dedicate this thesis to my mother and my sister, they have molded me to live well, believe in myself and work hard.
ACKNOWLEDGEMENT

I thank God for granting me the strength and the zeal to begin and complete this study. I acknowledge all the people who had anything to do with this project. The list is long and the gratitude immense. I would like to thank Kenyatta University for paying part of my tuition fees and approving my study, my supervisors Dr. Margaret Muturi and Dr. Minda Okemwa for their encouragement, advice, support and standing by me at the time of hardship during the course of my work. God bless them.

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<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen Receptor</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BPH/BPE</td>
<td>Benign Prostate Hyperplasia/ Benign Prostate Enlargement</td>
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<tr>
<td>BPSA</td>
<td>Benign PSA</td>
</tr>
<tr>
<td>CBA</td>
<td>Cytometric Bead Array</td>
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<tr>
<td>CT Scan</td>
<td>Computerized Tomography Scan</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital Rectal Examination</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>fPSA</td>
<td>Free PSA</td>
</tr>
<tr>
<td>GLOBOCAN</td>
<td>Global Cancer Statistics</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Haematoxylin and Eosin staining</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>Insulin like Growth Factor 1 receptor</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
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<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>--------------------------------</td>
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<tr>
<td>NC</td>
<td>Negative Control</td>
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<td>NK</td>
<td>Natural Killer cell</td>
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<td>PCa</td>
<td>Prostate Cancer</td>
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<td>PIN</td>
<td>Prostatic Intraepithelial Neoplasia</td>
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<td>PSA</td>
<td>Prostate Specific Antigen</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Science</td>
</tr>
<tr>
<td>STD</td>
<td>Sexually Transmitted Disease</td>
</tr>
<tr>
<td>T&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Cytotoxic T lymphocyte</td>
</tr>
<tr>
<td>Th&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Thelper1</td>
</tr>
<tr>
<td>Th&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Thelper2</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, Nodes and Metastasis</td>
</tr>
<tr>
<td>tPSA</td>
<td>Total PSA</td>
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<tr>
<td>Treg</td>
<td>T Regulatory Cells</td>
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<tr>
<td>TRUS</td>
<td>Trans Rectal Ultrasound</td>
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Prostate cancer (PCa) is Kenya’s most frequently diagnosed cancer of men with an incidence rate of 32 per 100,000 and mortality rate of 12 per 100,000. It is also the leading cancer in terms of incidence and mortality in men from Africa and the Caribbean and the numbers may double by 2030. Prostate Specific Antigen (PSA) is the main diagnostic biomarker used for screening patients currently. Biopsy histology is the only confirmatory test, but it is invasive and tedious to perform. There is an urgent need to develop other accurate biomarkers that can be used for screening and diagnosis. Cytokines have potential but have not been extensively studied as biomarkers. The objectives of this study were to evaluate the cytokine profile, determine the PSA levels in PCa patients and the risk factors that lead to the development of PCa. The study profiled six cytokines (interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-Gamma) and tumor necrosis factor alpha (TNF-Alpha) and the total PSA levels in the serum of 45 PCa patients, 7 benign prostate enlargement (BPE) patients and 7 healthy males as controls. Cytokines were measured using a highly sensitive cytometric bead array technique. PSA levels were determined using enzyme linked immunosorbent assay (ELISA) technique. A questionnaire was used to determine the demographic and risk factors that lead to the development of PCa. Statistical analysis was performed using statistical package for social sciences (SPSS) version 21. Analysis of variance (ANOVA) was used to compare the means of the cytokines and PSA levels in the different Gleason scores of the PCa patients. Pearson correlation was used to correlate the six individual cytokines and PSA levels. Statistical significance was set at the level of p<0.05 for both the ANOVA and the Pearson correlation test. The results showed that serum levels of all cytokines and PSA were significantly (p<0.05) higher in the PCa and the BPE patients. The PCa patients with a Gleason score of 8-10 had the highest levels of all the cytokines followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. TNF-Alpha and IL-6 were the cytokines with the highest levels while IL-4 and IL-10 had the lowest levels. The cytokine levels among the BPE patients were less than those of the PCa patients. The PSA levels were also significantly higher (p=0.002) in the BPE and PCa patients and they were significantly correlated (p=0.01) with the six cytokines in the two study groups. Kenyan men who are above 50 years are at risk of suffering from PCa. These results indicated that cytokine profiles in PCa patients are distinct by the stage of the disease. TNF-Alpha, IL-6, IFN-Gamma, IL-2 and IL-4 may be potential early diagnostic biomarkers for prostate cancer. The current data forms a basis for further investigations.
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The International Agency for Research on Cancer (IARC) estimates that prostate cancer (PCa) is the leading cancer in terms of incidence and mortality in men from Africa and the Caribbean. Men of Sub-Saharan Africa descent around the world appear to suffer disproportionately from PCa compared to men of other races and ethnicities (Odedina et al., 2009). IARC also estimates that PCa is a growing problem in Africa having caused approximately 28,006 deaths in 2010, and estimates approximately 57,048 deaths by 2030 (Ferlay et al., 2010 b). This represents a 104% increase in the number of PCa deaths in Africa over the next two decades.

The incidence of PCa is on the increase in Kenya (Ngugi and Byakika, 2007). According to the Kenya National Cancer Control Strategy 2011-2016, (2011) the most common cancers in Kenyan men are PCa, oesophagus cancer and Kaposi Sarcoma. Prostate cancer is the top male cancer in Kenya according to GLOBOCAN, (2012) with an estimate of 2,527 new cases and causing approximately 2,048 deaths each year. The standardized incidence rate of PCa is 31.6/100,000. Based on 2002 data from the Nairobi cancer registry, PCa accounted for 9.4% of the total cancers registered. Little is known about the epidemiology of PCa among men in Kenya. Many patients present with late and clinically advanced PCa, which is difficult to treat leading to an increase in mortality (Wasike and Magoha, 2007).
The main diagnostic biomarker in current use is prostate-specific antigen (PSA) which is found in serum/plasma. The PSA test is not specific because the levels are elevated in other conditions like Benign Prostatic Hyperplasia (BPH) and prostatitis. The test can also miss out on PCa cases because some men with low PSA levels (less than 4ng/mL) have been found to harbor PCa; hence it has been difficult to set the threshold limit (Crawford et al., 2011). Decisive diagnosis of PCa is based on prostate biopsy, but has a disadvantage of being very invasive. Thus the discovery of less-invasive PCa biomarkers remains urgent. The need to improve accuracy of diagnosis has led to research into a number of promising new biomarkers. These include genetic, for example nucleotide polymorphisms and gene fusions, blood, including urokinase plasminogen activator, interleukin 6, and circulating tumor cells and urine based biomarkers which include, cytokeratin, P63 (tumor protein 63), and Ki-67 (Artibani, 2012).

The utilization of urine has emerged as an attractive option for the less-invasive detection of PCa; the problem is that none of the urine biomarkers which include: adenomatous polyposis coli (APC), matrix metallo proteinase 9 (MMP9) and human prostatic acid phosphatase (PAP) has been validated (Rigau et al., 2013). Other emerging biomarkers under study include: cytokines, kallikrein-related peptidase 2 (KLK2), early prostate cancer antigens (EPCA), PCA3, hepsin, prostate stem cell antigen, and alpha-methyl acyl-CoA racemase (AMACR) (Sardana et al., 2008).

The prostate epithelial cells have been shown to produce cytokines that influence the growth and differentiation of normal and prostate cancer cells (Ricote et al., 2004). It
has been suggested that these cytokines are associated with the pathophysiology of prostate carcinoma (Tazaki et al., 2011). The cytokines that have been evaluated include IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IFN-Gamma and TNF-Alpha (Tazaki et al., 2011 and Maria et al., 2005). However the cytokine profile in the different stages of PCa has not been compared to the PSA levels and the role of the cytokines in the pathophysiology of prostate carcinoma is not yet clear. None of the cytokines that have been studied has been validated as a marker of prostate cancer. A comprehensive cataloguing of cytokines from the patients in the different stages of PCa may provide further insight into the mechanisms of PCa initiation and progression and may facilitate the exploration of new biomarkers. This study evaluated cytokines as alternative biomarkers and determined the PSA levels in the different stages of the disease. The study also established various demographic and risk factors in the development of PCa.

1.2 Problem Statement

Prostate Specific Antigen (PSA) test is the main method used for screening prostate cancer in Kenya. There is controversy about using the PSA test to look for PCa in men with no symptoms of the disease. Although the PSA test has been shown to lower death rates from the disease by finding PCa earlier, it also has increased the number of unnecessary prostate biopsies and treatments. The PSA test is non-specific and it can miss out on positive cases when the levels are low. There is no information on the variation of PSA levels in the different stages of PCa. There is need to develop other biomarkers that can effectively screen for PCa. Cytokines have been suggested to be useful biomarkers of various diseases (for example IL-7 in rheumatoid arthritis). Since
the cytokine profile in different stages of PCa has not been studied, a comprehensive cataloguing of the cytokine profile in the different stages of PCa may determine the cytokines that may play a role as biomarkers in the early stages of PCa. This study therefore investigates cytokines as diagnostic biomarkers for PCa.

1.3 Justification

The incidence of PCa is on the increase and it is predicted that the number of cases will almost double by 2030 (Ferlay et al., 2010 c). The use of PSA as a marker for screening and diagnosis of PCa is controversial because of its lack of specificity. Normal levels have been observed in patients with tumor pathology; hence PSA is not indicative of the evolution grade of the disease (Roddam et al., 2005). Histology is still widely used because of its accuracy although it is tedious and invasive. There is need to develop accurate and precise non-invasive biomarkers that can be used to screen for PCa. Cytokines have been proposed as potential biomarkers for PCa. Several diseases use cytokines as biomarkers (for example use of interleukin 1 beta in cardiovascular diseases). Specific cytokine levels rise during the progression of different diseases. Since cytokines have been found to play a role in the progression of PCa, a comprehensive cataloguing of cytokines from the patients in different stages may provide further insight into the mechanisms of PCa initiation and progression and may facilitate the exploration of new biomarkers. This will help in understanding the cytokine profile in relation to PSA levels in the progression of PCa.
1.4 Research Questions

1. What is the cytokine profile in the different stages of prostate cancer?

2. What are the Prostate Specific Antigen levels in the different stages of prostate cancer?

3. What are the demographic and risk factors that could lead to the development of PCa?

1.5 Hypotheses

HO₁: The cytokine levels do not significantly differ in the different stages of prostate cancer.

HO₂: The PSA levels do not significantly differ in the different stages of prostate cancer.

1.6 Objectives

1.6.1 General Objective

To evaluate the cytokine profile and determine the PSA levels in the different stages of prostate cancer.

1.6.2 Specific Objectives

1. To determine the cytokine profile in the different stages of prostate cancer.

2. To determine the prostate specific antigen levels in the different stages of prostate cancer.

3. To determine the social demographic factors and risk factors that lead to the development of prostate cancer.
1.7 Expected Study Output

A comprehensive cytokine cataloguing may provide information on cytokines that can be used as biomarkers for PCa. The biomarkers might in turn assist in diagnosis of PCa during the early stages of the disease and this will lower the mortality rates. Correlating the individual cytokines and PSA levels will help in understanding the cytokine profile in relation to PSA levels in the progression of PCa.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Prostate Cancer

Prostate cancer (PCa) is the second most frequently diagnosed cancer of men (1.1 million new cases in 2012, 15% of the total cancers diagnosed in men) and the fourth most common cancer overall worldwide (GLOBOCAN, 2012). Almost 70% of the registered cases occur in developed regions (759,000 cases) (GLOBOCAN, 2012). Incidence rates of PCa vary by more than 25-fold worldwide, the highest rates are in Australia/New Zealand and Northern America (111.6 and 97.2 per 100,000 respectively) and in Western and Northern Europe, largely because the practice of prostate specific antigen (PSA) testing and subsequent biopsy has become widespread in those regions (GLOBOCAN, 2012). Incidence rates are relatively high in certain developing regions such as the Caribbean, South America and sub-Saharan Africa. The lowest age-standardized incidence rate is estimated in South-Central Asia (4.5 per 100,000) (GLOBOCAN, 2012).

There was an estimated 307,000 deaths in 2012 due to PCa (GLOBOCAN, 2012). Since PSA testing has a much greater effect on incidence than on mortality, there is less variation in mortality rates worldwide (10-fold) than is observed for incidence (25-fold), and the number of deaths from PCa is almost the same in developed and developing regions (GLOBOCAN, 2008). Mortality rates are generally high in predominantly black populations (Caribbean, 29 per 100,000 and sub-Saharan Africa, age standardized rates...
(ASRs) 19-24 per 100,000), very low in Asia (ASR 2.9 per 100,000 in Eastern Asia for example) and intermediate in Europe and Oceania (GLOBOCAN, 2012).

2.2 Tumor Immunology

The immune response to tumors includes cytotoxic T lymphocytes mediated lysis, natural killer cell activity, macrophage-mediated tumor destruction and destruction mediated by antibody-dependent cell-mediated cytotoxicity. Several cytotoxic factors, including TNF-Alpha and TNF-Beta, help to mediate tumor cell killing. A study by Poutahidis et al (2009) suggest that lymphocytes protect against cancer and that protection from PCa resides in anti-inflammatory CD4+CD25+ T regulatory (Treg) cells that down regulate inflammatory cytokines. He showed that chronically elevated pro-inflammatory cytokines promoted carcinoma in Apc (Min/+) mice. Treg lymphocytes down regulated inflammation - associated carcinogenic processes and contributed to immune and epithelial homeostasis. Tumors use several strategies to evade the immune response. Cytokines play a role in the destruction of tumor cells hence they have potential to become biomarkers of different tumors (Dranoff, 2004).

2.3 Biomarkers

A biomarker is a distinct biochemical, genetic, or molecular characteristic or substance that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention (Gutman and Kessler, 2006). Genetic, epigenetic, proteomic, glycomic, and imaging biomarkers can be used to help diagnose conditions (diagnostic), to forecast how aggressive a condition is, as in the case of determining a patient's ability to fare in the
absence of treatment (prognostic) and to predict how well a patient will respond to treatment (predictive) (Mishra and Verma, 2010).

2.3.1 Biomarkers in Cancer

The ideal cancer marker would be one which is specifically made by a tumor or its precursor morphologic lesion or a specific response of the body to the presence of cancer and which enters the circulation at an early enough stage to be a biomarker useful in the early detection of cancer (Sardana et al., 2008). Another requirement is that the biomarker should be highly specific for the tissue of origin because if other tissues also produce this biomarker, then its background level in normal healthy individuals will likely be high. Another caveat for non–tissue specific biomarkers is that, if the level of a biomarker is affected by a non-cancer disease, then its utility for cancer detection may also be compromised (Diamandis, 2010). Ideally, biomarkers should be easily accessible such that they can be sampled non-invasively. Therefore biomarkers that can be sampled from body fluids, such as serum or urine, are particularly desirable (Gilad et al., 2008).

While numerous challenges exist in translating biomarker research into the clinical space; a number of biomarkers have already been used at some point in patient care in different types of cancer; including, alpha-feto protein (AFP) in liver cancer (Behne and Copur, 2012), break point cluster region - abelson leukemia protein (BCR-ABL) in chronic myeloid leukemia, breast cancer genes 1 and 2 (BRCA1/BRCA2) in breast/ovarian cancer (Musolino et al., 2007), B-Raf proto-oncogene, serine/threonine kinase (BRAF V600E gene) in melanoma/colorectal cancer (Dienstmann and
Taberner, 2011), cancer antigen 125 (CA-125) in ovarian cancer, cancer antigen 19.9 (CA19.9) in pancreatic cancer, carcinoembryonic antigen (CEA) in colorectal cancer, epidermal growth factor receptor (EGFR) in non-small-cell lung carcinoma (Lamparella et al., 2013), human epidermal growth factor receptor 2 (HER-2) in breast cancer (Orphanos and Kountourakis, 2012), V-Kit hardy-zuckerman 4 feline sarcoma viral oncogene homolog (KIT) in gastrointestinal stromal tumor (De Primo et al., 2009), prostate specific antigen (PSA) in prostate cancer (Bantis and Grammaticos, 2012) and soluble protein (S100 protein) in melanoma (Kruijff and Hoekstra, 2012). The use of most of these biomarkers is still not definite hence research is going on to discover more sensitive and specific biomarkers.

Prostate specific antigen is the main biomarker used to diagnose prostate cancer, but with its use becoming increasingly questionable several other PCa biomarkers are under study. Other emerging biomarkers under study include: cytokines, kallikrein-related peptidase 2 (KLK2), early prostate cancer antigens (EPCA), PCA3, hepsin, prostate stem cell antigen, and alpha-methyl acyl-CoA racemase (AMACR) (Sardana et al., 2008).

2.3.2 Cytokines as Biomarkers in Cancer

Cytokines play an important role in cancer pathogenesis and act as potential biomarkers for cancer (Dranoff, 2004). The mixture of cytokines that is produced in the tumor microenvironment has an important role in cancer pathogenesis. Cytokines that are released in response to infection, inflammation and immunity can function to inhibit tumor development and progression. Alternatively, cancer cells can respond to host-
derived cytokines that promote growth, attenuate apoptosis and facilitate invasion and metastasis (Dranoff, 2004).

Cytokines that promote inflammation and act to make disease worse and are called pro-inflammatory cytokines, whereas cytokines that serve to reduce inflammation and promote healing and are called anti-inflammatory cytokines (Yosra et al., 2009). Cytokines directly stimulate immune effector cells and stromal cells at the tumor site and enhance tumor cell recognition by cytotoxic effector cells (Sylvia and Kim, 2011). According to Lee and Margolin, (2011), animal tumor model studies have demonstrated that cytokines have broad anti-tumor activity which has been translated into a number of cytokine based approaches for cancer therapy.

Cytokines are used as diagnostic, prognostic and predictive biomarkers in different stages of the cancer. Cui et al (2013) showed that high levels of IL-17 expression in the tumor tissues may be a good prognostic marker for patients with glioblastoma multiforme. Li et al (2013) showed that cysteine-cysteine chemokine ligand 15 (CCL-15) may be a specific proteomic biomarker of hepatocellular carcinoma, which has an important role in tumorigenesis and tumor invasion. A study by Yuan et al., 2013, showed that high cysteine-cysteine chemokine ligand 18 (CCL-18) level might be an independent biomarker for predicting better survival of patients with colorectal cancer.

2.3.3 Cytokines in Prostate Cancer

The prostate epithelial cells have been shown to produce pro-inflammatory cytokine in androgen-dependent and androgen-independent prostate cells and influence the growth and differentiation of normal and prostate cancer cells (Ricote et al., 2004). These
cytokines have the potential to become more effective biomarkers of PCa as compared to PSA whose use as a biomarker has become increasingly questionable.

Chronic or recurrent inflammation is responsible for the development of many human cancers including prostate cancer (De Marzo et al. 2004, Dennis et al. 2002 and Roberts et al., 2004). Cytokines are key mediators of inflammation and also play an important role in initiation and progression of PCa (Fujita et al., 2008). Various cytokines for example: IL-1, IL-2, & IL-6 play a role in enhancing or suppressing immunological responses to tumors (Tazaki et al., 2011). Cytokines have a direct inhibitory or growth promoting effect on tumor growth (Dranoff, 2004). Elevated serum levels of IL-1, IL-6 and TNF-Alpha have been previously described in PCa, BPE specimens and in prostate cancer cell lines (Chen et al., 2000 and Mizokami et al., 2000).

Agarwal et al (2013), showed that serum cysteine-cysteine chemokine ligand 11 (CCL-11) levels may provide a useful diagnostic tool to help distinguish between prostatic enlargement and prostate cancer among men demonstrating low, but detectable, serum PSA values. A study by Yong et al (2014), showed that serum levels of macrophage colony stimulating factor (M-CSF) and CC chemokine ligand 18 (CCL-18) were remarkably higher in PCa patients than those in BPH patients, while serum levels of insulin-like growth factor-binding protein 6 (IGFBP-6) and Fas receptor (Fas), also called tumor necrosis factor receptor superfamily member 6 (TNFRSF6), were significantly. Serum levels of these four cytokines could distinguish PCa from BPH with high sensitivity and high specificity. CCL-18 and IGFBP-6 are new potential
serum biomarkers for PCa (Yong et al., 2014). Other cytokines expressed in PCa include: IL-1, IL-2, IL-4, IFN- Gamma, IL-6, IL-8, TNF-Alpha and IL-10 (Tazaki et al., 2011).

Interleukin-1 Beta (IL-1β) has been shown to support the skeletal colonization and metastatic progression of PCa cells with an acquired neuroendocrine phenotype (Liu et al., 2013). Bone tropic phenotypes of PCa cells up-regulate genes encoding for the cytokine IL-1 β. In a study conducted on mice, the exogenous overexpression of IL-1 β in non-metastatic cancer cells promoted their growth into large skeletal lesions, whereas its knockdown significantly impaired the bone progression of highly metastatic cells (Liu et al., 2013).

Interleukin-2 (IL-2) is a glycoprotein with a molecular weight of 15–17 kDa made up of 133 amino-acids and it is produced by Thelper1 (Th₁) lymphocytes but also by cytotoxic T-lymphocytes (Tc) after antigenic stimulation (Dolman et al., 1998). It has an effect on proliferation and activation of some other T-lymphocytes (which produce some other cytokines such as IL-4, IFN and lymphokines) and also on natural killer (NK) cells, these latter having the capacity of lysing several types of cells including the tumoral ones (Royuela et al., 2000). It is the major growth factor for T cells. It also promotes the growth of B cells and can activate NK cells and monocytes.

Interleukin-2, an immune-regulatory cytokine with potentially anti-tumor effect plays a role in PCa progression (Dolman et al., 1998). It was reported to effectively inhibit growth and dissemination of lung and bone marrow metastases of human prostate carcinoma in a study of severe combined immune-deficient mice (Dolman et al., 1998).
A comparative semi-quantitative immuno-histochemical study by Royuela et al., (2000) found that immunoreactions of IL-2 were much higher in PCa samples than in normal prostates.

Interleukin-4 (IL-4) is produced by macrophages and Thelper2 (Th2) cells. It stimulates the development of Th2 cells from naïve Thelper cells and it promotes the growth of differentiated Th2 cells resulting in the production of an antibody response. It also stimulates Immunoglobulin class switching to the Immunoglobulin E isotype (Roca et al., 2012). IL-4 plays a critical role in the regulation of immune responses and has been detected at high levels in the tumor microenvironment of cancer patients where it correlates with the grade of malignancy (Roca et al., 2012). The direct effect of IL-4 on cancer cells has been associated with increased cell survival (Roca et al., 2012). A study conducted by Wise et al (2000) demonstrated that IL-4 levels are significantly elevated in hormone-refractory PCa compared with values in hormone-sensitive PCa, and that IL-4 levels are directly correlated with elevated levels of serum PSA.

Interferon Gamma (IFN-γ) is an important cytokine produced by primarily by Th1 cells, although it can also be produced by Tc and NK cells to a lesser extent (Lee et al., 2008). It is mainly involved in cell growth/activation and it enhances major histocompatibility complex (MHC) expression. IFN-γ has been found to reduce the binding affinity for bone matrix stroma in PCa cells, indicating that this cytokine may be effective in reducing the secondary skeletal tumors common in PCa (Sokoloff et al., 1996). IFN-γ tumor suppressor genes have been shown to be deleted in PCa which leads to a decreased tumor suppressor activity of the IFN- Gamma (Lee et al., 2008).
IL-6 is a protein having a molecular weight between 21-45 kDa made up of 184 amino-acids synthesized into the body by several cells: fibroblasts, activated macrophages or monocytes, activated T and B cells, endothelial cells, stromal cells, and a variety of cancer cells (Maria et al., 2005). IL-6 is involved in co-stimulation, cell growth/activation and it is an acute phase reactant. IL-6 is secreted by both normal and neoplastic prostatic epithelial cells and can act as a growth factor for normal prostatic epithelial cells as well as for PCa cells (Giri et al., 2001).

Studies of Interleukin 6 (IL-6) show that it has been characterized as a prostate exocrine gene product that interacts with its receptor in prostate cells, regulating proliferation and differentiation into cancer cells, and activating androgen receptor (Michalaki et al., 2004). A review by Nguyen et al., (2014) suggested that IL-6 is able to promote prostate cancer cell proliferation and inhibit apoptosis in vitro and in vivo.

A study done by Yosra et al (2009) showed that IL-6 levels were increased in PCa and BPE patients. The study suggested that the pro-inflammatory cytokine IL-6 contributes in a paracrine and autocrine fashion to neoplastic cell proliferation and increase survival of initiated and damaged epithelial cells. Shariat et al (2001) showed that elevated serum IL-6 levels were associated with PCa and high PSA levels. Commonly used PCa lines (PC3, DU145, and LNCaP) express high-affinity receptors for IL-6, and PC3 and DU145 cell lines secrete IL-6 (Chung et al., 1999). IL-6 protein concentrations are increased (approximately 18-fold) in localized PCa when compared to normal prostate tissue (Okamoto et al., 1997).
Tumor Necrosis Factor Alpha (TNF-α) is a 17 kDa protein secreted by cells such as: monocytes/macrophages, T, B and NK lymphocytes, neutrophils, astrocytes, endothelial cells, smooth muscle cells; the protein has proved to be an important antitumor agent with cytotoxic, cytostatic and immuno-modulating effects (Michalaki et al., 2004). TNF-α is an acute phase reactant which participates in the vascular phases of the inflammation (Muenchen et al., 2000). TNF-alpha is a pleiotropic cytokine which has been shown to be associated with cancer progression (Michalaki et al., 2004). Many androgen-insensitive PCa cells are TNF-alpha insensitive (Muenchen et al., 2000). This may be because of the up-regulation of a series of anti-apoptotic genes involved in a network of paracrine and autocrine loops that modulate prostate carcinoma cell activity, and these include the nuclear factor-kappa b (NF-kb) family of transcription factors (Muenchen et al., 2000).

Interleukin 8 (IL-8) promotes the growth of tumor cells, and has been shown to influence the progression of solid tumors such as PCa (Haverkamp et al., 2008). Increased levels are associated with higher Gleason scores and metastatic disease (Haverkamp et al., 2008).

Interleukin-10 (IL-10) is produced by activated macrophages and Th2 cells. It is predominantly an inhibitory cytokine (inhibits antigen presenting cells and inhibits production of other cytokines). It inhibits production of IFN-γ by Th1 cells, which shifts immune responses toward a Th2 type. It also inhibits cytokine production by activated macrophages and the expression of class II MHC and co-stimulatory molecules on macrophages, resulting in a dampening of immune responses (Badger et al., 2008).
Interleukin 10 (IL-10) is a pleiotropic cytokine with both anti-inflammatory and anti-angiogenic properties (Badger et al., 2008). The anti-inflammatory properties of IL-10 are hypothesized to have pro-tumorigenic potential by enabling tumor cells to escape immune surveillance (Huang et al., 1996). Paradoxically, IL-10 also has anti-tumorigenic properties, since in both animal and in vitro models IL-10 has been shown to reduce both tumor growth and angiogenesis (Stearns and Wang, 1998).

2.4 Risk Factors in Prostate Cancer

Prostate cancer has a low overall incidence in men younger than 50 years of age, who represent less than 0.1% of all affected patients (Gronberg, 2003). Approximately 85% of cases of PCa are diagnosed after the age of 65 years. PCa incidence strongly increases with age. Based on US Surveillance, Epidemiology and End Results Program statistics from 2000-2008, the incidence rate of prostate cancer is 9.2/100,000 for men aged 40–44 years. That rate increases sharply to 984.8/100,000 in men aged 70–74 years, after which it slightly decreases (US Surveillance, Epidemiology, and End Results Program of the National Cancer Institute) (SEER, 2011).

According to Gronberg, (2003) PCa typically develops slowly and the cancer may be preceded by dysplastic lesions for many years, or even decades. Extrapolations from autopsy studies suggest that most men would have PCa if they lived to be more than 100 years old. The number of prostate cancers found incidentally at autopsy, which had been asymptomatic and not a cause of death, suggests that small, localized prostate cancers can remain unrecognized for many years before progressing to clinically significant disease (Frankel et al., 2003). Although the lifetime risk of developing
microscopic PCa for a man of 50 years is 42%, the risk of his dying of prostate cancer is only about 3% (Etzioni et al., 2002).

The incidence of PCa varies widely between different ethnic groups and countries. The lowest rates of PCa are found in Asia probably as a result of a lower genetic risk (Gronberg, 2003). Incidence rates of PCa tend to be higher in northern and central European countries than in southern and eastern European countries (Ferlay et al., 2010a). In 2008, in Europe as a whole, the incidence rate of prostate cancer was 93.4/100,000, ranging from a low of 27.7 per 100,000 in the Ukraine to a high of 183.1 per 100,000 in Ireland. In the USA, the incidence of PCa is several times higher than in Japan. Also, USA rates are 1.6 times higher among African–American men than among Caucasian men (Ferlay et al., 2010c).

Epidemiologic studies performed as early as 1960 proposed a familial aggregation of PCa, which suggested that the risk was increased in men with an affected first-degree relative (Woolf, 1960). A study by Singh et al. (2000) showed that some high-risk genes had been identified, which when present, may predispose a carrier to development of the PCa. These high risk genes were shown to be passed down within the family lineage making members of a specific family to be more prone and increasing the risk of developing PCa. Examples of PCa susceptibility genes include: HPC1 on chromosome 1q24-25, HPCX on Xq27-28, BRCA1 on 17q21 and BRCA2 on 13q12, CAPB at 1p36, PCAP on 1q42.2-43 and ELAC2/HPC2 on chromosome 17p (Tavtigian et al., 2001).

Epidemiologic, genetic, and molecular evidence also suggests that infection-associated inflammation and hyper-proliferation contributes to the development of PCa (Platz and
Exposure to environmental factors such as infectious agents and dietary changes, and hormonal imbalances lead to injury of the prostate and the development of chronic inflammation and regenerative risk factor lesions which could in turn lead to the development of PCa (Angelo et al., 2007).

Inflammation caused by microbial pathogens, host genetics, and the environment contribute to the development of PCa. Persistent inflammation causes increased proliferation to replace damaged tissue accompanied by an elaborate production of cytokines, which alters the prostatic microenvironment and promotes tumor genesis (Mukherjee et al., 2014). In the majority of PCa biopsies, inflammation is observed either within the tumor or in close proximity to it (De Nunzio, 2011). A study conducted by MacLennan, (2006) among 180 men with suspected PCa who were biopsied at baseline and after 5 years of follow-up showed that the 5-year PCa incidence was 20% for men with biopsy specimens showing inflammation at baseline compared with 6% for men with no inflammation in baseline biopsies.

A high body mass index (BMI) associated with obesity has been suggested to be a risk factor for PCa. High level of physical activity is also associated with decreased risk of PCa (MacInnis and English, 2006). Certain metabolic alterations sustained in obese men, such as increased levels of insulin, insulin-like growth factor-1 (IGF-1), and leptin may increase PCa risk (Hsing et al., 2001).

Individuals with type 2 diabetes are characterized by hyper-insulinemia. Hyper-insulinemia is associated with reduced levels of insulin-like growth factor-binding protein (IGFBP) and sex hormone-binding globulin (SHBG) and enhanced levels of
circulating IGF-1 and testosterone (Giovannucci, 2003). IGF-1 stimulates prostate tumor cell growth and is related to increased prostate cancer risk (Chan et al., 1998).

Smoking and excessive consumption of alcohol have been suggested to increase the chances of developing PCa (Schoonen, 2005). Smoking may promote the development of more aggressive, hormone-sensitive tumors through numerous mechanisms, including effects on sex steroid hormone levels, mutations in tumor suppressor genes such as p53, and continued exposure to carcinogens such as polycyclic aromatic hydrocarbons contained in cigarette smoke (Zu and Giovannucci, 2009). The first metabolite of alcohol, acetaldehyde, is a potent carcinogen, and alcohol consumption is considered to be a risk factor for many cancers. As PCa possibly develops over many decades, it may be long-term alcohol drinking habits that are related to PCa risk rather than drinking behavior close to diagnosis (AICR, 2007).

High consumption of meat and dairy products has been linked to a greater risk of developing PCa (Wolk, 2005). High meat consumption has been found to be correlated with increased cancer incidence since it is rich in saturated fats and cholesterol (AICR, 2007). Dairy products are also known to be rich in saturated fats. Increased intake of vitamin E and selenium (from supplements) has been shown in intervention studies to decrease the risk (Wolk, 2005).

Sexual behavior has been thought to be associated with PCa for several reasons. Some factors that might produce this reasoning include an increased likelihood of acquiring a sexually transmitted disease (STD), having a high number of sexual partners, and having higher circulating testosterone levels (Dennis and Dawson, 2002). Frequent
ejaculation has been reported to have a protective effect against PCa, although the biological basis of this effect is unknown (Dennis and Dawson, 2002). High risk human papilloma virus (HPV) and Epstein-Barr virus are thought to be collaborating with each other in most male PCa cases (Whitaker et al., 2013). Microorganisms that cause prostatitis are believed to promote chronic inflammation by inflicting cellular damage, cellular hyper-proliferation, and increased production of cytokines (Coussens and Werb, 2002). While cytokines are required to limit tissue damage by replacing damaged cells, angiogenesis, and tissue repair, uncontrolled response promotes the progression from benign prostatitis to intraepithelial neoplasia (PIN) and cancer (De Marzo et al, 1999, Putzi and De Marzo, 2000). Therefore, preventing or reducing inflammation would serve as an attractive mechanism in the chemoprevention of PCa.

2.5 Anatomy of the Prostate

Prostate cancer is cancer of the prostate gland found in men. The prostate is a small, walnut shaped (and sized) gland, which is located below the bladder in humans (Devens et al., 2000) (Figure 2.1). In a young man, the normal prostate gland is the size of a walnut (<30g). During normal aging, however, the gland usually grows larger. This hormone-related enlargement with aging is called benign prostatic hyperplasia (BPH), but this condition is not associated with PCa.
The gland surrounds the urethra and has a fibro muscular function which acts to restrict urine flow, but its principal function is secretory, producing a number of essential proteins for the functioning of sperm, such as acid phosphatase, citric acid and bioavailable zinc (Devens et al., 2000). It makes some of the highest amounts of polyamines, which regulate the pH of sperm, preserving a mildly alkaline environment for the sperm within the acidic female cervix (Devens et al., 2000).

There are four major zones within the normal prostate: the peripheral zone, the central zone, the transition zone, and the anterior fibro-muscular stroma (Mc Neal, 1981) (Figure 2.2). The peripheral zone extends postero-laterally around the gland from the apex to the base and represents the most common site in the prostate for developing prostate carcinomas. The central zone surrounds the ejaculatory duct apparatus and makes up the majority of the prostatic base. The transition zone constitutes two small
lobules that abut the prostatic urethra and represent the region where benign prostatic hyperplasia primarily originates (McNeal et al., 1988). Compared to peripheral zone cancers, carcinomas that originate in the transition zone have been suggested to be of lower malignant potential; however, other studies have suggested that there is no difference in outcome when controlled for grade and stage (Reissigl et al., 1997).

**Figure 2.2: Prostate Gland Zones (American journal of Roentgenology)**

### 2.6 Pathology of Prostate Cancer

Prostate cancer is classified as an adenocarcinoma which begins when normal prostate gland cells mutate into cancer cells (Humphrey, 2003). Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma *in situ* or prostatic intraepithelial neoplasia (PIN) (Bostwick and Cheng, 2012). Over time, these cancer cells begin to multiply and spread to the surrounding prostate tissue forming a tumor. Eventually, the tumor may grow large enough to invade nearby organs. It commonly metastasizes to the bones, lymph nodes, and may invade rectum, bladder and lower ureters after local progression (Bostwick and Cheng, 2012).
The following are the most common symptoms of PCa according to Miller et al., (2003): Weak or interrupted flow of urine, urinating often (especially at night), difficulty urinating or holding back urine, pain or burning when urinating, blood in the urine or semen, nagging pain in the back, hips, or pelvis, trouble getting an erection and painful ejaculation.

Detrimental variants in genes involved in the inflammatory pathway and immune response could increase the risk of chronic inflammatory stimulation of growth mechanisms in the prostate gland, which could lead to the development of uncontrolled epithelial growth and, ultimately, PCa. A study by Beuten, (2010) showed that mutations in the RNASEL (encodes 2-5A-dependent ribonuclease) and MSR1 (encodes macrophage scavenger receptor types 1 and II) genes - both of which are involved in the response to infection - are associated with increased risk of prostate cancer. Increased levels of plasma C-reactive protein - a marker of low-grade systemic inflammation - have been associated with progression of PCa (Saito & Kihara, 2011).

Proliferative inflammatory atrophy - which is thought to be a precursor of high-grade intraepithelial neoplasia and PCa - arises from areas of the prostate where cells are actively regenerating following tissue injury caused by various pathological processes, including infection (Merrimen et al., 2010). Given the possible influence of inflammation, it has been hypothesized that use of anti-inflammatory drugs could reduce the risk of PCa. In a recent observational population-based trial, use of certain nonsteroidal anti-inflammatory drugs (NSAIDs) was associated with a 10% reduction in PCa risk (Mahmud, 2011). In one meta-analysis of studies relating to the use of
NSAIDs and risk of PCa, this effect was even greater for advanced PCa, with risk reduction approaching 25% (Mahmud et al., 2010).

2.7 Benign Prostate Enlargement

Benign Prostate Enlargement (BPE) is histologically defined as microscopic or macroscopic nodules with hyperplasia of stromal cells and, to lesser extent, of epithelial cells (most often located in the transition zone of the prostate gland) (Guess, 2001). When sufficiently large, the nodules impinge on the urethra and increase resistance to flow of urine from the bladder. This is commonly referred to as "obstruction," although the urethral lumen is no less patent, only compressed. Resistance to urine flow requires the bladder to work harder during voiding, possibly leading to progressive hypertrophy, instability, or weakness (atony) of the bladder muscle (Guess, 2001). BPE is not a premalignant lesion (Alcaraz et al., 2009). Although BPE can cause morbidity and reduce quality of life, it is not a lethal disease per se.

Adenomatous prostatic growth is believed to begin at approximately age 30 years. An estimated 50% of men have histologic evidence of BPE by age 50 years and 75% by age 80 years; in 40–50% of these men, BPE becomes clinically significant. BPE affects approximately 70% of men over age 70 years (McVary, 2006). Benign prostate enlargement and PCa share some important features, including hormone-dependent growth and response to anti-androgen therapy and high PSA levels (Andriole, 2010 and De Nunzio, 2011). Furthermore, studies have indicated that chronic inflammation, metabolic disruption, and genetic variation are common risk factors for both diseases.
De Nunzio et al., 2012), and large epidemiological studies have reported a positive association between the two conditions.

2.8 Diagnosis of Prostate Cancer

Prostate cancer can be detected using various methods namely: digital rectal examination, measurement of serum PSA levels, trans-rectal ultra sound and a prostate biopsy.

2.8.1 Digital Rectal Examination (DRE)

A doctor inserts a gloved finger into the rectum to feel the condition of the prostate that lies close to the rectal wall. Age related enlargement is not a particular concern but if the gland feels abnormally firm or hard, it may sometimes be an indication of an abnormal growth in the prostate gland (Reuben et al., 2012). The DRE is quick, inexpensive and easy to perform, allowing access to the dimensions, shape and boundaries of the prostate, as well as the presence of deformities, bulging, changes in the consistency and mobility of the gland (Bruno et al., 2011).

2.8.2 Prostate Specific Antigen (PSA)

Prostate specific antigen is a glycoprotein secreted into the seminal fluid by the luminal epithelial cells of the prostatic ducts, acini, and peri-urethral glands (Adhyam and Gupta, 2012). PSA (also called hK3) is a member of the human kallikrein gene family and is a 33-kilodalton serine protease (Lilja, 1985). The main role of PSA is the liquefaction of the seminal coagulum. Prostate epithelial cells are the only cells that
secrete PSA. It is expressed by both normal and neoplastic prostate tissue and it is elevated in benign prostatic hyperplasia (BPH) and prostatitis (Chang et al., 2006).

Prostate specific antigen in blood can exist as either complexed (bound) or free (unbound) form. The complexed form (cPSA) is bound to protease inhibitors (α-2 macroglobulin bounded PSA and α-1-anti-chymotrypsin bounded PSA) which deactivate PSA while it is in the serum. Complexed PSA is primarily eliminated by hepatic mechanism (Adhyam and Gupta, 2012). Free PSA (fPSA) is composed of three forms of inactive PSA, which are: proPSA, benign PSA (BPSA) and intact inactive PSA, proPSA and BPSA being the best described (Hori et al., 2012). BPSA is expressed in the transitional zone of the prostate and is associated with BPE, it is a degraded form of PSA. On the other hand, proPSA is expressed almost entirely in the peripheral zone of the prostate where most prostate cancers arise. Free PSA is eliminated through renal excretion (Adhyam and Gupta, 2012).

Under normal conditions, PSA is produced as a pro-enzyme and secreted into the lumen, where the pro-peptide is removed to generate active PSA. The active PSA can then undergo proteolysis to generate inactive PSA, of which a small portion then enters the bloodstream and circulates in an unbound state (free PSA) (Mikolajczyk et al., 2002). Alternatively, active PSA can diffuse directly into the circulation where it is rapidly bound by protease inhibitors (Lilja et al., 1991). PSA can cleave insulin-like growth factor binding protein-3 (IGFBP-3), extra cellular matrix glycoproteins such as fibronectin and laminin (Webber et al., 1995).
In men with a normal prostate the majority of free PSA in the serum reflects the mature protein that has been inactivated by internal proteolytic cleavage (Björk et al., 1996). In contrast, this cleaved fraction is relatively decreased in PCa. Thus, the percentage of free or unbound PSA is lower in the serum of men with PCa compared with those who have a normal prostate or BPE (Balk et al., 2003).

Prostate specific antigen is used extensively as a biomarker to screen for PCa, to detect recurrence following local therapies, and to follow response to systemic therapies for metastatic disease (Adhyam and Gupta, 2012). Early studies established that the reference range of PSA was 0 to 4ng/mL, this was based on the finding that healthy men aged 40 years and younger and 97% of men above 40 had PSA levels equal to and less than 4ng/mL (Campbell, 2012).

Currently, first-line screening of PCa consists of annual digital rectal examination (DRE) and determination of serum PSA levels. The upper limit of normal PSA values is generally considered to be 4ng/mL; between 4-10ng/mL is considered borderline and more than 10ng/mL is considered high. Patients with a PSA value greater than 4ng/mL, regardless of DRE results, generally undergo biopsy (Caplan and Kratz, 2002).

The usefulness of PSA as a screening biomarker has become increasingly questionable with the finding that some men whose PSA levels never exceeded the normal value (which is ≤4ng/mL) had PCa, including high grade cancer (Thompson et al., 2004). This has led to calls for the lowering of the upper limit of normal to 2.5ng/mL (Welch et al., 2005). Thompson et al., (2004) also noted that men with benign DRE and very low levels of PSA (below 0.5ng/Ml) could still have high grade PCa, hence there is no PSA
value at which a man can be assured of not having cancer. There is a risk at all values with higher PSA values associated with a higher PCa risk.

A study by Thompson et al., (2004) reported results on 2,950 men whose PSA never exceeded 4 ng/mL and had prostate biopsy. The prevalence of PCa was 23.9 percent of men with PSA values of 2.1–3.0 ng/mL and 26.9 percent of those with PSA levels of 3.1 to 4.0 ng/mL. Twenty five percent of men in the 3.1–4.0 ng/mL range had high grade cancers. These results demonstrated that PCa, including high grade cancers, is not uncommon in men with PSA levels of 4 ng/mL or less, which are below levels routinely used as a threshold for performing further diagnostic studies such as prostatic biopsy.

PSA has a low positive predictive value (PPV). When PSA is 4–10 ng/mL, the PPV is 18% to 25% (mean, 21%), and when PSA is >10ng/mL, the PPV is 58% to 64% (mean, 61%), when combined with a DRE as a screening tool this still results in approximately 66% negative prostate biopsies (Catalona et al., 1994 and Makarov et al., 2009). These patients are often subjected to repeat PSA measurements and prostate biopsies (the “over-diagnosis” problem). “Over-treatment,” through the detection of non-life-threatening tumors (Tuma, 2010), especially in the so-called gray zone (serum PSA between 4–10ng/mL), represents yet another dilemma, as it is difficult to discriminate between patients with PCa and those with benign prostatic enlargement (BPE) or between those patients suffering from prostatitis and the results of urethral manipulation, which can also increase PSA levels (Thompson et al., 2005).

Major problems in PSA testing arise as a result of both over and under-diagnosis. Some 15% of men whose PSA levels are regarded as normal (4ng/mL or less), do in fact
harbor prostate cancer, including high-grade carcinoma (Greene et al., 2009). By increasing the limit to a level considered clinically borderline (4-10ng/mL), some 25% of men are found to be affected by PCa (Andriole et al., 2009). Prevalence of PCa in men with tPSA ranging 4-10ng/mL in the western world is 22% (Catalona et al., 1991). In Africa, the prevalence of PCa among males with tPSA 4-10ng/mL is 13.3% (Ezenwa et al., 2012 and Kirimi et al., 2009). Wakwabubi noted that 6.6% of patients at Kenyatta national hospital (KNH) with tPSA ranging 0-4ng/mL had PCa (Wakwabubi et al., 2008). This was based on the samples obtained via prostatectomy which usually leaves out the peripheral zone where most of the cancers arise from. Thus, the obtained prevalence could be higher.

Enhanced protease activity has been documented experimentally to facilitate both malignant transformation as well as progression to a metastatic phenotype according to Williams et al., (2007). Thus PSA may be playing a role in the pathobiology of PCa since it is an extracellular protease (Rubin, 2003). Serum PSA measurements show variable reliability when it comes to diagnosis of PCa, given the dynamics of PSA physiology. Surrogate measures like PSA density, PSA velocity, free-to-total PSA ratio, complexed PSA, Age-specific PSA and percentage Pro-PSA, have been used to improve the predictive utility of this assay for PCa (Adhyam and Gupta, 2012). The most definitive roles of PSA appears to be in diagnosing recurrences after adequate surgical treatment, and in evaluating response to treatment (Adhyam and Gupta, 2012).
2.8.3 Biopsy Histology

Histology remains the only method for confirmation of a suspicion of PCa (Gardiner, 2011). In conjunction with serum PSA and DRE, the tumor can then be stratified with the patient’s cancer being categorized as low, medium, or high-risk disease (Gardiner, 2011). Trans-rectal Ultrasound (TRUS) guidance is used to permit spatial positioning of biopsy needles so that the sites more likely to contain cancer can be targeted preferentially.

The procedure is imprecise, unpleasant, and invasive (Gardiner, 2011). Modern needle biopsy techniques have low morbidity, result in fewer ambiguous diagnoses, and provide more specific information about the grade and extent of the tumor than fine-needle aspiration. The difficulty with needle biopsy not only stems from the small amount of tissue available for histological examination, but also arises because biopsies often identify only a few malignant glands among many benign glands (Gardiner, 2011). Morphologically, PCa is difficult to diagnose because the clues to malignant disease can be subtle, increasing the risk of under diagnosis. Although a few histological findings are specific for PCa for example: perineural invasion, glomerulations, and collagenous micro-nodules—in general, diagnosis is made on the basis of architectural, cytological, and ancillary findings (Humphrey, 2004). The tissue that is obtained is then stained using the Haematoxylin and Eosin (H&E) (Appendix IV) stain and then the diagnosis is made according to the Gleason grade.

The Gleason grading system is based entirely on the histologic pattern of arrangement of carcinoma cells in H & E-stained prostatic tissue sections (Humphrey, 2004). The
histologic score can range from 2 to 10. Increasing Gleason grade is directly related to a number of histopathological end points, including tumor size, margin status, and pathologic stage. The score is obtained by adding the primary grade pattern and the secondary grade pattern (Gleason, 1977). The primary pattern is the one that is predominant in the area by simple visual inspection while the secondary pattern is the second most common pattern. If only one grade is in the tissue sample, that grade is multiplied by two to give the score (Gleason, 1992).

The internationally approved system for staging PCa is the tumor, nodes and metastasis system (TNM) (Cheng et al., 2012). It evaluates the size of the tumor, the extent of involved lymph nodes, and any metastasis and also takes into account cancer grade. Staging means trying to find any evidence of spread outside the prostate. Often a Computerized Tomography Scan (CT Scan), Ultrasound or Magnetic Resonance Imaging (MRI) is used to inspect the abdominal cavity in order to attempt to detect any spread to lymph nodes or metastasis to other organs (Cheng et al., 2012).

2.8.4 Prostate Imaging

The three main imaging methods in PCa detection are Ultrasound, CT scan and Magnetic Resonance Imaging (MRI) (Mocarska et al., 2012). According to Bonekamp et al., (2011) MRI examines the prostate and nearby lymph nodes, distinguishing between noncancerous and cancerous areas. Trans-rectal ultrasound (TRUS) examination of an organ is applied to obtain systematic core biopsies for a histological examination (Mocarska et al., 2012).
Previous studies had evaluated certain cytokines independently and more specifically in the pathological perspective and not as biomarkers, however they did not determine how the cytokines differ in the different stages of PCa and also they did not compare PSA levels with the cytokine profile. Out of all the cytokines reviewed IL-2, IL-4, IFN-Gamma, IL-6, IL-8, TNF-Alpha and IL-10 could be potential PCa biomarkers because ongoing studies have shown that each of the cytokines plays a role in the progression of PCa. This study aimed to evaluate cytokines as alternative biomarkers and determine the PSA levels in the different stages of the disease. The study also established various demographic and risk factors in the development of PCa since Kenya is a developing country and all other existing reports on risk factors were done in developed countries.
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site
The study was conducted at Kenyatta National Hospital (KNH) at the urology outpatient clinic between April 2014 and August 2014. The clinic is attended by patients referred from KNH casualty and other hospitals in the country who are suspected to suffer from PCa and all other urogenital system disorders.

3.2 Study Population
The target population was all male patients coming to the KNH urology outpatient clinic suspected to suffer from prostate cancer while the study population was the male patients who were confirmed to be suffering from prostate cancer.

3.3 Research Design
This was a cross sectional study among male patients suspected to suffer from PCa.

3.4 Sampling
The patients who participated in the study were sampled using criterion purposive sampling technique. Patients who were suspected to suffer from prostate cancer with a positive digital rectal examination and elevated prostate specific antigen levels and met the inclusion criteria were enrolled after they gave a written consent to participate in the study.
3.5 Patient Enrolment

Male patients suspected to suffer from PCa were requested to participate in the study. Patients who consented filled a consent form (Appendix Ia or Ib). The consent was signed after the full details of the study were explained to the each patient privately and the patient consented. A detailed medical examination by a urologist accompanied by biopsy histology was then carried out to confirm whether they were suffering from PCa. The grade of the cancer was then determined by examining the histology biopsies and giving the specific Gleason score. The scores correlated to the stage of the disease, the higher the scores the later the stage of the disease. The BPE patients were those patients who had been suspected to suffer from prostate cancer but their biopsies revealed that they had benign prostate enlargement. The control group comprised of healthy male blood donors at the hospital blood donation unit aged between 30-39 years without histories of reproductive and endocrine system disorders or related diseases.

3.5.1 Inclusion Criteria:

1. Male patients suspected to have been suffering from PCa.
2. Patients who consented to participate in the study.
3. Healthy male blood donors aged 30-40 years who consented.

3.5.2 Exclusion Criteria:

1. Patients who did not consent to participate in the study.
2. Healthy male blood donors aged 30-40 years who did not consent.
3.6 Selection of BPE Patients and the Control Group

The BPE patients were those patients who had been suspected to suffer from prostate cancer but their biopsies revealed that they had benign prostate enlargement. The purpose of enrolling the BPE patients was to determine if they expressed cytokines similar to those expressed by PCa patients and how their PSA levels differed from those patients diagnosed with PCa. The control group comprised of healthy male blood donors at the hospital blood donation unit aged between 30-39 years without histories of reproductive, endocrine or related diseases. The purpose of enrolling the controls was to determine if the six cytokines (IL-2, IL-4, IFN- Gamma, IL-6, IL-8, TNF-Alpha and IL-10) were expressed by men without enlarged prostates and to determine their PSA levels.

3.7 Ethical Considerations

Informed consent was obtained from every patient (Appendix Ia or Ib). The patients were informed about the objectives of the study and the risks and benefits of the study. The benefits of the study were that the patients would get results of their cytokine profile and the PSA levels and also any new information obtained from the study may be implemented to help in the early diagnosis of prostate cancer. The only risk that could be experienced by the patients would be bruising during the blood sample collection but that would be taken care of incase it happened. The patients interview and sample collection were done in a private examination room. Anonymity of records and confidentiality on any information gathered from the patients was also maintained.
3.7.1 Ethical Approval

Ethical approval was sought from the Kenyatta University Ethics Review Committee (Appendix III) and Kenyatta National Hospital/University of Nairobi Ethics and Research Committee.

3.8 Sample Size Determination

The minimum sample size of 45 was determined using an online sample size calculator (source:www.Raosoft.com/samplesize.html) using the prevalence of 12.8% (Ngugi and Byakika, 2007), however this study enrolled 52 patients.

\[
n = \frac{N \times (N-1) E^2 + x}{(N-1) E^2 + x} \quad (source:www.Raosoft.com/samplesize.html)
\]

\[
x = Z(c/100)^2 \times (100-r)
\]

Where: \( n \) =sample size, \( N \) = Population size (60)  \( E \) = Margin of error (5%) \( r \) = fraction of responses you are interested in. \( Z(c/100) \) = critical value for confidence level c

Prevalence= 12.8% (Ngugi and Byakika, 2007) Confidence interval= 95%

3.9 Blood Collection

Five (5ml) of venous blood samples was collected from the patients at the clinic using venipuncture technique into serum separator vacutainer tubes by a medical laboratory technologist. The tubes were then coded to conceal the identity of the patient and taken to the laboratory. The blood samples were then centrifuged at 1,500 × g for 10 minutes at 4°C within 30 minutes of collection. The serum was placed in pre-labelled cryovials
and stored at -80°C pending analysis. Samples were thawed overnight at 4°C prior to analysis, centrifuged at 1,500 × g to remove debris, and then aliquoted and analyzed in duplicate. After analysis serum in the vials was disposed into biohazard safety bins and incinerated together.

### 3.10 Structured Questionnaire

A structured questionnaire was used to determine social demographic factors and risk factors that could lead to the development of prostate cancer (Appendix IIa or IIb).

### 3.11 Biopsy Histology

A trans-rectal 12 core prostate biopsy was collected by a doctor guided by the trans-rectal ultrasound through the anus into the rectum. The biopsy cores obtained were preserved in formalin and the containers were labeled with the patients details and taken to the laboratory. The biopsy was then processed appropriately and then stained using the Haematoxylin & Eosin (H&E) staining technique (Appendix IV). The tissue samples were then examined under a microscope by a pathologist to determine the Gleason score (refer to modified Gleason score by the international society of urological pathology). The biopsies of the BPE patients were negative for cancer but had some benign changes. A sample histology grading image is shown on Appendix V.

### 3.12 Cytokine Assay

To determine the cytokine profile in the different stages of PCa, a Cytometric Bead Array assay was carried out on a flow cytometer. BD™ Cytometric Bead Array (CBA) Human Th1/ Th2 cytokine kit II (Catalog No. 550749) was used on a dual-laser flow
cytometer equipped with a 532-nm and a 635-nm laser and the test was run according to the manufacturer’s instructions (bdbiosciences.com). The cytokine kit is shown on Appendix VI. The assay was seeking to quantify the following cytokines in the different stages of PCa: IL-2, IL-4, IL-6, IL-10, TNF-Alpha and IFN-Gamma.

### 3.13 PSA ELISA Assay

To determine the levels of Total PSA in the different stages of PCa, a sandwich ELISA test was carried out. The ELISA kit was obtained from Human Diagnostics Worldwide and the PSA test was carried out according to the manufacturer’s instructions (www.human.de/data/gb/vr/el-psa.pdf). The PSA ELISA kit is shown on appendix VI.

### 3.14 Data Analysis

Data was coded and entered into a computer. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) statistical software package version 21 for Windows. ANOVA was used to compare the means of the cytokines and the PSA levels in the different Gleason scores of the prostate cancer patients. Pearson correlation was used to correlate the six individual cytokines and the PSA levels. Statistical significance was set at the level of p<0.05 for both the ANOVA and the Pearson correlation test.
CHAPTER FOUR

4.0 RESULTS

A total of 52 patients suspected to have PCa were sampled of these, 45 were found to have prostate cancer (PCa) while 7 had benign prostate enlargement (BPE). The negative control group (NC) comprised of 7 healthy adult males and they were recruited from KNH blood donation unit aged 30-39 years with no enlarged prostate and without histories of reproductive, endocrine or related diseases.

The summary of statistics for PCa patients according to their Gleason scores, BPE patients and the control group is shown on Table 4.1. There were no missing values and no obvious errors. There appeared to be no oddities in the data set and therefore analysis was done.

Table 4.1: The number of PCa and BPE Patients.

<table>
<thead>
<tr>
<th>Study subject category</th>
<th>PCa (Gleason scores)</th>
<th>B.P.E</th>
<th>N.C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts</td>
<td>7</td>
<td>18</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>

**PCa**  Prostate Cancer

**B.P.E**  Benign Prostate Enlargement

**N.C**  Negative Control
4.1 Demographic Information and Risk Factors

4.1.1 Age

All the PCa and the BPE patients who were recruited in the study were above 50 years and they were all Kenyans. The ages of the study participants were as follows; PCa patients were between 54 and 102 years, BPE patients were between 55 and 86 years and the NC group was aged between 30 and 39 years (Table 4.2).

Table 4.2: Age of the Study Participants

<table>
<thead>
<tr>
<th>Study Subject Category</th>
<th>Mean Age (Years)</th>
<th>Number of patients (N)</th>
<th>Standard Deviation</th>
<th>Minimum Age</th>
<th>Maximum Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score 2-4</td>
<td>70.14</td>
<td>7</td>
<td>9.118</td>
<td>59</td>
<td>80</td>
</tr>
<tr>
<td>Gleason score 5-7</td>
<td>71.56</td>
<td>18</td>
<td>10.590</td>
<td>54</td>
<td>90</td>
</tr>
<tr>
<td>Gleason score 8-10</td>
<td>76.70</td>
<td>20</td>
<td>10.043</td>
<td>61</td>
<td>102</td>
</tr>
<tr>
<td>B.P.E</td>
<td>70.00</td>
<td>7</td>
<td>9.781</td>
<td>55</td>
<td>86</td>
</tr>
<tr>
<td>N.C</td>
<td>35.29</td>
<td>7</td>
<td>3.638</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>68.64</td>
<td>59</td>
<td>15.671</td>
<td>30</td>
<td>102</td>
</tr>
</tbody>
</table>

**B.P.E:** Benign Prostate Enlargement, **N.C:** Negative Control

4.1.2 Marital Status

Out of all the study subjects 49 comprising 83.1% were married while 10 of the study subjects comprising 16.9% were widowed.
4.1.3 Education Level

Thirty (30) study subjects comprising 50.84% had below secondary level education. Nineteen (19) study subjects comprising 32.20% had secondary level education while 10 of the study subjects comprising 16.94% had post-secondary level of education. Figure 4.1 is a clustered column chart showing the education level of the study subjects.

![Clustered Column Chart](image)

**Figure 4.1: Education Level of Study Subjects**

4.1.4 Smoking Cigarettes

Out of all the study subjects 30.5% were smokers or had smoked at some point of their lives while 69.5% were nonsmokers. Of the total number of smokers, 89% were PCa patients while 11% were BPE patients (Table 4.3).
Table 4.3: Study Participants who Smoked Cigarettes

<table>
<thead>
<tr>
<th>Study Subject Category</th>
<th>Smokers</th>
<th>Non Smokers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score 2-4</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Gleason score 5-7</td>
<td>4</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Gleason score 8-10</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>BPE</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>NC</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18</td>
<td>41</td>
<td>59</td>
</tr>
</tbody>
</table>

**B.P.E:** Benign Prostate Enlargement, **N.C:** Negative Control

4.1.5 Drinking Alcohol

Out of all the study subjects 42.40% drank alcohol or had taken alcohol at some point of their lives while 57.60% did not drink alcohol. Of the total number of those who drank alcohol 88% were PCa patients while 12% were BPE patients (Table 4.4).

Table 4.4: Study Participants who Drunk Alcohol

<table>
<thead>
<tr>
<th>Study Subject Category</th>
<th>Number that drunk alcohol</th>
<th>Number that did not drink alcohol</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score 2-4</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Gleason score 5-7</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Gleason score 8-10</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>BPE</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>NC</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>34</td>
<td>59</td>
</tr>
</tbody>
</table>

**B.P.E:** Benign Prostate Enlargement, **N.C:** Negative Control
4.1.6 Relatives with Prostate Cancer

Out of all the PCa patients only 5 (11.11%) had relatives suffering from PCa. Prostate cancer high risk genes are passed down within the family lineage making members of specific families to be more prone to PCa.

4.1.7 Number of Wives

Majority of the study subjects (91.5%) had one wife while 8.5% had two wives.

4.1.8 Sexual Partners

None of the patients reported to be having other sexual partners apart from their wives.

4.1.9 Casual Sex

None of the patients agreed to have taken part in casual sex at any point of their lives. Sexual activity might expose the prostate to infectious agents, which could lead to chronic inflammation of the prostate which could over time lead to the development of PCa.

4.1.10 Hypertension and Diabetes

Among the PCa patients with a Gleason score of 2-4, 3 patients had both hypertension and diabetes. Gleason 5-7, 1 patient had hypertension while 2 had both hypertension and diabetes while in Gleason 8-10 category 4 patients had hypertension while 2 had both hypertension and diabetes. Among the BPE patients 3 had both hypertension and diabetes. Hypertension and diabetes are both risk factors for the development of PCa (Figure 4.2).
None of the patients had a wife suffering from cervical cancer. Human Papilloma Virus (HPV) is the virus which causes cervical cancer and is transmitted sexually. HPV causes chronic inflammation and prostatitis in the prostate gland, hence it is a risk factor for PCa.

4.1.12 HIV/AIDS Status

Out of the 59 study subjects, 20 (34%) knew their HIV status while 39 (66%) did not know their status. Out of the 20 who knew their status, they said they were all negative. HIV/AIDS is a risk factor in many cancers and various other diseases.
4.2 Cytokine Profile of the Study Participants

TNF-Alpha was the highest cytokine present, (42.31) while IL-10 was the lowest cytokine present, (3.89). Generally the cytokine levels were highest in the prostate cancer patients followed by the BPE patients and the levels were lowest in the control group. Table 4.5 shows the summary statistics for the cytokines in pg/mL.

Table 4.5: Summary Statistics of the Cytokines in pg/mL

<table>
<thead>
<tr>
<th>CYTOKINE</th>
<th>N</th>
<th>Grand mean(pg/mL)</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-Alpha</td>
<td>59</td>
<td>42.31</td>
<td>0.10</td>
<td>80.00</td>
<td>22.68</td>
</tr>
<tr>
<td>IL-6</td>
<td>59</td>
<td>35.99</td>
<td>0.20</td>
<td>73.00</td>
<td>20.99</td>
</tr>
<tr>
<td>IFN-Gamma</td>
<td>59</td>
<td>30.53</td>
<td>0.10</td>
<td>60.00</td>
<td>17.04</td>
</tr>
<tr>
<td>IL-2</td>
<td>59</td>
<td>27.61</td>
<td>0.10</td>
<td>57.00</td>
<td>17.90</td>
</tr>
<tr>
<td>IL-4</td>
<td>59</td>
<td>9.61</td>
<td>0.10</td>
<td>24.00</td>
<td>6.79</td>
</tr>
<tr>
<td>IL-10</td>
<td>59</td>
<td>3.89</td>
<td>0.10</td>
<td>10.00</td>
<td>2.52</td>
</tr>
</tbody>
</table>

IL: Interleukin, IFN: Interferon, TNF: Tumor necrosis factor, Min: Minimum, Max: Maximum, SD: Standard deviation
The individual cytokine levels were distinct among the various study groups. Generally the cytokine levels were highest in the prostate cancer patients followed by the BPE patients and the levels were lowest in the control group. The cytokine means increased with increasing Gleason score (Table 4.6).

**Table 4.6: Individual Cytokine Levels in the Study Participants in pg/mL**

<table>
<thead>
<tr>
<th>Study subject category</th>
<th>IL-6 (Mean)</th>
<th>TNF-Alpha (Mean)</th>
<th>IL-2 (Mean)</th>
<th>IFN-Gamma (Mean)</th>
<th>IL-4 (Mean)</th>
<th>IL-10 (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gleason score 2-4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23.29</td>
<td>32.14</td>
<td>15.71</td>
<td>23.71</td>
<td>5.00</td>
<td>2.29</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>2.69</td>
<td>3.29</td>
<td>2.21</td>
<td>4.46</td>
<td>2.00</td>
<td>1.11</td>
</tr>
<tr>
<td><strong>Gleason score 5-7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>39.06</td>
<td>48.89</td>
<td>29.22</td>
<td>35.11</td>
<td>9.39</td>
<td>4.61</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>SD</td>
<td>7.64</td>
<td>11.34</td>
<td>9.88</td>
<td>7.47</td>
<td>4.10</td>
<td>1.37</td>
</tr>
<tr>
<td><strong>Gleason score 8-10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.20</td>
<td>62.35</td>
<td>46.70</td>
<td>45.05</td>
<td>16.85</td>
<td>5.70</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>SD</td>
<td>11.46</td>
<td>9.40</td>
<td>5.88</td>
<td>10.98</td>
<td>3.87</td>
<td>2.54</td>
</tr>
<tr>
<td><strong>BPE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>15.29</td>
<td>20.14</td>
<td>7.86</td>
<td>14.43</td>
<td>3.43</td>
<td>1.14</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>4.31</td>
<td>10.45</td>
<td>3.24</td>
<td>7.23</td>
<td>1.40</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>N.C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.87</td>
<td>0.46</td>
<td>0.53</td>
<td>0.21</td>
<td>0.31</td>
<td>0.49</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>0.34</td>
<td>0.35</td>
<td>0.41</td>
<td>0.12</td>
<td>0.33</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>35.98</td>
<td>42.30</td>
<td>27.61</td>
<td>30.53</td>
<td>9.61</td>
<td>3.80</td>
</tr>
<tr>
<td>N</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>SD</td>
<td>20.99</td>
<td>22.68</td>
<td>17.91</td>
<td>17.04</td>
<td>6.79</td>
<td>2.61</td>
</tr>
</tbody>
</table>
The levels of the six cytokines were elevated in the prostate cancer patients. Generally TNF-Alpha and IL-6 were the cytokines with the highest levels present within the various Gleason scores while IL-10 and IL-4 had the lowest within the various Gleason scores among the prostate cancer patients (Figure 4.3). The levels of the six cytokines (IL-2, IL-4, IL-6, IL-10, TNF-Alpha and IFN-Gamma) increased as the prostate cancer progressed.

**Figure 4.3: Individual Cytokine Levels in the Study Participants.**

**GS:** Gleason score, **IL:** interleukin, **TNF:** Tumor necrosis Factor Alpha, **IFN:** Interferon Gamma
The IL-6 levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of IL-6, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IL-6 among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.4). The levels of IL-6 increased as the prostate cancer progressed.

**Figure 4.4: IL-6 Levels in the Study Participants**

**GS:** Gleason score, **IL:** Interleukin, **BPE:** Benign prostate enlargement
The TNF-Alpha levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of TNF-Alpha, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of TNF-Alpha among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.5). The levels of TNF-Alpha increased as the prostate cancer progressed.

GS: Gleason score, TNF: Tumor necrosis factor, BPE: Benign prostate enlargement

Figure 4.5: TNF-Alpha Levels in the Study Participants
The IL-2 levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of IL-2, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IL-2 among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.6). The levels of IL-2 increased as the prostate cancer progressed.

**Figure 4.6: IL-2 Levels in the Study Participants**

**GS:** Gleason score, **IL:** Interleukin, **BPE:** Benign prostate enlargement
The IFN-Gamma levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of IFN-Gamma, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IFN-Gamma among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.7). The levels of IFN-Gamma increased as the prostate cancer progressed.

**Figure 4.7: IFN-Gamma Levels in the Study Participants.**

**GS:** Gleason score, **IL:** Interleukin, **BPE:** Benign prostate enlargement
The IL-4 levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of IL-4, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IL-4 among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.8). The levels of IL-4 increased as the prostate cancer progressed.

GS: Gleason score, IL: Interleukin, BPE: Benign prostate enlargement

Figure 4.8: IL-4 Levels in the Study Participants.
The IL-10 levels were distinct in the 3 study groups. The PCa patients with a Gleason score of 8-10 have the highest amount of IL-10, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IL-10 among the BPE patients were less than those of the PCa patients. The control group had the least amounts of the cytokine (Figure 4.9). The levels of IL-10 increased as the prostate cancer progressed.

GS: Gleason score, IL: Interleukin, BPE: Benign prostate enlargement

**Figure 4.9: IL-10 Levels in the Study Participants.**
4.2.1 Hypothesis Testing

The means of the cytokine levels were compared by ANOVA. The aim was to determine whether there is any difference between the mean cytokine levels at the different stages of PCa. Therefore, the null hypothesis was that there is no difference between the mean of the cytokine levels in the different stages of prostate cancer and the alternative hypothesis is that there is a true difference between at least two of the Gleason score category.

H0: \( \mu_1 = \mu_2 = \ldots = \mu_{10} = \mu \)

Against

H1: At least two cytokine means in different stages of the prostate cancer are not equal.

The p-values for IL-6, TNF-Alpha, IL-2, IFN-Gamma, IL-4 and IL-10 from the hypothesis test were 0.0001\( (F_{44} = 41.08) \), 0.0001\( (F_{44} = 27.09) \), 0.0001\( (F_{44} = 53.17) \), 0.0001\( (F_{44} = 16.08) \), 0.0001\( (F_{44} = 33.05) \) and 0.001\( (F_{44} = 7.90) \) respectively. Therefore the null hypothesis was rejected because the data provided evidence against the null. There was a significant difference in the means of the six individual cytokines within the various Gleason score categories of prostate cancer.
4.3 Prostate Specific Antigen Levels in the Study Participants

Prostate cancer patients had the highest PSA levels followed by the BPE patients while the control group had the lowest PSA levels. The highest PSA levels in PCa patients were in the Gleason Score category of 8-10 (98.97) while the lowest levels were in the Gleason score category of 2-4 (38.98). The negative control patients had the lowest PSA values (0.764) (Table 4.7).

Table 4.7: PSA Levels in the study participants.

<table>
<thead>
<tr>
<th>Study subject category</th>
<th>Total PSA Mean (ng/mL)</th>
<th>N</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score 2-4</td>
<td>38.98</td>
<td>7</td>
<td>29.58</td>
<td>35.65</td>
<td>0.10</td>
<td>92.00</td>
</tr>
<tr>
<td>Gleason score 5-7</td>
<td>91.89</td>
<td>18</td>
<td>74.13</td>
<td>72.50</td>
<td>4.90</td>
<td>241.00</td>
</tr>
<tr>
<td>Gleason score 8-10</td>
<td>98.97</td>
<td>20</td>
<td>79.41</td>
<td>90.50</td>
<td>9.30</td>
<td>306.00</td>
</tr>
<tr>
<td>B.P.E</td>
<td>22.63</td>
<td>7</td>
<td>11.77</td>
<td>21.10</td>
<td>4.73</td>
<td>43.00</td>
</tr>
<tr>
<td>N.C</td>
<td>0.76</td>
<td>7</td>
<td>0.52</td>
<td>0.58</td>
<td>0.41</td>
<td>1.92</td>
</tr>
<tr>
<td>Total</td>
<td>68.99</td>
<td>59</td>
<td>72.00</td>
<td>49.00</td>
<td>0.10</td>
<td>306.00</td>
</tr>
</tbody>
</table>

The median was used to explain the variation in the PSA levels because the mean was highly affected by the outliers in the 8-10 category. The median is highest among the patients in the 8-10 category which is 90.50 and the lowest in the 2-4 category which is
35.65. The medians were highest among the PCa patients and lowest in the control group.

Among the three groups of the study subjects, prostate cancer patients had the highest PSA values, followed by the BPE patients and the control group had the lowest values (Figure 4.10). The PSA levels increased as the prostate cancer progressed.

**PSA**: Prostate specific antigen, **NC**: Negative control, **BPE**: Benign prostate enlargement

**Figure 4.10**: PSA Levels in the Study Participants.
4.3.1 Hypothesis Testing

To compare the PSA means in the different Gleason score categories ANOVA was used. The aim was to determine whether there is any significant difference between the mean PSA levels in the different stages of PCa. Therefore, the null hypothesis was that there is no difference between the mean of the PSA levels in the different stages of PCa and the alternative hypothesis was that there is a true difference between at least two of the Gleason score category.

\[ H_0: \mu_1 = \mu_2 = \ldots = \mu_{10} = \mu \]

Against

\[ H_1: \text{At least two PSA means in different stages of the prostate cancer are not equal.} \]

The \( p \)-value for the PSA from the hypothesis test is 0.167\((F_{44} = 1.868)\) so we accept the null hypothesis because there is no variation. There is no significant variation/difference in the PSA means within the various Gleason score categories of prostate cancer.

Individual cytokines were correlated against each other and against the PSA levels. The results indicated that all the cytokines and the PSA levels were significantly correlated (Table 4.8).
Table 4.8: Correlations of Individual Cytokines against Each Other and Against the PSA Levels.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>IL-6</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-10</th>
<th>PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>1</td>
<td>.892**</td>
<td>.912**</td>
<td>.896**</td>
<td>.774**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>TNF-Alpha</td>
<td>.892**</td>
<td>1</td>
<td>.878**</td>
<td>.871**</td>
<td>.782**</td>
</tr>
<tr>
<td></td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>IL-2</td>
<td>.912**</td>
<td>.878**</td>
<td>1</td>
<td>.896**</td>
<td>.900**</td>
</tr>
<tr>
<td></td>
<td>.0001</td>
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<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>IFN-Gamma</td>
<td>.896**</td>
<td>.871**</td>
<td>.896**</td>
<td>1</td>
<td>.813**</td>
</tr>
<tr>
<td></td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
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<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>IL-4</td>
<td>.864**</td>
<td>.782**</td>
<td>.900**</td>
<td>.813**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>IL-10</td>
<td>.774**</td>
<td>.720**</td>
<td>.695**</td>
<td>.720**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>PSA</td>
<td>.429**</td>
<td>.514**</td>
<td>.565**</td>
<td>.499**</td>
<td>.469**</td>
</tr>
<tr>
<td></td>
<td>.001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
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<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
</tbody>
</table>

**: Correlation is significant at the 0.01 level (2-tailed), PC: Pearson correlation.
CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Demographic data

The data from this study revealed that prostate cancer incidence strongly increases with age. The youngest PCa patient was 54 years old and the oldest was 102 years. The mean ages of the patients increased as the Gleason score of the disease increased. The data is comparable with that from a study carried out by the National Cancer Institute-USA, (1999) which showed that PCa is rare in men younger than 50 years of age and that the incidence significantly increased between 60–80 years. Other studies show that PCa has a low overall incidence in men younger than 50 years of age, who represent less than 0.1% of all affected patients. Approximately 85% of cases of PCa are diagnosed after the age of 65 years. At the age of 85 years, the cumulative risk of developing PCa ranges from 0.5% to 20.0%, worldwide (Grönberg, 2003).

Majority of the subjects in this study (83.1%) were married while 16.9% were widowed. Among the married men 91.5% had one wife while 8.5% had two wives. There was no association found between this PCa and marriage in this study. None of the patients admitted to have had other sexual partners apart from their wives or to have taken part in casual sex at any point of their lives. The data from this study does not show any association between sexual behavior and the development of PCa. Studies by Dennis & Dawson, (2002) and Giles, (2003) showed that having a high number of sexual partners
increases the chances of developing PCa. None of the patients agreed to have a wife suffering from cervical cancer hence cervical cancer may not have been a risk factor in this study. According to a study done by Whitaker et al. (2013) in Australia to determine if high risk human papilloma viruses (HPV) and Epstein Barr virus (EBV) are both present in the same prostate cancer specimens, Human Papilloma Virus type 18 was shown to cause chronic inflammation to the prostate which could eventually lead to the development of prostate cancer. Sexual activity might expose the prostate to infectious agents. Other studies have reported an increase in PCa risk associated with sexual intercourse at an early age, and a high number of sexual partners (Giles, 2003).

In the current study more than half of the patients had basic level education. Majority of the study subjects (50.84%) had their education level as primary and below, 32.20% had secondary level and 16.94% had post-secondary level of education. A patient’s education level may impact their understanding and perceptions of the benefits of seeking medical intervention early during the disease because they can be able to detect early signs and symptoms. This may lower the chances of suffering from late stages of the disease although this may not apply to cancer because awareness of the symptoms associated with cancer and early screening is the key thing. A study done by Rosenblatt et al. (2001) in Seattle on risk factor in prostate cancer showed that there was no relationship between religion, marital status, income, or education and the risk of developing prostate cancer.

The data from this study showed that 30.5% of the study subjects were smokers or had smoked at some point of their lives while 69.5% were non-smokers. The total number
of years smoked were not significant to the stage of the disease although on average the PCa patients had smoked for more years than the BPE patients. The results from this study showed a probable association between smoking and development of prostate cancer although the findings were not conclusive to term smoking cigarettes as a risk factor in the development of PCa.

Studies carried out by Schoonen, (2005) and Zu & Giovannucci, (2009) showed that smoking increased the chances of developing PCa. Smoking may promote the development of more aggressive, hormone-sensitive tumors through numerous mechanisms, including effects on sex steroid hormone levels, mutations in tumor suppressor genes such as p53, and continued exposure to carcinogens such as polycyclic aromatic hydrocarbons contained in cigarette smoke (Zu & Giovannucci, 2009). No clear dose-dependent relationship has been demonstrated between smoking and PCa risk. However, smoking is a source of cadmium exposure, increases oxidative stress, and increases circulating androgen levels, all of which represent potential mechanisms of prostate carcinogenesis (Bostwick, 2004).

Forty two percent (42.40%) of the study subjects took alcohol or had taken alcohol at some point of their lives while 57.60% were non-alcoholics. Most of the patients who took alcohol had drunk alcohol for many years which could have placed them at a higher risk of developing PCa. The total number of years they had drunk alcohol were not significant to the stage of the disease although on average the PCa patients had taken alcohol for more years than the BPE patients. The findings from this study indicate that long term and excessive drinking of alcohol could be a probable risk factor
to the development of PCa although the findings did not show that drinking of alcohol was a risk factor in the development of PCa. A prospective cohort study carried out by Sesso, (2001) demonstrated an increased risk of PCa in men who consumed more than three alcoholic drinks per day. However, other studies have shown no increase in the risk of PCa with high alcohol intake, but a protective effect of 1–3 glasses of wine per week (Schoonen, 2005). The first metabolite of alcohol, acetaldehyde, is a potent carcinogen, and alcohol consumption is considered to be a risk factor for many cancers. As PCa possibly develops over many decades, it may be long-term drinking habits that are related to PCa risk rather than drinking behavior close to diagnosis (AICR, 2007).

The data from this study showed that 11.11% of the study participants had relatives suffering from PCa. This shows that family history is a risk factor for the development of PCa. A study by Singh et al., (2000) showed that some high-risk genes identified when present, may predispose a carrier to development of PCa. These high risk genes were shown to be passed down within the family lineage making members of a specific family to be more prone and increasing the risk of developing PCa. A case–control study by Glover et al., (1998) in Kingston, Jamaica found that men who had an affected first-degree relative were 2 times more likely to develop prostate cancer than the general population.

Among the PCa patients with a Gleason score of 2-4, 3 patients had both hypertension and diabetes. Gleason 5-7, 1 patient had hypertension while 2 had both hypertension and diabetes while in Gleason 8-10 category 4 patients had hypertension while 2 had both hypertension and diabetes. Among the BPE patients, 3 had both hypertension and
diabetes. There is no correlation between hypertension and diabetes and the development of prostate cancer. This is contrary to other studies, for example a study done by Richard et al., (2010) showed that raised blood pressure was associated with an increased risk of developing PCa, particularly advanced cancers at diagnosis. This is further supported by Takeshita et al., (2011) who provided additional evidence that high blood pressure is associated with PCa risk. Studies by Chan et al., (1998) and Giovannucci, (2003) showed type 2 diabetes was a risk factor for prostate cancer.

The current results showed that out of the 59 study subjects, 20 knew their HIV/AIDS status while 39 did not know their status. All the 20 who knew their HIV/AIDS status, indicated that they were negative. HIV/AIDS is considered to be a risk factor for many cancers however, the results from this study did not show any association between HIV/AIDS and prostate cancer.

5.1.2 Cytokine Profile

The key cytokines in PCa and BPE patients that were evaluated in this study are IL-6, TNF-Alpha, IFN-Gamma IL-2, IL-4 and IL-10. The levels of the six cytokines (IL-6, TNF-Alpha, IFN-Gamma IL-2, IL-4 and IL-10) were higher in the PCa patients than in the BPE patients. The order of the cytokine levels in the PCa patients from the highest to the lowest was: TNF-Alpha, IL-6, IFN-Gamma, IL-2, IL-4 and IL-10. The levels of each of the cytokine increased as the Gleason score of PCa increased. This compares well with data by Tazaki et al., (2011) in a study that profiled ten serum cytokines in PCa patients and similar cytokines and more were found to be present and elevated.
The cytokine profile data obtained from this study indicated the presence of chronic inflammation in both the PCa patients and the BPE patients. A study by De Marzo et al., (2004) indicated that chronic or recurrent inflammation is responsible for the development of prostate cancer. According to a study done by Cohen et al. (2005), chronic and/or acute glandular inflammation was observed in many radical prostatectomy specimens, prostatic tissues resected during the treatment of BPE and tissue samples obtained from prostate needle biopsy, suggesting that inflammation may play a role in prostate carcinogenesis. The prostate epithelial cells have been shown to produce pro-inflammatory cytokines in androgen-dependent and androgen-independent prostate cells and influence the growth and differentiation of normal and prostate cancer cells (Ricote et al. 2004) Lippitz, 2013 and Hobisch et al., 2000).

Comparison of the cytokines levels in this study, revealed significant differences (p≤0.05) between the PCa and BPE patients. The six cytokines (IL-6, TNF-Alpha, IFN-Gamma IL-2, IL-4 and IL-10) were significantly (p≤0.01) correlated in the two study groups and the PSA values were also significantly (p≤0.01) correlated with the six cytokines in the two study groups. All the cytokine levels in the PCa patients were significantly higher than in the BPE patients. The finding are similar to those from a study conducted by Yosra et al., (2009) in a study to determine the profile of prostate epithelial cytokines and its impact on sera Prostate Specific Antigen levels among PCa and BPE patients, the data demonstrated a local production of pro-inflammatory cytokines by prostate epithelial cells and a cross talk between PSA and these cytokines in prostate pathologies.
The levels of the six cytokines were significantly high within the various Gleason scores. Prostate cancer patients with a Gleason score of 8-10 had the highest levels of the 6 cytokines followed by those patients with a Gleason score of 5-7 and those with a Gleason score of 2-4 had the lowest cytokine levels. The findings are similar to those from a study conducted by Tazaki et al., (2011) whereby an aberrance imbalance of cytokine production was found to be associated with the pathophysiology of PCa and the cytokine profiles in PCa patients was distinct by disease stage.

Nakashima et al., (2000) reported that IL-6 is independently associated with survival in a series of 74 patients with prostate cancer. On the other hand, Shariat et al., (2001) in a cohort of 120 patients treated with radical prostatectomy, reported that the preoperative IL-6 and sIL-6R predicted biochemical progression after surgery, suggesting an association with occult metastatic disease present at the time of radical prostatectomy. Results in the current study are in agreement with data published by these two groups. However, the IL-6 levels were different in the PCa and BPE patients. IL-6 was the cytokine with the second highest levels in the PCa patients. The PCa patients with a Gleason score of 8-10 had the highest amount of IL-6, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of IL-6 among the BPE patients were less than those of the PCa patients. Interleukin-6 levels were significantly high (p = 0.0001) within the various Gleason scores. This is similar to findings from two studies carried out by Kuroda et al., (2007) and Pfitzenmaier et al., (2003) which showed that IL-1, IL-6, IL-8 and TNF-α levels were found to be higher than normal in patients with advanced PCa.
IL-6 has been linked with IGF-IR (Insulin like growth factor 1 receptor) signaling in the prostate micro-environment to promote prostate tumorigenesis and progression (Rojas et al., 2011). The increase in IL-6 has been associated with bad prognosis in PCa (Shariat et al., 2001) and Liu et al., (2002) suggested that IL-6 could promote PCa cell growth. IL-6 has been characterized as a prostate exocrine gene product that interacts with its receptor in prostate cells, regulating proliferation and differentiation, and in prostate cancer cell lines it activates androgen receptor (AR) (Michalaki et al., 2004).

Another study that supports these findings was done by Yosra et al., (2009) and they found out that IL-6 levels were increased in PCa and BPE patients. A study conducted by Elizabeth et al., (2012) presented evidence that IL-6 plays a role in PCa pathogenesis, including a significant elevation in IL-6 transcriptional activity in the prostate (both cancerous and non-malignant) of individuals with PCa compared to BPE controls. Deeble et al., (2001) showed that local production of IL-6 by malignant cells significantly contributes to elevated serum levels.

The results from this study showed that high expressions of IL-6 were associated with TNF-Alpha and also with high PSA levels (>20 ng/mL). The finding are similar to those from a study conducted by Yosra et al., (2009) to determine the profile of prostate epithelial cytokines and its impact on sera Prostate Specific Antigen levels among PCa and BPE patients. The data demonstrated that high levels of IL-6 and TNF-Alpha were associated with high PSA levels. IL-6 has potential as a biomarker for prostate cancer.

The TNF-Alpha levels were different in the PCa and BPE patients. TNF-Alpha was the cytokine with the highest levels in the PCa patients among the six cytokines. The PCa
patients with a Gleason score of 8-10 have the highest amount of TNF-Alpha, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The levels of TNF-Alpha among the BPE patients were less than those of the PCa patients. TNF-Alpha levels were significantly elevated \((p = 0.0001)\) within the various Gleason scores. The findings are similar to those by Pfitzenmaier et al., (2003) which showed that levels of pro-inflammatory cytokine, TNF-Alpha, in cachectic PCa patients as well as organ-confined PCa patients were higher compared to the control. According to a study by Nakashima et al., (1998), TNF-Alpha is associated with PCa progression. Serum TNF-Alpha activity was positive in 76% of the patients with relapsed disease who had a significantly higher mortality rate than those with undetectable serum TNF-Alpha levels. TNF-Alpha has been reported to be involved in the initiation of PCa, inhibit neovascularization, induce apoptosis of PCa cells, and stimulate antitumor immunity. Serum TNF-Alpha levels have been shown to be reflective of tumor load in PCa patients being low in healthy men \((\text{mean } 1.1 \pm 0.5 \text{ pg/mL})\), higher in patients with bulky locally-advanced PCa \((3.9 \pm 3.4 \text{ pg/mL})\), and highest in those with metastatic disease \((\text{lymph node and bone involvement}) \,(6.3 \pm 3.6 \text{ pg/mL})\) (Michalaki, et al., 2004).

TNF-Alpha has been proposed as a tumor promoter (Balkwill, 2002) and its endogenous production has been associated with tumor invasion and development of metastasis (Beutler, 1999). The serum levels of TNF-Alpha in PCa patients have been related to the development of the disease and presence of metastasis (Michalaki et al., 2004). The functional duality of TNF-Alpha in tumor tissues is determined by the intracellular signs that contributed to its arrival to the cellular surface and binding its membrane receptors (TNF Receptor I and TNF Receptor II) and to activate different
transduction pathways. The data showed that TNF-Alpha also has potential as a biomarker for prostate cancer.

The results from this study showed that the IFN-Gamma levels were different in the PCa and BPE patients. The PCa patients with a Gleason score of 8-10 have the highest amount of IFN-Gamma, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. Men with advanced and hormone refractory PCa were found to have high plasma concentrations of IFN-gamma in other comparable findings (Platz and De Marzo, 2004, Maggio et al., 2006 and Wise et al., 2000). The levels of IFN-Gamma among the BPE patients were less than those of the PCa patients. IFN-Gamma levels were significantly high \((p = 0.0001)\) within the various Gleason scores. The findings are similar to those in a study by Tazaki et al., (2011) where IFN-Gamma levels were found to be higher than normal in cachexic prostate cancer patients. Interferon Gamma is a Th1 cytokine produced by phagocytic cells (monocytes, macrophages and neutrophils) and dendritic cells (DC), and activate natural killer (NK) cells (representative anticancer immune cells) leading to anticancer effects. IFN-Gamma accordingly plays crucial anticancer effects \(in vivo\) and it is thought to suppress cancer development and metastasis (Del Vecchio et al., 2007 and Sangro et al., 2005). The data showed that IFN-Gamma also has potential as a biomarker for prostate cancer.

The results from this study showed that the IL-2 levels were different in the PCa and BPE patients. The PCa patients with a Gleason score of 8-10 have the highest amount of IL-2, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. The findings are similar to those in a study by Tazaki et al., (2011) where IL-2
levels were higher than normal in cachexic prostate cancer patients. The levels of IL-2 among the BPE patients were less than those of the PCa patients. IL-2 levels were significantly high (p = 0.0001) within the various Gleason scores. Interleukin-2 (IL-2) just like IFN-Gamma is a Thelper1 cytokine and it is thought to suppress cancer development and metastasis (Del Vecchio et al., 2007). Interleukin-2 elicits IFN-Gamma from NK cells, CD4 cells and CD8 cells, and accordingly play crucial anticancer effects in vivo (Sangro et al., 2005). The data showed that IL-2 also has potential as a biomarker for prostate cancer.

The data from this study showed that IL-4 and IL-10 levels were different in the PCa and BPE patients. IL-4 and IL-10 were the two cytokines with the lowest levels among the six cytokines. The PCa patients with a Gleason score of 8-10 had the highest amount of IL-4 and IL-10, followed by those with a Gleason score of 5-7, then those with a Gleason score of 2-4. These findings are comparable to those from studies by Quatan et al., (2006) and Mocellin et al., (2005), which showed that these immunosuppressive and anti-inflammatory cytokines were most significantly elevated in the PCa patients with a Gleason score of 8-10. IL-4 and IL-10 which are both Th2 cytokines elicit an antibody kind of response against the cancer cells in the prostate. Both cytokines regulate the immune response by inhibiting and dampening various responses by effector cells which has been associated with increased survival of the PCa cells (Roca et al., 2012). Hence these cytokines were thought to have contributed to further cancer development. The levels of IL-4 and IL-10 among the BPE patients were less than those of the PCa patients. IL-4 and IL-10 levels were significantly high (p = 0.0001 and p = 0.001) respectively within the various Gleason scores. The data showed that IL-4 also
has potential as a biomarker for prostate cancer. IL-10 also has the potential to be a prostate cancer biomarker although its level of significance is less than that of the other five cytokines.

An ideal cancer biomarker is one that allows for early detection of disease and/or assessment of response to therapy and prognosis, is minimally invasive to the patient when sampled, and cost effective to be assayed (Ludwig and Weinstein, 2005). Since cytokines are often involved in the evolution of cancers, measuring their levels in bodily fluids may provide a reflection of the patient’s pathological state. The results of the study support the possibility that IL-6, TNF-Alpha, IFN-Gamma IL-2, IL-4 and IL-10 are all potential biomarkers of PCa. Among the six TNF-Alpha and IL-6 would probably be better biomarkers because they both had the most elevated levels in the different Gleason score categories of PCa.

5.1.3 Prostate Specific Antigen Levels

Results in the current study showed that prostate cancer patients had higher PSA levels than the BPE patients. A review by Adhyam and Gupta, (2012) previously described high PSA levels in PCa and BPE patients, with the PSA values being higher in the PCa patients. Data from this study showed that PCa patients in the Gleason Score category of 8-10 that highest PSA levels while the patients in the Gleason score category of 2-4 had the lowest levels. PSA levels were found to be proportional to tumor volume in the PCa patients and they correlated with the clinical and histological stage of the disease. The mean PSA increased as the Gleason score increased although it did not vary significantly (p= 0.167) in the different stages of the PCa. The mean PSA levels of the
BPE patients were lower than those of all the prostate cancer patients although they were significantly (p=0.002) elevated compared to the normal PSA levels. This compares with a study by Alcaide et al., (2009) in which a high expression of inflammatory cytokines and high PSA levels were observed in both PCa and BPE patients. The levels of both the cytokines and the PSA were higher in the PCa patients as compared to the BPE patients.

The data from this study showed no correlation between patient age and PSA levels. According to Rong et al., (2013) other studies support the notion that total PSA levels increase with age which increases the risk of having PCa and high grade PCa. Currently, age-specific PSA reference ranges used in the USA and European countries are 0–2.5 ng/mL for men 40–49 years old, 0–3.5 ng/mL for men 50–59 years old, 0–4.5 ng/mL for men 60–69 years old, and 0–6.5 ng/mL for men 70–79 years old.

The data from this study shows that there is no clear cut ranges of PSA in PCa and BPE, the results suggest that any elevation in PSA is a risk factor for PCa. Previous studies have shown that serum levels of PSA in patients with other prostatic diseases, such as BPE and prostatitis are normally increased, producing high rates of false positive results and leading to over-detection of PCa (Catalona et al., 1994 and Heijnsdijk et al., 2009). PCa cells, like normal prostate epithelial cells, produce high levels of the differentiation marker PSA. PSA is used extensively as a biomarker to screen for PCa, to detect recurrence following local therapies, and to follow response to systemic therapies for metastatic disease (Adhyam and Gupta, 2012).
Elevated serum levels of inflammatory cytokines in this study was associated with increased PSA. A study by Mizokami et al., (2000) also showed that high PSA levels were correlated with high levels of inflammatory cytokines in BPE, PCa and prostate cancer cell line. The six cytokines were significantly correlated in the PCa and BPE patients and the PSA levels were also significantly correlated with the six cytokines in the two study groups. The finding are similar to those from a study conducted by Yosra et al., (2009) in a study to determine the profile of prostate epithelial cytokines and its impact on sera Prostate Specific Antigen levels among PCa and BPE patients. The results demonstrated a local production of pro-inflammatory cytokines by prostate epithelial cells and a cross talk between PSA and these cytokines in prostate pathologies.

The results from this study suggests a possible link between PSA, and pro-inflammatory cytokines and its implication in tumor progression. The prostate epithelial cells produce both the cytokines and PSA, the two could be working together in the progression of the disease. Indeed, in PCa and BPE cases, the high pro-inflammatory cytokines expressions could be associated with high production of PSA. The high expression of cytokines with increased PSA levels could be involved in increased loss of epithelial cells polarity and basal cell numbers, therefore the pro-inflammatory cytokines could be implicated indirectly to leakage of PSA into the circulation as well increased PSA levels.
5.2 Conclusions

1. Kenyan men who are above 50 years of age are at a risk of suffering from prostate cancer.

2. Long term smoking and drinking of alcohol, chronic inflammation, diabetes and hypertension which have been shown to be risk factors in the development of prostate cancer in other studies have not been shown to be risk factors in this study.

3. TNF-Alpha, IL-6, IFN-Gamma, IL-2 and IL-4 are potential prostate cancer biomarkers.

4. There is a possible association between PSA and inflammatory cytokines and their implication in prostate tumor progression.
5.3 Recommendations

1. Men above the age of 40 years should be encouraged to go for annual prostate cancer screening.

2. More research into the clinical diagnostics and prognostic utility of TNF-Alpha, IL-6, IFN-Gamma, IL-2 and IL-4 in prostate cancer needs to be done.

5.4 Further Studies

1. Other studies with a bigger sample size to determine risk factors in the development of prostate cancer among Kenyan men should be carried out.
REFERENCES


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Tuma, R. (2010). New tests for prostate cancer may be nearing the clinic. *Journal of the National Cancer Institute, 102*: 752-754.


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APPENDICES

APPENDIX I a: INFORMED CONSENT FORM

DETERMINATION OF THE CYTOKINE PROFILE AND THE PSA LEVELS IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL.

This informed consent form is for male patients attending Urology Outpatient Clinic at KNH and have been invited to participate in the research “DETERMINATION OF THE CYTOKINE PROFILE AND THE PSA LEVELS IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL.”

Principal Investigator: Liza Kiente Mwirigi

Institution: Department of Medical Laboratory Sciences, School of Medicine, Kenyatta University.

This Informed Consent Form has three parts:

1) Information Sheet (to share information about the research with you).
2) Certificate of Consent (for signatures if you agree to take part).
3) Statement by the research/ person taking consent.

You will be given a copy of the full informed consent form.
PART I: Information Sheet

Introduction

My name is Liza Kiende Mwirigi, a post graduate student in Infectious Diseases at Kenyatta University. I am carrying out a research on “Determination of the Cytokine Profile and the PSA levels in Prostate Cancer Patients at Kenyatta National Hospital.”

Purpose of the Research

Prostate cancer is the second most frequently diagnosed cancer of men and the fifth most common cancer overall. It is the leading cancer in terms of incidence and mortality in men from Africa. Determining the cytokine profile and studying the PSA levels in the different stages could lead to the discovery of new biomarkers for early prostate cancer screening thereby reducing the morbidity and mortality rates that result from prostate cancer. The purpose of this study is to determine the cytokine profile and the PSA levels in the different stages of prostate cancer. I am going to give you information and invite you to be a participant in this research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will explain. After receiving the information concerning the study, you are encouraged to seek clarification in case of any doubt.

Questionnaire

The questionnaire is aimed at assessing the demographic and risk factors that can lead to the development of prostate cancer. If you agree to take part in the study, the age, education level, marital status, religion and various risk factors that could make you
susceptible to prostate cancer will be recorded. Blood samples will be collected and the cytokine profile and Prostate Specific Antigen levels will be analyzed in the laboratory.

**Type of Research Intervention**

Blood samples will be collected for cytokine profile and PSA levels analysis after administration of a questionnaire.

**Voluntary participation/ right to refuse or withdraw**

It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this hospital will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this hospital for your condition. You have the right to refuse or withdraw your participation in this study at any point.

**Confidentiality**

The information obtained will be treated with confidentiality and only be available to the principal investigator and the study team. Your name will not be used. Any information about you will have a number on it instead of your name. We will not be sharing the identity of those participating in this research.

**Sharing the results**

The knowledge that we get from this study will be shared with policy makers in the Ministry of Health and doctors through publications and conferences. Confidential information will not be shared.
Risks

You may experience temporary local pain or bruising when a needle is inserted into your vein to get venous blood.

Costs and compensation

There will be no extra cost incurred for participating in this study nor is there compensation offered.

This proposal has been reviewed and approved by the Kenyatta University Ethical Review Committee and UoN/KNH Ethics Committee, which are committees whose task is to make sure that research participants are protected from harm.

Who to contact

If you wish to ask any questions later, you may contact:

1. **Principal Researcher:**
   Liza Kiende Mwirigi
   Department of Medical Laboratory Science, School of Medicine, Kenyatta University
   P.O Box 43844, Nairobi 00100
   Mobile no. 0723 372 486

2. **Supervisors:**
   Dr. Margaret Muturi
   Lecturer
   Department of Medical Laboratory Science, School of Medicine, Kenyatta University
   P.O Box 43844, Nairobi 00100
   Mobile no. 0722 758 523
Dr. Minda P. Okemwa  
Pathologist/ Lecturer  
Department of Pathology, School of Medicine, University of Nairobi  
P.O Box 19676 KNH, Nairobi 00202  
Mobile No. 0722 790 678

If you have any ethical concerns, you may contact:

Secretary,  
KNH/UoN-ERC,  
P.O Box 20723 KNH, Nairobi 00202  
Tel + 254-020-2726300-9 Ext 44355  
Email: KNHplan@ken.Healthnet.org

Kenyatta University Ethical Review Committee,  
P.O Box 43844-00100, Nairobi  
Tel 8710901/12  
Email: kuerc.chairman@ku.ac.ke

PART II: Certificate of Consent

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant.................................................................
Signature of Participant...........................................................................
Date...........................................................................................................

If Non-literate:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Witness...........................................................................
Signature of Witness............................................................................
Date...........................................................................................................
PART III: Statement by the Researcher

I have accurately read out the information sheet to the participant, and to the best of my ability made sure that the participant understands that the following will be done:

Refusal to participate or withdraw from the study will not in any way compromise the care treatment.

All information given will be treated with confidentiality.

The results of this study might be published to facilitate early diagnosis of prostate cancer.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of researcher/ person taking consent....................................................

Signature of researcher/ person taking consent..............................................

Date..............................................................................................................
APPENDIX I b: FOMU YA MAKUBALIANO YA KUJIUNGA NA UTAFITI

DETERMINATION OF THE CYTOKINE PROFILE AND THE PSA LEVELS
IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL.

Fomu hii ya makubaliano ni ya wale wanaume ambao wanahudumiwa kwenye kliniki za Urology katika hospitali ya KNH na wamealikwa kujiunga na utafiti “DETERMINATION OF THE CYTOKINE PROFILE AND THE PSA LEVELS IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL.”

Mtafiti mkuu: Liza Kiende Mwirigi

Kituo: Kitengo cha Matibabu Maabara ya Sayansi, Shule ya Afya, Chuo Kikuu cha Kenyatta.

Fomu hii ya makubaliano ina sehemu tatu:
1) Habari itakayo kusaidia kukata kauli
2) Fomu ya makubaliano (utakapo weka sahihi)
3) Ujumbe kutoka kwa mtafiti

Utapewa nakala ya fomu hii.

SEHEMU YA KWANZA: Ukurasa wa habari

Kitambulizi

Jina langu ni Liza Kiende Mwirigi. Mimi ni mwafunzi ninaeke katika chuo kikuu cha Kenyatta. Ninafanya utafiti kwa anwani ya “Determination of the Cytokine Profile and the PSA levels in Prostate Cancer Patients at Kenyatta National Hospital.”
Nia ya Utafiti huu

Saratani ya tevi ni mojawapo ya saratani kuu kuathiri wanaume ambao wamezidi umri wa miaka 50 nchini Kenya. Wanaume wengi ambao wana saratani ya tevi, wanatambuliwaikiwa ugonjwa ushaenea kwa mwili. Nia ya utafiti huu ni kuchunguza aina mpya za kugundua saratani ya tevi kabla haijaenea kwa mwili.

Aina ya Utafiti

Utafiti huu utahusisha vipimo vya damu na kujibu kwa orodha ya maswali.

Haki ya kukataa utafiti

Kushiriki kwako kwa utafiti ni kwa hiari yako. Una uhuru wa kukataa kushiriki, na kukataa kwako hakutatumiwa kukunyima tiba. Uko na haki ya kujitoa katika utafiti wakati wowote unapoamua.

Tandhima ya siri

Ujumbe kuhusu majibu yako utahifadhiwa. Ujumbe kuhusu ushiriki wako katika utafiti huu utaweza kupatikana na wewe na wanaoandaa utafiti na wala si yeyote mwingine. Jina lako halitatumika bali ujumbe wowote kukuhusu utapewa nambari badili ya jina lako.

Hatari unayoweza kupata

Utahisi uchungu kidogo wakati utadungwa sindano kutolewa damu.

Anwani za wahusika

Ikiwa uko na maswali ungependa kuuliza baadaye, unaweza kuwasiliana na:

Mtafiti mkuu:

Liza Kiende Mwirigi

Kitengo cha Matibabu Maabara ya Sayansi, Shule ya Afya, Chuo Kikuu cha Kenyatta.
SLP 43844, Nairobi 00100
Simu: 0723 372 486

Wahadhiri wahuwika:
Dkt. Margaret Muturi

Mhadhiri
Kitengo cha Matibabu Maabara ya Sayansi, Shule ya Afya, Chuo Kikuu cha Kenyatta.
SLP 43844, Nairobi 00100
Simu: 0722 758 523

Dkt. Minda P. Okemwa

Mhadhiri
Kitengo cha Pathologia, Shule ya Afya, Chuo Kikuu cha Nairobi.
SLP 19676 KNH, Nairobi 00202
Simu: 0722 790 678

Wahusika wa maslahi yako katika utafiti:
Karani,

KNH/UoN-ERC,
SLP 20723 KNH, Nairobi 00202
Simu + 254-020-2726300-9 Ext 44355
Barua pepe: KNHplan@ken.Healthnet.org
Kenyatta University Ethical Review Committee,
SLP 43844-00100, Nairobi
Simu 8710901/12
Barua pepe: kuerc.chairman@ku.ac.ke

**SEHEMU YA PILI: Fomu ya makubaliano**


**Jina la Mshiriki**


**Sahihi ya Mshiriki**


**Tarehe**


---

**Kwa wasiweza kusoma na kuandika**


**Jina la shahidi**


**Sahihi la shahidi**


**Tarehe**


---
SEHEMU YA TATU: Ujumbe kutokea kwa mtafiti

Nimemsomea mshiriki ujumbe kiwango ninavyoweza na kuhakikisha kuwa mshiriki amefahamu yafuatayo:

- Kutoshiriki au kujitoa kwenye utafiti huu hautadhuru kupata kwake kwa matibabu.
- Ujumbe kuhusu majibu yake yatahifadhiwa kwa siri.
- Matokeo ya utafiti huu inaweza chapishwa kusaidia utambuzi wa saratani ya tevi.

Ninathibitishe kuwa mshiriki alipewa nafasi ya kuuliza maswali na yote yakajibiwa vilivyo. Ninahakikisha kuwa mshirikialitoa ruhusa bila ya kulazimishwa. Mshiriki amepewa nakala ya fomu hii ya makubaliano.

Jina la Mtafiti........................................................................................................................................

Sahihi ya Mtafiti........................................................................................................................................

Tarehe........................................................................................................................................
APPENDIX II a: QUESTIONNAIRE

This questionnaire is aimed at assessing the demographic and risk factors that can lead to the development of prostate cancer.

I am Liza Mwirigi, an MSc student in the department of Medical Laboratory Science, School of Medicine, Kenyatta University. I am investigating the demographic and risk factors that can lead to the development of prostate cancer. I kindly request you to fill the questionnaire below to the best of your knowledge.

Serial No:…………………… Date:………………………………

Please tick in the appropriate bracket.

SECTION A: DEMOGRAPHIC INFORMATION

1. Age: 50-54 years ( ); 55-59 years ( ); 60-64 years ( ); 65-69 years ( ); ≥70 years ( ).

2. Marital status: Single ( ); Married ( ); Widower ( ); Divorced ( ).

3. Educational level: Primary and below ( ); Secondary ( ); Post-secondary ( ).

SECTION B: RISK FACTORS

1. Do you smoke? Yes ( ); No ( ).

2. Do you take alcohol? Yes ( ); No ( ).

3. Do you have any relative suffering from prostate cancer? Yes ( ); No ( ).

4. How many wives do you have? None ( ); 1 ( ); 2 ( ); More than 2 ( ).
5. Do you have any other sexual partners? Yes ( ); No ( ).

6. Have you ever been paid for sex? Yes ( ); No ( )

7. Have you suffered from any lifestyle disease e.g. Diabetes, high blood pressure etc. before? Yes ( ); No ( ).

8. Has your wife been diagnosed with cervical cancer in the recent past? Yes ( ); No ( ).

9. Do you know your HIV/ AIDS status? Yes ( ); No ( )

10. If yes, are you HIV/ AIDS Positive ( ) or Negative ( )?
APPENDIX II b: DODOSO (ORODHA YA MASWALI)

Dodoso hii inawania kuchunguza habari ya idadi watu na yanayoweza kusababisha saratani ya tevi.

Jina langu ni Liza Kiende Mwirigi. Mimi ni mwanafunzi ninaesomea katika chuo kikuu cha Kenyatta. Ninafanya utafiti kwa anwani ya “Determination of Prevalence, Cytokine Profile and PSA levels in Prostate Cancer Patients at Kenyatta National Hospital.”

Ninachunguza habari ya idadi watu na yanayoweza kusababisha saratani ya tevi. Nakusihi ujaze dodoso lifuatalo kwa umakini.

Namba tambulishi:…………………………… Tarehe:………………………………………

Tafadhali weka alama ya (x) kwenye kijisanduku kinachofaa

SEHEMU A: HABARI YA IDADI WATU

1. Miaka: 50-54 years (  ); 55-59 years (  ); 60-64 years (  ); 65-69 years (  ); ≥70 years (  ).

2. Hali ya ndoa: Sijaoa (  ); Nimeoa (  ); Mjane (  ); Nimetaliki (  ).

3. Cheo cha masomo: Shule ya msingi (  ); Shule ya upili (  ); Chuo Kikuu (  ).

SEHEMU B: YANAYOWEZA KUSABABISHA SARATANI YA TEVI

1. Je, wewe huvuta sigara? Ndio (  ); La (  ).

2. Je, wewe hunywa pombe? Ndio (  ); La (  ).
3. Je, kuna yeyote katika familia yako ambaye amewahi ama anugua ugonjwa wa saratani ya tevi? Ndio ( ); La ( ).

4. Je, una wake wangapi? Sina ( ); 1 ( ); 2 ( ); Zaidi ya wawili ( ).

5. Je, una mpenzi mwingine unaye husiana naye isipokuwa bibi yako?
Ndio ( ); La ( ).

6. Je, umewahi kujihusisha kwa ngono kwa ajili ya malipo? Ndio ( ); La ( ).

7. Je, umewahi kuugua magonjwa ya maisha? kwa mfano: kisukari, shinikizo la damu...
Ndio ( ); La ( ).

8. Je, bibi yako amewahi kuugua ama anugua maradhi ya saratani ya kizazi? Ndio ( ); La ( ).

9. Je, unaijua hali yako ya ukimwi? Ndio ( ); La ( ).

10. Kama waijua, Unayo ( ) ama Hauna ( )?
APPENDIX III: ETHICAL REVIEW APPROVAL

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12
Tel: 8710901/12

Our Ref: KU/R/COMM/51/275

Date: 10th January, 2014

APPLICATION NUMBER PKU/ 175 I 153 - "DETERMINATION OF PREVALENCE, CYTOKINE PROFILE AND THE PSA LEVELS IN PROSTATE CANCER PATIENTS AT KENYATTA NATIONAL HOSPITAL" - Version2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic “Determination of prevalence, cytokine profile and the PSA levels in prostate cancer patients at Kenyatta national hospital” dated 10th January, 2013.

2. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 10th January, 2014.

3. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

[Signature]

10 JAN 2014

LIZA KIENDE MWIRIGI accept the advice given and will fulfill the conditions therein.

Signature: ........................................ Dated this day of ........................................ 2014

cc. Vice-Chancellor
    Director: Institute for Research Science and Technology
APPENDIX IV: HEMATOXYLIN AND EOSIN STAINING PROTOCOL

1. Deparaffinize in Xylene I and II and III (5 minutes)
2. Rehydrate
   - Ethanol 100% (3 minutes)
   - Ethanol 100% (3 minutes)
   - Ethanol 95% (3 minutes)
   - Ethanol 95% (3 minutes)
   - Ethanol 70% (3 minutes)
3. Rinse in distilled water (5 minutes)
4. Stain in hematoxylin (6 minutes) *Filter before each use to remove oxidized particles*
5. Rinse in running tap water (20 minutes)
6. Decolorize in acid alcohol (1 second) *can go up to 3 seconds. Longer = Lighter Discard after each use *
7. Rinse well in tap water (5 minutes)
8. Immerse in Lithium Carbonate (3 Seconds) *Longer time = floating tissue *
9. Rinse in tap water (5 minutes)
10. Counterstain in Eosin (15 seconds)
11. Dehydrate
    - Ethanol 95 % (3 minutes) *Discard after each use *
    - Ethanol 95% (3 minutes)
    - Ethanol 100 % (3 minutes)
    - Ethanol 100 % (3 minutes)
12. Clear in Xylene I and II (5 minutes)

**Stock Solutions – EOSIN:**

Stock – 1% aqueous Eosin-Y
Stock – 1% aqueous Phloxin B

**Working Solutions – Eosin:**

100ml stock Eosin
10 ml stock Phloxin B
780 ml 95% Ethanol
4 ml glacial Acetic Acid

**Working Solution: - Hematoxylin**

Harris Hematoxylin, Sigma, HHS-32, 1 Liter

**Working Solution: - Lithium Carbonate 1.36%**

Lithium Carbonate, 47g, dH2O, 3500 ml

**Working Solution: - 0.25% Acid Alcohol (95% Ethanol, 2578 ml, dH2O, 950ml, HCL, 9ml)**
APPENDIX V: SAMPLE HISTOLOGY GRADING

PATIENTS A: GLEASON 4+5=9

Gleason 4 (Magnification X40)  
Gleason 5 (Magnification X40)

PATIENT B: GLEASON 2+2= 4

Gleason 2 (Magnification X40)  
Gleason 2 (Magnification X40)
APPENDIX VI: ASSAY KITS

CYTOKINE KIT

PSA KIT