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A Comparison of Testosterone With Prostate Specific Antigen and Prostatic Acid Phosphatase For the Serodiagnosis of Prostate Cancer In Adult Males

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A COMPARISON OF TESTOSTERONE WITH PROSTATE SPECIFIC ANTIGEN AND PROSTATIC ACID PHOSPHATASE FOR THE SERODIAGNOSIS OF PROSTATE CANCER IN ADULT MALES

by

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ABSTRACT

Prostate cancer is the second most common cause of death from cancer in men. The American Cancer Society has estimated that there were 240,890 newly diagnosed cases in 2011 and 33,720 deaths from prostate cancer. Diagnosis of this disease has traditionally been done by measuring prostate specific antigen (PSA) levels through sero-chemical testing. The purpose of this study is to compare serum testosterone levels to PSA levels and Prostatic Acid Phosphatase (PAP) levels in the search for new and more accurate technology. This study shows that testosterone was not efficient in correctly diagnosing those with prostate cancer. It had a sensitivity of 0% compared to PSA at 30.12% and PAP at 20.73%. However, the percentage of true negative results in those without prostate cancer was significantly higher in testosterone than PSA or PAP testing. It had a specificity of 96.80% versus PSA at 91.29% and PAP at 80.38%. Possible explanations for 0% true positives may be due to skewed results from patients already receiving treatment. The reference values for this test may also require revision. The manufacturer's suggested reference ranges were used in this study and may not properly represent the patient demographic.

KEY TERMS: Testosterone, Prostate Cancer, Prostate Specific Antigen, Prostatic Acid Phosphatase, Tumor Marker

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INTRODUCTION

In 2011, The American Cancer Society (ACS) released a report including statistics on all new cases of cancer as well as mortality related to cancer in the United States of America (U.S.). Excluding basal and squamous cell carcinomas, prostate cancer had the highest estimated new cases of cancer diagnoses in males of all races. There were 240,890 new prostate cancer diagnoses out of 822,300 total new diagnoses in males, which is approximately 29 percent (North American Association of Central Cancer Registries, 2011). In regards to estimated mortalities, it was second only to lung cancer. ACS reported 33,730 mortality cases due to prostate cancer out of 300,430 cancer mortalities reported, which is approximately 11 percent (North American Association of Central Cancer Registries, 2011).

In American men, adenocarcinoma of the prostate (PCA) is one of the two most frequent types of cancer (American Medical Association, 1991). With such a high prevalence in the population, studies have been conducted to try to screen for this disease. It is suggested that with earlier detection, metastasis of the tumor can be contained or prevented and mortality rates would be decreased.

The purpose of this study was to compare potential tumor markers for prostate cancer. PCA is the first cancer with a tissue-specific tumor marker, known as prostate specific antigen (PSA). Though specific to the prostate gland, this tumor marker is a poor indicator of cancerous growth and does not differentiate between benign and malignant tumors (Wu, Individual Tumor Markers, 1997). Therefore, the PSA blood test is a screening tool and not diagnostic for PCA. With the development of new, more advanced technology in the laboratory, it is useful to re-evaluate other potential tumor markers in

order to develop a better method of detection for this disease. Testosterone, prostatic acid phosphatase (PAP), and PSA were re-evaluated for specificity and sensitivity to prostate cancer. These analytes were chosen due to their direct correlation with the prostate gland.

Prostate cancer can be linked to age, familial inheritance, ethnic differences, and lifestyle choices (Haese, et al., 2002). This disease is more prevalent among males above the age of 50 years. The risk of developing this cancer increases significantly from ages less than 39 years old to ages greater than 79. However, the disease progression is slow, and most patients who are diagnosed late in life do not die from metastatic complications. Though there are many new cases of prostate cancer, there are significantly fewer occurrences of death from prostate cancer.

According to one source, the risk of developing PCA is higher in African-American males compared to Caucasian males. The risk of developing PCA is the lowest in Asian countries; however, migration of Asian populations to the U.S. shows an increase in risk that may be correlated with dietary changes (Haese, et al., 2002).

Study Objective

The objective of this study was to compare testosterone with prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) in regards to its performance as a superior tumor marker for the diagnosis of prostate cancer. Five hundred and fifty-one patient samples were collected for testing. Tumor markers for prostate cancer should be re-evaluated periodically due to improving technology in the laboratory. PSA measurements were taken at the patients' respective hospitals, and values were provided for each sample. Testosterone and PAP values were obtained in our laboratory.

Hypothesis

It was hypothesized that testosterone would prove to be a superior tumor marker for the serodiagnosis of prostate cancer.

LITERATURE REVIEW

CANCER OVERVIEW:

One of the first observations in the field of oncology was made by Sir Percival Pott, who linked young chimney sweepers to high incidences of scrotum cancer due to the carcinogen present in their work. This study both emphasized that a certain agent caused the cancerous growth and that the effect can occur many years after exposure (Harrington, et al., 2005). In 1846, Bence-Jones described the first identified cancer marker as a monoclonal immunoglobulin chain that can be detected in urine of multiple myeloma patients. Approximately 120 years later, alpha-fetoprotein and carcinoembryonic antigen were discovered as major tumor markers. In 1960, radioimmunoassays were introduced into the field of clinical science. This method allowed analysis of minute amounts of analytes in substances. In 1975, monoclonal antibodies allowed many more tumor markers to be discovered and analyzed. In 1980, prostate specific antigen (PSA) became the first tissue-specific tumor marker to be discovered. Research expanded into the area of oncogenes and tumor suppressor genes.

The origin of cancer is unknown. Though the pathways of development have been extensively researched, one common direct link has not been found among oncology cases. Most sources have agreed that mutations at the DNA level are the more probable cause of oncology cases (Conklin, 1949).

Epidemiology

In 1949, cancer was the second leading cause of death, after heart ailments, in the United States (Conklin, 1949). In 2000, a study was conducted on the worldwide

prevalence and incidence of cases for all types of cancers, excluding non-melanoma skin cancer. The study showed that in 2000, there were 22,407,000 new cases of cancer worldwide (McLaughlin & Gallinger, 2005). As the population is exposed to more chemicals, the chances for genetic cancerous mutations increase. Scientists are still discovering new carcinogens and markers; new testing methods have improved their search capabilities.

Most statistics arise from observational experiments; moral ethics do not allow human subjects to be purposefully exposed to cancer in order to study its effects. Studies can be categorized based on occupational hazards, demographic locations, lifestyle choices, or genetic inheritances (Conklin, 1949). Quantitative reports such as a prevalence rate can be given – this figure estimates the amount of current cancer cases that have been reported to date. An incidence rate defines the number of new cases to be reported in a given time frame. These numbers can be compared to the same population to assess whether a risk is involved that increases the likelihood of disease. However, other interfering lifestyle choices may skew the results of an observation experiment.

Lifestyle Risks

Some carcinogens require repeated episodes of exposure before they cause a detrimental mutation. Very strong factors (for example, radiation poisoning after a nuclear bomb event) may require only one dose before taking effect. Six lifestyle risk factors for developing cancer have been identified and include obesity, physical activity, folic acid intake, consuming red meat, and tobacco usage (McLaughlin & Gallinger,

2005). A pattern seen with carcinogens is that the damaging exposure may have occurred long before the side effects of cancer are seen.

Smoking has been repeatedly linked to the manifestation of lung adenocarcinoma cases. The enzymes CYP2A6 and CYP2A13 are capable of activating a tobacco smoke carcinogen, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Okey, et al., 2005). Patients with these genes may present with a higher risk of developing lung cancer. Occupational hazards that involve potential carcinogenic chemicals, such as aromatic amines, may also be a contributing factor in initiating cancerous growth.

Definition of a Carcinogen

Some substances, such as chemicals, are considered carcinogenic because they can cause a direct mutation in DNA strands that subsequently affects cell synthesis and apoptosis (Upton, 1982). Such changes usually occur in various differentiation stages where cells do not fully reach apoptosis. They can also affect DNA replication components, such as polymerase enzymes, which would lead to an indirect mutation on the DNA strands. Another potential target site would be the translation molecules that form the various proteins. These malformed proteins may be a part of necessary regulating substances in feedback loops, and their alteration could lead to uncontrolled growth. All of these effects can be corrected if the polymerases can detect and repair the mistake. However, if the mistake is overlooked and integrated into the next strand to be replicated, that mutation becomes a permanent part of the DNA strand. Subsequent replications will yield the same mutation in each strand and potentially cause a cancer case.

Environmental Carcinogens

To date, three-fourths of human cancer cases have been attributed to carcinogenic material found in the environment. A study done by the International Agency for Research on Cancer tested approximately 300 various substances for carcinogenicity and approximately 21 substances have been identified as human carcinogens (Holbrook, 1980). Some examples of environmental factors are located in the food and water supply. In the food supply, the nitrite compound that preserves the color of processed meats may potentially react with acidic stomach contents to form nitroso compounds. Another carcinogenic pathway involves cooking nitrite-containing foods at high temperatures to allow for a reaction with amines to form nitrosamines. These nitrite derivatives have been tested and shown to be carcinogenic to some animals (Holbrook, 1980).

The water supply may also harbor potential carcinogens in the form of carbon tetrachloride or other chlorinated substances. Laboratories that do not dispose of benzene properly may also inadvertently expose the public to harmful chemicals. Cases have been reported of an epidemic of cancer in a downstream area of a dumping site where epoxide-containing chemicals were dumped. Other types of environmental hazards include the aflatoxins from *Aspergillus* species that may coat food products. These aflatoxins have been linked to increased incidences of hepatic tumors (Holbrook, 1980). Metal salts such as arsenic, cadmium, chromium, nickel, beryllium, and lead have been implicated as human carcinogens (Holbrook, 1980). They disrupt DNA replication by causing mismatched base pairing.

Occupational Carcinogens

Some careers carry a higher risk of carcinogen exposure than others. These are usually polynuclear aromatic hydrocarbons, such as benzene-containing compounds. In the cultivation of fossil fuels, carcinogen benzopyrene may be formed. Radiation exposure during x-rays and in the field of nuclear medicine may also increase the likelihood of DNA mutations and subsequent tumor formation (Upton, 1982).

Cancer Risks in Infectious Diseases

Multiple viruses such as cytomegalovirus, herpesvirus 6, and the Epstein-Barr virus (EBV) have been implicated in Hodgkin lymphoma (HL), which is a blood cancer (Hyder, 2009). Out of these three viruses, EBV has been shown to be present in approximately 80% of HL cases. It is hypothesized that a previous suppression of the immunology system has allowed altered responses to the EBV infection, and this may cause oncogenic development (Hyder, 2009). EBV has also been found in Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma. Infection is known as infectious mononucleosis and is transmitted via the saliva of infected individuals.

Schistosoma haematobium, a parasite commonly found in Africa and the Middle East, has been linked to occurrences of bladder cancer (Leventhal & Cheadle, 1979). This is due to the fact that the organism lives primarily in the veins surrounding the bladder. Snails are the natural hosts to this organism, and infection rates are increasing due to more areas coming into development for snails to breed. Chronic infection with another parasite, clonorchis sinensis, may develop into cholangiocarcinoma; however, this route

of pathology is rare (Leventhal & Cheadle, 1979). Humans can be infected after eating raw or undercooked fish (Smith, 2011). This organism is commonly found in the Far East.

Oncogenes and Tumor Suppressors

An oncogene is a gene that directly or indirectly affects the growth of a potential tumor. During the normal processes of cell replication, these genes are closely monitored with either a negative or positive feedback loop. Though some may be vital for normal cell function, they also have the potential to initiate cancerous growth if the feedback loop has been altered. For example, the disruption of regulated cell growth can be caused by a mutation in which the stop signal for replication has been dismantled. Thus, the oncogene that initiates cell replication is constitutively expressed, and further growth occurs (Wu, Emerging Tumor Markers, 1997). Another example of these growth factor oncogenes comes from the study of platelet-derived growth factor and its *sis* oncogene. In this scenario, oncogenes of the cell can stimulate growth factors that send signals to stimulate growth for itself in an auto-regulatory fashion (Weinberg, 2007).

In a human tissue cell, there are receptors for growth factors that signal for various activities such as proliferation and apoptosis, or programmed cell death. These receptor/growth factor interactions have been studied as a potential origin of carcinogenic activity. An increase in receptors would allow for more sites to bind with various growth factors to begin signaling for proliferation. As they become more concentrated in the bloodstream, measurements can be taken to possibly detect a forming tumor (Wu, Emerging Tumor Markers, 1997).

Some receptors have been used to aid in the prognosis of several cancers, such as breast or lung cancer. For example, an overexpression of interleukin-4 (IL-4) receptors has been linked to a poor prognosis in both types of cancer. Overexpression of estrogen receptors (ER) has been linked to a good prognosis in breast cancer (Wu, 1997). Because of its role in conducting signals for the proliferation of DNA, an increased expression of receptors may be the original cause of a developing tumor (Weinberg, 2007).

Tumor angiogenesis factor (TAF) is an important growth factor secreted by certain tumor cells to support their proliferation. It stimulates nearby blood vessels to generate new pathways towards the tumor. When these pathways are complete, the tumor will have access to more nutrients and be able to grow at an increased rate. From this point, the tumor can invade nearby tissue or have cells break off into the bloodstream to set up satellite tumors elsewhere (Folkman, 1976).

On the other side of the same perspective, mechanisms affecting inhibiting substances or their receptors may generate carcinogenesis. For example, the purpose of transforming growth factor beta (TGF-β) is to regulate cell growth by inhibiting proliferation. This cytokine can cause down-regulation of systems such as the inflammatory process after it is no longer beneficial to the body (Stegall, 2010). It shuts down the expression of the *myc* gene and allows expression of various inhibitors to act on the cell (Weinburg, 2007). An alteration in its inhibitory pathway may allow potential tumor cells to circumvent the effects of TGF-β. The negative feedback loop will have been disrupted, and the cell will have lost its signal to stop generating new growth.

Depending on the type of tissue from which a tumor cell is derived, some cancers may secrete an inhibitory substance against their own growth. This observation arose

when a procedure to remove a primary tumor resulted in aggressive metastases of secondary growths (Sturk & Dumont, 2005).

Role of Radiation in Vivo

Radiation is the emission of energy measured in photons. There are two types – ionizing and non-ionizing radiation. Ionizing radiation includes emissions that can cause an ionization of atoms and are considered more harmful per dose than the latter type (Upton, 1982). Non-ionizing radiation includes radio waves, visible light, and heat. These emissions are damaging due to the amount of thermal heat they produce in an individual, but at low doses they are considered generally safe.

Technically, ultraviolet (UV) light falls into the non-ionizing category; however, the damages caused by UV light are more significant than others in the same category. UV light has the capacity to alter chemical bonds even though the amount of energy required to ionize an atom is not present. A way to numerically distinguish between the two utilizes electron volts. Those photons with 2-10 electron volts are placed in the non-ionizing category. Any photons with electron volts greater than 10 are considered ionizing radiation (Bristow & Hill, 2005).

Radiation exposure occurs from natural sources, and everyone is essentially exposed to it at a continuously low dose. These compounds include cosmic rays, decaying radioactive elements found in the environment, and decaying elements found in vivo. However, those who have X-rays taken are delivered the entire lifetime amount of background radiation in one graph. To emphasize the level of radiation exposure, a measurement of each was taken; the average citizen of the United States is exposed to 0.8

millisieverts per year from natural sources, but a dental X-ray exposes the same person to approximately one millisieverts per graph (Upton, 1982). Since radiation exposure is largely due to medical diagnostic testing, an investigation into the effects of medical radiation was conducted. Two theories occurred which addressed whether lifetime radiation or radiation at a threshold amount causes carcinogenesis. Both of these theories have been supported by differing radiation wavelengths. For example, gamma rays and X-rays require frequent doses because they do not frequently cause ion formation as they penetrate cell membranes. However, over time, they can cause cancerous growth to form. On the other hand, particulate radiation, such as electrons and alpha particles, may only require one dose before causing cancerous growth. This is because particulate radiation forms a much higher amount of ions upon one application (Upton, 1982).

There are several ways for radiation to cause damage to the DNA structure. One method is by forming ions with molecular elements in the body. These ions are then responsible for reactions with water to pull off a hydrogen atom. Free radicals are formed that cause the oxidation of various cellular components and damages either enzymes or proteins by binding to them and causing conformational changes. DNA damage is the main concern of in vivo oxidation. Other methods of modification involve the photons emitted directly interacting with DNA strands. The chemical bonds of the double helical structure may be broken through translocations and inversions, or there may be alternate binding of DNA base pairs that are mismatched. Most of these effects result in DNA alterations which are the basis of cancer cell formation (Okey, et al., 2005).

Radiation Therapy

Low energy X-rays have been frequently used to treat various skin cancers because they undergo an initialization in the first layer of skin. This concentration ensures that the dose is not penetrating beyond the depth of the skin cancer. High energy X rays generated bypass the skin attenuation to penetrate deeper into the body. The advantage of high energy X-rays is that they spare the skin from unnecessary dosages of radiation (Bristow & Hill, 2005). Electrons and particles administered in the form of beams also have medical purposes.

Nomenclature of Tumor Growths

In England and America, the term "tumor" is generally regarded as synonymous with "neoplasm" – (the plural form is "neoplasia"). However, there is a difference in definition between the two terms. Tumors have been defined as any swelling or mass of tissue that occupies a space. This term does not specifically associate with cancerous cells, but may also include scars and hematomas. Neoplasia, however, are characterized by their uncontrolled growth rate along with the ability to generate new cells of the same alterations. Therefore, neoplasia would be more accurate in describing possible cancer cells within the human body. For the purpose of this research, the terms shall be interchangeable (Friedberg, 1986).

Neoplasia can be categorized as either benign or malignant. A benign classification indicates that the neoplasm will remain encapsulated within its originating organ. The cells remain differentiated, and growth is slow. A malignant neoplasm could also be described as a malignancy or cancer. Two malignant characteristics can occur.

Expansion is described as being able to grow into neighboring tissues. Invasion, however, describes the tumor cells as being carried to various areas of the body for the production of satellite tumors. These cells are typically undifferentiated with a rapid rate of growth. There are four types of cell growth: hyperplasia, metaplasia, dysplasia, and anaplasia (Hall, Nomenclature of Oncology, 2010).

Hyperplasia describes an increased cell growth of normally functioning, non-neoplastic cells. Because this type of growth can be reversed, it is not commonly considered cancerous. Puberty or pregnancy can cause hyperplasia; removal of the stimulating hormone would allow the cells to revert to their original rate of growth (Hall, Nomenclature of Oncology, 2010). Hyperplastic growth may develop into a tumor when these cells proliferate in excess amounts (Weinberg, The Nature of Cancer, 2007).

Metaplasia occurs when a substitution is made in regards to fully differentiated cells due to irritation or inflammation. An example of this is callous formation in the hands or feet. Usually specialized functions are affected in the replacement cells, such as decreased sensation (Hall, Nomenclature of Oncology, 2010).

Dysplasia is usually associated with variations in cell sizes and shapes, also known as pleomorphism. This type of growth is also reversible, but advanced stages may mimic cancer. The internal structure of a cell may also be skewed and have marked variety from the normal internal cellular structures. Dysplasia is also considered the transitional phase between benign tumors and malignant tumors (Weinberg, The Nature of Cancer, 2007).

Anaplasia includes a higher rate of pleomorphism along with the loss of differentiation within the cells. This type of growth is seen only in cancerous cells;

characteristics include abnormally large cells, large and multiple nuclei, increased amounts of deoxyribonucleic acid (DNA), and enlarged nucleoli (Hall, Nomenclature of Oncology, 2010). Cells within this category can also develop more growth receptors to continue or increase the rate of growth. The increased growth rate can cause nuclei to appear misshapenned, and the internal structure of the cell can appear disorganized. In 1-2% of cases of tumor analysis, the cells being investigated have lost all differentiating characteristics of their original tissue location (Weinberg, The Nature of Cancer, 2007).

The nomenclature of neoplasias includes a specific suffix to indicate a benign or malignant designation. Benign neoplastic growths usually combine the type of cell with the ending "–oma." For example, "adenoma" indicates a benign growth of glandular tissue. Malignant neoplasias are further classified according to their tissue of origin. If they originate from mesenchymal tissue that forms connective tissue, they are designated as "-sarcomas." If they originate from epithelial cells, they are designated as carcinomas. Cancer of the prostate gland, for example, can be defined as prostatic adenocarcinoma. A few exceptions exist such as melanomas and hepatomas. Though these are actually considered carcinomas, revision has been difficult due to the fixation of these terms in literature (Hall, Nomenclature of Oncology, 2010).

PROSTATE CANCER OVERVIEW

Epidemiology

The American Cancer Society (ACS) released a report in 2011 that stated prostate cancer had the highest estimated number of new cases of cancer diagnosed in males of all races, excluding basal and squamous cell carcinomas. Out of 822,300 total new diagnoses, 240,890 of these cases were due to cancerous growth in the prostate (North American Association of Central Cancer Registries, 2011). Mortalities due to prostate cancer ranked second only to those from lung cancer. Mortality due to prostate cancer was reported by ACS to total 33,730 deaths out of 300,430 total cancer mortalities (North American Association of Central Cancer Registries, 2011).

Symptoms

Clinical symptoms usually do not appear until after the tumor has already invaded nearby tissue or the lymph nodes (Haese, et al., 2002). These symptoms typically involve urinary irregularities, which is due to the location of the prostate gland wrapped around the urethra. A tumor which induces swelling at this location can cause the urethra to become constricted or blocked off. The patient may begin to experience a delayed urinary stream or have trouble fully relieving the contents of his bladder. Other symptoms include a slow leakage of urine after the first initial voiding. With additional strain, blood may be seen in urine or semen samples. Upon metastasis, bone pain may be felt often in the lower back (A.D.A.M. Medical Encyclopedia, 2011). With the advent of regular prostate specific antigen (PSA) screening in males approximately 50 years of age, the

suspicion of prostate cancer can be addressed before any observation of patient symptoms and make available the opportunity for curative treatments.

Routine Screening

Testing for prostate cancer includes digital rectal exams (DRE), measuring tumor markers such as prostate specific antigen, and imaging techniques.

Routine screening involves a DRE and testing serum PSA levels. A DRE is used to evaluate the size of the prostate and the presence of abnormal growths. Usually the prostate is the size of a walnut, with two palpable lobes. With swelling, a third middle lobe may be felt. However, the DRE is not very sensitive as a screening tool, nor is it ideal in regards to specificity. Very small growths may be overlooked during the exam and result in a false negative. However, these lesions could still be aggressive in their progression. Other conditions such as chronic inflammatory processes may cause nodules on the prostate, which can be misinterpreted as a false positive (Haese, et al., 2002).

Tumor markers such as prostate specific antigen have been used traditionally to evaluate prostate growth. Since PSA is tissue specific for the prostate gland, it can give a good indication of growth and activity. Elevation usually occurs approximately six months before metastasis occurs. PSA-ACT (PSA complexed to protein) has been found to be elevated more often in patients with prostatic adenocarcinoma (PCA) than in patients with benign prostatic hyperplasia (BPH). Commercial assays generally set the normal reference interval for total PSA as follows: 0-4 ng/mL is normal, 4-10 ng/mL may indicate BPH or PCA confined to the prostate, and greater than 10 ng/mL may indicate metastasis outside of the prostate (Wu, 1997). Free PSA testing may be useful in trying

to determine risk if the total PSA falls within the 4-10 ng/mL zone, also known as the gray zone. Elevated levels of free PSA may be associated with a lower probability of tumor growth, whereas lower levels of free PSA may increase the probability of prostate cancer (American Association for Clinical Chemistry, 2012).

There are various methods of utilizing PSA to increase detection sensitivity for PCA. First, PSA density can be calculated by dividing PSA concentration with the prostate gland volume. This is usually obtained by performing an ultrasound of the prostate gland to measure the volume and a serum test to measure PSA levels in the blood. Adjustments are made for variation in the development of the prostate, since levels correlate with individual size. Next, PSA velocity is obtained by testing PSA levels over a period of time and its concentration variation charted to indicate the rate of change. A significant rise may correlate with cancer. The higher the rate of change, the more aggressive of a tumor is assumed to be present (American Association for Clinical Chemistry, 2012). Another method is the PSA doubling time, which is a variation of measuring PSA over time. Last, the ratio of free to complexed PSA can be measured as a differentiation between PCA and BPH (Wu, 1997). However, a high PSA level does not necessarily correlate with the definite presence of a malignancy. False positives could occur with benign conditions. The reference intervals for PSA can also be adjusted according to the ages of the patients to make the test more specific for each individual. Additional testing to support a PCA diagnosis is recommended.

Diagnostic Testing

Once the suspicion of PCA is raised, a biopsy of the prostate gland may be performed to detect any abnormal cellular growth. Using a transrectal ultrasonography (TRUS), the physician can be guided to the correct area for obtaining samples. A sample is taken from each of the six prostatic regions, with the anterior portion of the prostate being a better area due to the frequency of growths originating from that area. This is considered the gold standard in PCA detection (Haese, et al., 2002). However, there are limitations to the technique. The initial biopsy may not have obtained a sample of any abnormal growth if the lesion is still minute, and a second biopsy may be required. Chances also increase in an enlarged prostate to obtain negative biopsies due to an increased sampling area.

Prostate Cancer Staging

Prostate cancer is staged according to the Gleason Grading System of PCA. There are two parts to this system – the Gleason grade and the Gleason score. The Gleason grade ranges from 1 to 5 in order of decreasing differentiation and is based on the biopsies. Grade 1indicates the most differentiated type of cancer. Grade 5 indicates the anaplastic stage (Haese, et al., 2002). Two observations are made; the primary grade is the most frequent type of differentiation seen, and the secondary grade is the next most common differentiation seen.

These two grades are then combined to produce the Gleason score. The Gleason score gives an indication of the rate of cancer growth and metastasis. The scores range from 2 to 10 and correlate with prognosis. A score of two to four indicates a low-grade

prostate cancer. A score of six to seven indicates intermediate-grade cancer, under which many prostate cancer diagnoses fall. A score of eight to ten indicates a high-grade cancer (A.D.A.M. Medical Encyclopedia, 2011). The risk of already having metastasis past the prostate is more likely with a higher Gleason score. After staging, additional testing can be performed to verify whether the cancer has spread and the extent of the invasion.

The World Health Organization (WHO) has another grading system in place for PCA, but this system is less precise in describing the extent of differentiation in cancers. The system includes Grades I, II and III. Grade I indicates a well-differentiated cancer and correlates to Gleason score 2-4. Grade III correlates to Gleason score 8-10. Grade II does not correlate as well to the Gleason system due to differences in better prognosis with Gleason score 5 than Gleason score 7 (Haese, et al., 2002).

Prostate Cancer Treatments

If the cancer is well contained within the prostate, and studies seem to indicate that the growth is slow, the patient and the doctor may decide to monitor any new developments instead of an immediate removal of the growth. This is termed active surveillance, also known as “watchful waiting”. It may be used on both patients who have a contained growth and those diagnosed later in life (American Association for Clinical Chemistry, 2012). For the latter, the prognosis of prostate cancer after diagnosis may involve a decade before the tumor has metastasized to cause significant health complications. Those diagnosed with it and whose prognosis is less than their life expectancy may die of other natural causes. Therefore, treatment for this cancer may not be necessary. For example, the average life expectancy of a Caucasian male living in the

United States of America is 76.05 years (Central Intelligence Agency, 2012). If the patient is diagnosed at 75 years old, his chances of mortality from other causes will be greater, and the rigors of cancer therapy may not be favored.

Tumor radiation is another alternative to surgery. However, the side effects of this treatment are not favored. They can include nausea and vomiting, hair loss, fatigue, skin changes, joint problems, infertility, and a possible secondary cancer (National Cancer Institute, 2007). There are several different approaches to radiation therapy. One type uses an external source of radiation that is targeted at the specific location of the tumor. This method requires application of a measured dose over a period of several weeks. Currently, photons or protons can be used to deliver the amount needed to destroy cancerous growth. High-intensity focused ultrasound therapy (HIFU) is another type of radiation therapy and can be used for patients who do not qualify for radiation therapy. In this method, the suspected abnormal tissues are subjected to intense heat in order to destroy cancerous growth. With patients who have already experienced back pain due to metastasis, hormone agonists can be combined with radiation to help alleviate symptoms.

The ideal curative procedure would be surgical intervention before metastasis occurs. The difficult part is determining whether a tumor is benign or malignant, and if surgery is advantageous due to the risk of infection and complications. Radical prostatectomy involves the complete surgical removal of both the prostate tumor and the prostate gland. Though efficient, this type of surgery has complications. Due to the location of the surgical procedure, nerves for erectile function may be damaged. The patient may also experience impotency or urinary incontinence. This procedure is both painful and invasive, but it can be curative if performed early (Preuss & Adderly, 1998).

In some methods, rapid freezing and thawing of tissues can be utilized to remove cancerous cells. One example is cryosurgery, which uses liquid nitrogen to freeze affected tissue and effectively destroy them (Preuss & Adderly, 1998). After surgical removal, PSA levels should drop significantly. In serum, it has a half-life of 3 days. Therefore, any detection of PSA in the serum after 6 months is a good indication of PCA reoccurrence (Wu, 1997).

If the tumor has metastasized beyond the prostate gland, the first line of treatment is androgen withdrawal (Rennie, et al., 2005). The effect of this treatment would be to stop or shrink any growth of hormone-dependent tissue, such as the prostate. Since testosterone is known to stimulate and regulate the growth of this gland, its reduction and removal from the bloodstream has been considered a primary goal in treatment. This can be achieved through various means. Physical castration by removing the testes is very effective in withdrawing and blocking androgen production. Afterwards, testosterone levels have been reported to be lower than 20 ng/mL (Tombal & Berges, 2005). However, this procedure is not favored due to its impact on the physiological response of the patient.

The traditional method involves using estradiol as an inhibitor of the testosterone feedback loop. Estradiol is structurally similar to testosterone but does not have the same effect of stimulating the prostate to grow. Because of this similarity, it can bind the same receptors and trick the hypothalamus into believing that testosterone levels are elevated and production is temporarily ceased. The inhibition of this feedback loop is an effective method to lower testosterone levels in the bloodstream as well as its effects on the prostate (Hall, Hormone Therapy For Prostate Cancer, 2010).

An alternate method is to use luteinizing hormone-releasing hormone (LHRH) agonists, which act to block LH secretion from the pituitary gland. This, in turn, blocks testosterone production in the testes. Leuprolides are one example of a class of the drugs that is available to inhibit the production of hormones. Though this method is very effective initially, testosterone levels do not reach the same amount of decreased production in the physical method; instead, initial generations of this drug allowed serum levels of at least 50 ng/dL of testosterone production (Tombal & Berges, 2005).

Furthermore, initial administration of hormone agonists may also cause an uptake in androgen receptor expression. Testosterone levels previously depressed by the drug have been known to spike later and cause an acute-on-chronic development (Tombal & Berges, 2005). With increased receptors binding to the excess testosterone, tumor growth is then greatly stimulated and can result in metastasis. To help eliminate this scenario, anti-androgens can be administered concurrently to bind and block the activation of androgen receptors on tumor cells.

With additional pharmaceutical developments, this issue has been addressed. The current generation of leuprolide drugs, one of which is known as Eligard®, are administered in single doses that last for three months. It is injected subcutaneously, and the administered dose has a slow release mechanism for the drug (Tombal & Berges, 2005). This drug has been found to mimic physical castration by lowering levels of testosterone to less than 20 ng/dL. With Eligard®, the breakthrough surges in testosterone have also declined.

Chemotherapy has been studied as another method of treatment for prostate cancer. Typically, this route is opened after androgen withdrawal therapy fails to sustain

an adequate response against metastasis. For patients with hormone-refractory prostate cancer (HRPC), docetaxel has been reported as having significant improvement in patient prognosis as compared to other standards of care (Shelley, et al., 2006).

TESTOSTERONE OVERVIEW

Testosterone is categorized as an androgen whose main source of production is in the testes. Approximately 90% of the body's supply is produced there, with trace amounts produced indirectly through the adrenal glands (Rennie, et al., 2005). This hormone controls masculine sexual differentiation, such as a deepening voice, hair growth, and development of a masculine skeletal frame. It also aids in maturing secondary spermatocyte by acting on the primary spermatocytes. In regards to its role in prostate tumor growth, testosterone also has a growth effect on the seminal vesicles and prostate gland (Kudolo, 2003).

Pathway

Testosterone is synthesized through a series of enzyme catalysts located in smooth endoplasmic reticulum of cells. Cholesterol molecules are the starting basis for testosterone. The body can obtain cholesterol both endogenously and exogenously. Exogenous cholesterol taken from the diet contributes to 150 – 300 milligrams daily while endogenous cholesterol produced by the human body creates 1.5 grams per day (Mroz, 2003).

Production of testosterone begins in the brain. It is synthesized through the Hypothalamus-Pituitary-Gonadal (HPG) system and regulated through negative feedback. The hypothalamus secretes gonadotropin-releasing hormones (GnRH) in a periodic manner every 65 to 90 minutes (Haese, et al., 2002).This feeds into the pituitary gland to stimulate the release of luteinizing hormones (LH) and follicle stimulating hormones (FSH). In turn, LH stimulates Leydig cells to catalyze the conversion of

cholesterol into testosterone. Then, testosterone is transported to either the Sertoli cells within the seminiferous tubules or to the peripheral circulation. Most of the testosterone hormone is bound to albumin or sex hormone-binding globulin (SHBG). This binding renders the hormone biologically inactive. Two percent remains unbound and biologically active, and this causes sexual differentiation to occur (Rennie, et al., 2005). Testosterone can also be converted to 5α-dihydrotestosterone (DHT) in the liver or skeletal muscles, which is a more potent form of the hormone. Eventually, this increased level will send a signal to the hypothalamus by binding to receptors and down-regulate GnRH. In turn, the pituitary gland decreases activity and the pathway is inhibited.

Testosterone Levels

The normal reference interval for total testosterone levels in a male range from 300 to 1000 ng/dl (Kudolo, 2003). Decreased levels may cause a decreased libido and complications in reproduction. As an adult male becomes older, the level of testosterone may decrease and a physician may recommend hormone replacement therapy. However, this may indirectly affect the prostate cancer. Since the decline of testosterone and the beginning indications of prostate cancer occur in approximately the same age group, this study has been done to investigate whether a correlation between the two can be established for diagnostic purposes.

To test for testosterone production defects, several radioimmunoassay and enzyme immunoassays are available. Usually the sample is collected in a heparin tube, and the sample can be tested within one week if refrigerated or up to 6 months if frozen. The gonadotropin-releasing hormone (GnRH) assay tests whether the pituitary gland will

show an adequate response when stimulated with GnRH. An unresponsive pituitary gland may show a secondary defect in the production of testosterone. The human chorionic gonadotropin (hCG) stimulation assay tests for primary testicular defects by using hCG as a replacement for luteinizing hormone (LH). The replacement is given, and testosterone levels should show an increase. If there is no response, a defect in the testes may be present. Defects in the synthesis of gonadotropins can also lead to decreased testosterone levels by not producing a signal for production. Some diseases associated with a decreased testosterone level include hypogonadism and decreased sexual differentiation (Braunstein, 2004).

For testosterone levels that are abnormally high, a tumor may be present in tissue that secretes testosterone. For example, the adrenal cortex where androgens are secondarily synthesized may exhibit a growth of select cells in which testosterone is constitutively produced without regard to any feedback inhibition. Another suggestion would be that there is a disruption along the testosterone production pathway in which a stimulating factor has been continually expressed, causing the conversion of all cholesterol molecules to testosterone. The detrimental effect of excess testosterone in relations to the prostate gland would be an increased growth and stimulation of these cells – including both normal, differentiated cells and potentially, malignant undifferentiated cells. High testosterone levels can usually be detected using blood tests and a urinary 17-ketosteroids test, which tests for testosterone metabolites (Kudolo, 2003). Increased testosterone can be an indicator that cells may be under the influence of a stimulating growth factor.

Androgen Receptors

Androgen receptors (AR) are the primary targets for testosterone and its more potent derivative, dihydrotestosterone (DHT). The androgen binds to the prostate gland's cell receptors and initiates gene transcription. This promotes DNA replication and growth. With continued testosterone stimulation, the prostate gland may exhibit swelling. Any cell that may have undergone mutation is also stimulated to grow. Thus, an increased level of testosterone may indirectly cause PCA. Measuring this analyte in the serum can raise suspicions that the prostate gland has been affected. However, a high level of testosterone is not specific for cancer in any tissue. Other types of cancers, such as testicular cancer, can raise testosterone levels (Kudolo, 2003). Hormone therapy to correct falling testosterone levels in aging adults may also indirectly stimulate any tumors present. Along with growth of the cells, proteins such as PSA are synthesized. Therefore, an increase in PSA can be correlated to a growth in the prostate.

Cell receptors can also be activated without stimulation from androgens; some initiators include vitamin D, retinoic acid, interleukin-6 (IL-6), transforming growth factor-beta (TGF-β), and epidermal growth factor (EGF) (Haese, et al., 2002). When the cancerous cells are able to grow in the absence of androgens, this development is termed hormone refractory PCA. Typically, failure of androgen withdrawal treatment is due to these other growth factors. One source hypothesized that various growth factors were the original carcinogenic material used to initiate cancerous growth (Wu, 1997).

PROSTATIC ACID PHOSPHATASE OVERVIEW

Acid phosphatase can be found in various organs and tissues, such as the prostate, the bones, the spleen, and in red blood cells (Miteva, et al., 2010). There are four isoenzymes of acid phosphatase: erythrocytic, lysosomal, prostatic, and macrophagic (Haese, et al., 2002). PAP is used in the hydrolysis of tyrosine phosphate esters. It is secreted during ejaculation to aid in liquefaction of semen. These enzymes are nonspecific for any tissues. Therefore, the presence of PAP in serum does not necessarily indicate the presence of a tumor in the prostate region. Furthermore, analysis of this substance is hindered by its instability as an enzyme and its diurnal variation in vivo. Though the prostatic portion of ACP can be identified by chemical means – it is resistant to tartrate – the significance of this molecule has been considered inferior to PSA as a tumor marker for prostate cancer. (Haese, et al., 2002)

ACP has not been reliably noted as a good screening tool for detecting prostate cancer; however, it has been used to differentiate among patients with extracorpuscular or encapsulated diseases (Hall, 2003). Testing for this analyte is also difficult due to its isoenzymes and their similar reactivity. This causes interference from acid phosphatases that are not derived from the prostate. ACP can also experience diurnal variation, making it harder to correlate rising and falling levels with disease progression. A good use for ACP has been indicated in considering surgical candidates. After a diagnosis of prostate cancer has been established via other measures, ACP concentrations can be an indicator of a more aggressive disease. This would help screen patients whose disease has progressed past the stage where surgery would not be beneficial (Haese, et al., 2002).

PROSTATE SPECIFIC ANTIGEN OVERVIEW

Biological Role

PSA is a serine protease secreted by the epithelial cells of the prostate gland and is used biologically to aid in reproduction. It causes the liquefaction of the semen in order for sperm to travel easier through the vaginal canal. It also helps to neutralize vaginal secretions and allow spermatozoa to achieve a longer lifespan for the purpose of fertilization (Arneth, 2009). In the serum, the majority of PSA is bound to α1-antichymotrypsin (ACT) and is known as the PSA-ACT complex with a small amount existing as free PSA. This portion is biologically inactive.

Laboratory Detection

There are several methodologies available to clinical laboratories that would quantitate PSA levels. Each method is performed on a compatible machine analyzer. Two examples of these PSA assays are the ADVIA Centaur PSA Assay and the Architect I Total PSA assay. Currently, total PSA is the preferred screening test; free PSA is usually measured if the total PSA shows increased levels. (Arneth, 2009). If the free PSA is elevated in relation to total PSA, a benign condition may be suspected.

Biological False Positives

Conditions other than cancer that affect the prostate may cause PSA levels to rise temporarily. An infection in this area is an example of a source of increased PSA levels. Therefore, it is recommended that PSA levels be repeated between 6 weeks to 3 months to ensure a correct diagnosis. Rigorous exercise in which inadvertent manipulation of the

prostate occurs can also cause an increase in PSA. Riding a bike or collecting a sample after a DRE may cause this reaction. A waiting period of 6 weeks after manipulation is recommended. Also, samples should not be collected if the patient has ejaculated within the last 24 hours (American Association for Clinical Chemistry, 2012).

MATERIALS AND METHODS

Patient Samples

This study was given approval by the USM IRB. The procedures used followed the ethical guidelines set forth by the University of Southern Mississippi (USM). Patient samples were provided by Memorial Hospital at Gulfport, Singing River Hospital, and Wilford Hall Hospital. Diagnoses were made by the attending physicians based on pathological examinations. Five hundred and fifty-two patients were evaluated. Eighty-two patients were diagnosed with prostate cancer and four hundred and sixty-nine patients were diagnosed without prostate cancer. One hundred and two healthy adult control patients were also collected from the airforce hospital in Wilford Hall Hospital at San Antonio. The serum samples were collected and stored frozen at -20°C. Each sample was identified by a code number (numerical value) that was linked only to collected PSA values and prostate cancer evaluation (diagnosis) and had no correlation with patient identification information. All patients evaluated were greater than or equal to 18 years of age.

Prostate Specific Antigen

Testing was performed on the patient samples using Beckman Coulter Synchron LXI 725 technology at Memorial Hospital at Gulfport and Singing River Hospital. This company has office locations in California. A list was provided with sample values as well as the status of each patient in regard to a prostate cancer diagnosis.

The ADVIA Centaur PSA Assay measures total PSA in serum. This testing method is a sandwich immunoassay which utilizes two antibodies that bind to different

sites on the PSA molecule. The first reagent, known as the Lite Reagent, is a polyclonal goat anti-PSA antibody conjugated with acridinium. The second reagent, known as the Solid Phase, is a monoclonal mouse anti-PSA antibody. The Acid Reagent and Base Reagent are used to initiate the chemiluminescent reaction. This measurement is quantitated in relative light units (RLUs) and is proportional to the amount of PSA detected in the sample.

The Access Hybritech free PSA assay is a chemiluminescent sandwich assay, performed on the Synchron LXI 725, which quantitates free PSA in serum. The sample is added to a well with mouse monoclonal anti-free PSA conjugated to alkaline phosphatase and immobilized paramagnetic particles bound to a second mouse anti-free PSA antibody. The patient's serum PSA reacts with the immobilized antibody while the conjugated anti-PSA reagent competes for a different site on the patient's serum PSA molecule. This bound complex is held by magnetic energy, and unbound material is washed away. The chemiluminescent substrate, Lumi-Phos* 530, is added. The light generated is measured with a luminometer and is directly proportional to the free PSA levels in serum. This quantitation is derived from a calibration curve (Beckman Coulter, Inc., 2010).

Testosterone Enzyme Immunoassay

This method utilizes competitive binding between serum testosterone and reagent testosterone-Horseradish Peroxidase (HRP) conjugate to rabbit anti-testosterone antibody. During incubation, wells coated with goat anti-rabbit antibodies are incubated with 10 uL of patient samples, 100 uL of reagent testosterone-HRP conjugate, and 50 uL

rabbit anti-testosterone reagent. As the concentration of serum testosterone increases, the reagent testosterone- enzyme conjugate has more competition for binding to the rabbit anti-testosterone. With more serum testosterone present to bind to the antibody, there are fewer sites for the reagent testosterone conjugate to bind. Any unbound conjugate will be washed away, leaving only bound serum and reagent testosterone bound to the rabbit anti-testosterone antibody. This antibody is not washed away due to its binding affinity to the goat anti-rabbit coated wells. The TMB-substrate is then added and a blue color is allowed to develop for a monitored time period. Color development is stopped with 1N hydrochloric acid. The amount of color development is detected with a spectrophotometer at 450nm wavelength. There is an inverse relationship between the spectrophotometric value and the serum testosterone value.

The kits used were produced by Diagnostic Automation, Inc. The reference number for the kit is 20952.

Testosterone EIA Procedure (obtained from the manufacturer's insert):

1. Bring all reagents to room temperature. Serum is used.
2. Secure the desired number of coated wells in the holder.
3. Dispense 10 uL of standards, specimens, and controls into appropriate wells.
4. Dispense 100 uL of Testosterone-HRP Conjugate Reagent into each well.
5. Dispense 50 uL of rabbit anti-testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at 37˚C for 90 minutes.
7. Rinse and flick the microwells 5 times with distilled or deionized water.

8. Dispense 100 uL of TMB Reagent into each well. Gently mix for 5 seconds.
9. Incubate at room temperature (18-25˚C) for 20 minutes.
10. Stop the reaction by adding 100 uL of Stop Solution to each well.
11. Gently mix 30 seconds. Ensure all the blue colors changes to yellow completely.
12. Read absorbance at 450 nm within 15 minutes.

Expected normal values for the males were also obtained from this insert. The NRI of prepubertal males ranges from 0.1 - 0.2 ng/ml. The NRI of an adult male ranges from 3.0 - 10.0 ng/ml. This test method can detect testosterone levels greater than 0.05 ng/ml.

Prostatic Acid Phosphatase Enzyme Immunoassay

This test method utilizes a solid phase enzyme linked immunosorbent assay (ELISA) method to detect PAP levels in blood. The microwells are coated with rabbit anti-PAP antibodies that bind to PAP in the serum sample. Then, mouse anti-PAP antibody conjugated to HRP is added and binds to a different epitope of PAP. Any unbound conjugate is washed away. The TMB substrate is added, which is acted on by the HRP to give a colorimetric reaction. The amount of color development is proportional to the level of PAP in the sample.

This kit was produced by Diagnostic Automation, Inc. The catalogue number for the kit is 42272.

PAP EIA Procedure (obtained from the manufacturer's insert):

1. Bring all reagents to room temperature. Serum is used.
2. Secure the desired number of coated wells in the holder.
3. Dispense 25 uL of standards, specimens, and controls into appropriate wells.
4. Dispense 100 uL of Enzyme Conjugate Reagent into each well. Incubate for 30 minutes at room temperature.
5. Remove incubation mixture and rinse the wells 5 times with washing buffer (300ul/well/each rinse).
6. Dispense 100 uL of TMB Solution into each well. Incubate for 15 minutes at room temperature.
7. Stop the reaction by adding 50 uL of Stop Solution to each well.
8. Read absorbance at 450 nm in 5 minutes.

Expected normal values for the males were also taken from this insert. The NRI of adult males ranges from 0.0 – 5.0 ng/ml. Elevated levels can indicate prostate cancer or BPH. A low level does not necessarily indicate a lack of cancer.

Statistical package

Results were analyzed using the SPSS version 18 statistical software package.

Miscellaneous Instrumentation

Washing of the microwell plates was performed with the Stat Fax 2600 microplate washer. Absorbances for each well were then measured using a Beckman

Coulter AD 340 microwell plate reader. Incubation was achieved via the Thermo Scientific General Purpose Incubators Series 2076.

RESULTS

Table 1. Assay Precision

Precision values for the test procedures used during this study are given in Table 1. The amount of runs completed is represented by the letter "n." Control sera with known analyte values were used to determine within-run and between-run assay precision for each analyte measured. The mean (x), standard deviation (SD), and the percent coefficient of variability (%CV) were calculated. These values serve to indicate how much variability was detected for each procedure and allow comparison between different testing methodologies. In the clinical laboratory a %CV equal to or less than 10% is considered good. A %CV less than 20% is acceptable, and values above 20% are questionable.

Two levels of testosterone controls were monitored. Level 1 was a low control and Level 2 was a high control. The %CV was poor for both within-run and between-run precision using the low control (23%CV and 91.96%CV respectively). By contrast, the within-run assay precision was good (10.74%CV) for the high control. The prostatic acid phosphatase (PAP) assay had poor precision both within-run (41.78%CV) and between-run (58.40%CV). The prostate specific antigen (PSA) assay was automated and had excellent precision both within-run (2.00%CV) and between-run (2.20%CV). Manual pipetting could significantly alter the standard volume dispensed into each microwell, causing higher or lower spectrophotometric values.

Table 2. Assay Linearity

This table compares the assay linearity for PSA, PAP, and testosterone. As the substance increases in serum, the spectrophotometric value should also increase in the same regard. Thus, a correlation can be established between the spectrophotometrically measured value and the true value (Tholen, et al., 2003). For example, an analyte which has doubled in value would be expected to also double in the observed value. For these three procedures, the linearity (represented as R-squared) is very good. Perfect linearity would be represented by the value "1." All three tests come very close to this value, which assures the laboratory that our values are being interpreted accurately from the spectrometric value to the diagnostic value. These values were taken from a linearity graph (Figures 1 through 3).

Table 3. Normal Reference Intervals

This table represents the healthy adult reference ranges from our supporting hospitals. It studies our demographic to compile a more reliable picture of the values usually seen in the southeastern states. The letter "n" represents the number of healthy patients who were tested with each of the various procedure; healthy is defined here as having no symptoms or cause to suspect disease. The PSA assay, processed on the Beckman Coulter Synchron LXI 725, gave a range of 0-2.90 ng/mL as the normal reference range for a healthy adult. The PAP and testosterone assays demonstrated a larger normal reference ranges. If these reference values were to be adopted in a laboratory, these values would be used to interpret the presence or absence of disease for the surrounding community. The process of calculating the normal reference ranges uses

the mean value of all the test values for that assay and the standard deviation. Then, twice the standard deviation plus the mean gives the upper value of the normal reference range. The mean minus twice the standard deviation gives the lower value of the normal reference interval; in this case, the range stops at zero. Thus, Table 3 represents the cut-off values that our demographic would use as an indicator of health versus disease. However, for the purpose of this study, the manufacturer's recommended values were used.

Table 4. Assay Analytical Sensitivity

Analytical sensitivity represents the lowest concentration of a chemical substance that can be detected by an assay. Approximately 20 replicates of a "zero control" were tested for each assay procedure. The zero control consisted of buffer without the analyte. The mean plus and minus two standard deviations ($\bar{x} \pm 2$ S.D.) represents the analytical sensitivity range. Similarly, the mean plus two standard deviations ($\bar{x} + 2$ S.D.) represents the cutoff between results which are essentially zero and those which have numeric value. Results are found in Table 4.

The Beckman Coulter Synchron LXI 725 required at least 0.008 ng/ml before detection began. The PAP and testosterone assays required a higher concentration before detecting each substance. For testosterone, which has a NRI of 3.0-10.0 ng/mL, a value of 2.330 ng/ml analytical sensitivity would still pick up any significant increases. However, the PAP analytical sensitivity started at 1.980 with an NRI of 0.0-3.5 ng/mL. It would require the PAP levels to be higher before it is detected and used for diagnosis.

Table 5. Diagnostic Parameters

Five hundred fifty-one patients were tested for PSA, PAP, and testosterone levels (Table 5). Out of the three substances, the PSA values had the highest diagnostic sensitivity (30.12%). This value indicates the portion of individuals with prostate cancer who test positive for the disease; however, the percentage for this analyte is still very low. Approximately 70% of those who had the disease were not detected by this assay. PAP had the second best % patient sensitivity, and testosterone had the lowest % patient sensitivity percentage. Testosterone detected no cases of prostate cancer, which gave it a diagnostic sensitivity of 0%.

For the diagnostic specificity, testosterone had the best percentage (96.80%). This indicated that the analyte was very good in ruling out prostate cancer in patients who do not have the disease. PSA had the second best specificity (91.29%), and PAP had the lowest specificity (80.38%).

The positive predictive value (PV) reflects the fraction of positive results that were a true positive versus a false positive. The negative PV detects the fraction of negative tests that are true negatives. PSA had the best value for both positive and negative PV. Since testosterone did not indicate any positive results, it had a positive PV of 0%. The negative PV of testosterone (84.70%) closely followed that of PSA (87.58%). Testosterone also had the best diagnostic efficiency (82.40%), though PSA closely followed this value (81.73%). This calculates the accuracy of each test in detecting true negatives and true positives out of all the test results. The cut-off values for each test to be considered a positive result are also listed.

Table 6. Combination Testing

After using the given statistics to investigate whether a combination of these tumor markers could increase the diagnostic sensitivity of prostate cancer screening tests, it was found that PSA combined with PAP had better results (43.37%) than when PSA is combined with testosterone (30.12%). Once again, testosterone does not contribute any type of sensitivity to the test. Furthermore, it lowers the specificity of the PSA test. When PSA and PAP are combined, the diagnostic sensitivity increases by approximately 13 percent. However, the specificity has dropped compared to only testing PSA.

DISCUSSION

The findings in this research did not support the hypothesis that testosterone would prove to be a superior tumor marker for the detection of prostate cancer, as compared with prostate specific antigen or prostatic acid phosphatase. Though it had the best specificity for patients who truly did not have the disease, it had no sensitivity for ruling in prostate cancer and was not useful as a screening tool. Several other studies have also tried to correlate testosterone to prostate cancer with varying results.

One particular study by a different research group was conducted on total testosterone in an attempt to improve the prostate cancer screening tests. This study measured both total testosterone levels and total testosterone/PSA ratios as a predictive index for determining good candidates for prostate biopsies. Approximately 1570 participants who had an abnormal DRE and/or elevated PSA levels were chosen to complete the study. The researchers found that there was no significant difference in total testosterone levels when compared among PCA, BPH, or a non-cancer diagnosis. They also noted that there was no significance in total testosterone levels when compared to patients with varying Gleason scores. Their study concluded that measuring total testosterone levels was not useful for the selection of biopsy candidates or for the prediction of cancer aggression in existing cases (Botelo, et al., 2012).

This research was supported by another group that measured serum testosterone levels after radical prostatectomy in 579 patients with PSA levels < 20 ng/mL. They also assessed the prostate size via magnetic resonance imaging before the operation. Their study showed an initial link between lower serum testosterone levels and Gleason scores of greater than 8; however, after covariates were accounted for, the link was eliminated.

Their study concluded that a more aggressive case of prostate cancer may be linked to smaller prostate sizes but not to serum testosterone levels (Kwon, et al., 2010).

Another study investigating the same hypothesis, however, concluded that testosterone levels did appear to indicate more aggressive diseases according to the Gleason score. Their study divided the patients into two groups based on total testosterone. Group 1 contained patients with less than 3 ng/mL, and group 2 contained patients with greater than or equal to 3 ng/mL. Their research showed that group 1 correlated with higher Gleason scores of >7 while group 2 correlated with confined, localized diseases (Xylinas, et al., 2011). Their findings may have differed due to the separation of testosterone levels and the independent evaluation of each study group, as well as the distinction for prostate size.

A different study used a ratio of free testosterone to total testosterone, also known as percent free testosterone, to determine the significance of this substance in prostate cancer screenings. A 12-core biopsy was performed on approximately 812 white Italian men with no prior history of PCA. Patients with PCA were separated into two categories. The low grade group was based on a Gleason score of less than or equal to 6, and the high grade group was based on a Gleason score of greater than or equal to 7. Though the percent free testosterone levels did not correlate with the detection of low grade PCA, it was a significant factor for the prediction of high-grade PCA. The researchers concluded that percent free testosterone rather than total testosterone would be a better testing method for detecting more aggressive PCA cases (Albisinni, et al., 2012).

The varying results in these studies seem to indicate that testosterone may have importance in the prognosis and staging of prostate cancer instead of in screening tests.

Different measurements of serum testosterone, such as the percent free testosterone, may show a better correlation to PCA than measuring total serum testosterone alone. Further topics of research would be to evaluate the various measurements of testosterone as a screening tool. Though the ratios of testosterone, PSA, and PAP did not show a satisfactory predictive value for PCA, testosterone as a ratio or another derivative may improve this number. Another possible topic for future research in this field may include quantitating testosterone cell receptors located on potential tumor cells. A hypothesis would be that a decreased amount could correlate with disease progression, with significant decrease possibly signaling the phase when tumor cells become androgen-independent.

CONCLUSION

The analytical parameters were good for all three testing methods. Therefore, values obtained using these assays should be reliable. The results of this study reject my hypothesis and indicate that testosterone was not effective in correctly diagnosing those with prostate cancer. Testosterone had the lowest diagnostic sensitivity of the three procedures, but it had the highest diagnostic specificity for ruling out prostate cancer. Possible explanations for having 0% PV may be due to skewed results from patients already undergoing treatment. Also, the reference values used may not reflect the demographic of our samples and would require revision.

Table 1. Assay Precision

Comparison of PSA, PAP, and Testosterone Precision using Control Sera.

	n	X (ng/mL)	SD (ng/mL)	CV (%)
Within-Run				
Testosterone Level 1	20	4.25	0.99	23.29
Testosterone Level 2	24	19.65	2.11	10.74
PSA	2	1.00	0.02	2.00
PAP	20	2.13	0.89	41.78
Between-Run				
Testosterone Level 1	15	5.11	4.69	91.96
Testosterone Level 2	n/a	n/a	n/a	n/a
PSA	40	1.00	0.02	2.20
PAP	22	3.51	2.05	58.40

Table 2. Assay Linearity

Comparison of Linearity with PSA, PAP, and Testosterone.

Assay	R squared
Testosterone	0.9830
PSA	0.9996
PAP	0.9850

Table 3. Normal Reference Intervals

Comparison of Healthy Adult Reference Intervals for PSA, PAP, and Testosterone.

Assay	n	X (ng/ml)	SD (ng/ml)	Range (ng/ml)
Testosterone	102	4.44	3.40	0-11.24
PSA	80	0.98	0.96	0-2.90
PAP	101	7.79	14.99	0-37.77

Table 4. Assay Sensitivity

Comparison of Sensitivity with PSA, PAP, and Testosterone.

Assay	n	X (ng/ml)	SD (ng/ml)	Range (ng/ml)
Testosterone	20	1.21	0.560	0-2.330
PSA	20	0.00	0.004	0-0.008
PAP	19	0.32	0.830	0-1.980

Table 5. Diagnostic Parameters

Comparison of Diagnostic Parameters in PSA, PAP, and Testosterone for Prostate Cancer in 551 Patients.

Assay	Sensitivity (%)	Specificity (%)	PV (+)	PV (-)	Efficiency (%)	Cut-off (ng/mL)
Testosterone	0.00	96.80	0.00	84.70	82.40	10.00
PSA	30.12	91.29	39.06	87.58	81.73	4.00
PAP	20.73	80.38	15.60	85.29	71.51	5.00

Table 6. Combination Testing

Comparison of Diagnostic Parameters after PSA is combined with Testosterone and/or PAP to detect Prostatic Cancer

Assay	Sensitivity (%)	Specificity (%)	PV (+)	PV (-)	Efficiency (%)	Cut-off (ng/mL)
PSA / Testosterone	30.12	89.25	32.89	87.84	80.29	n/a
PSA / PAP	43.37	75.11	23.53	88.25	70.34	n/a
PSA / Testosterone/ PAP	43.37	72.77	21.95	87.92	68.35	n/a

Figure 1. Testosterone Linearity

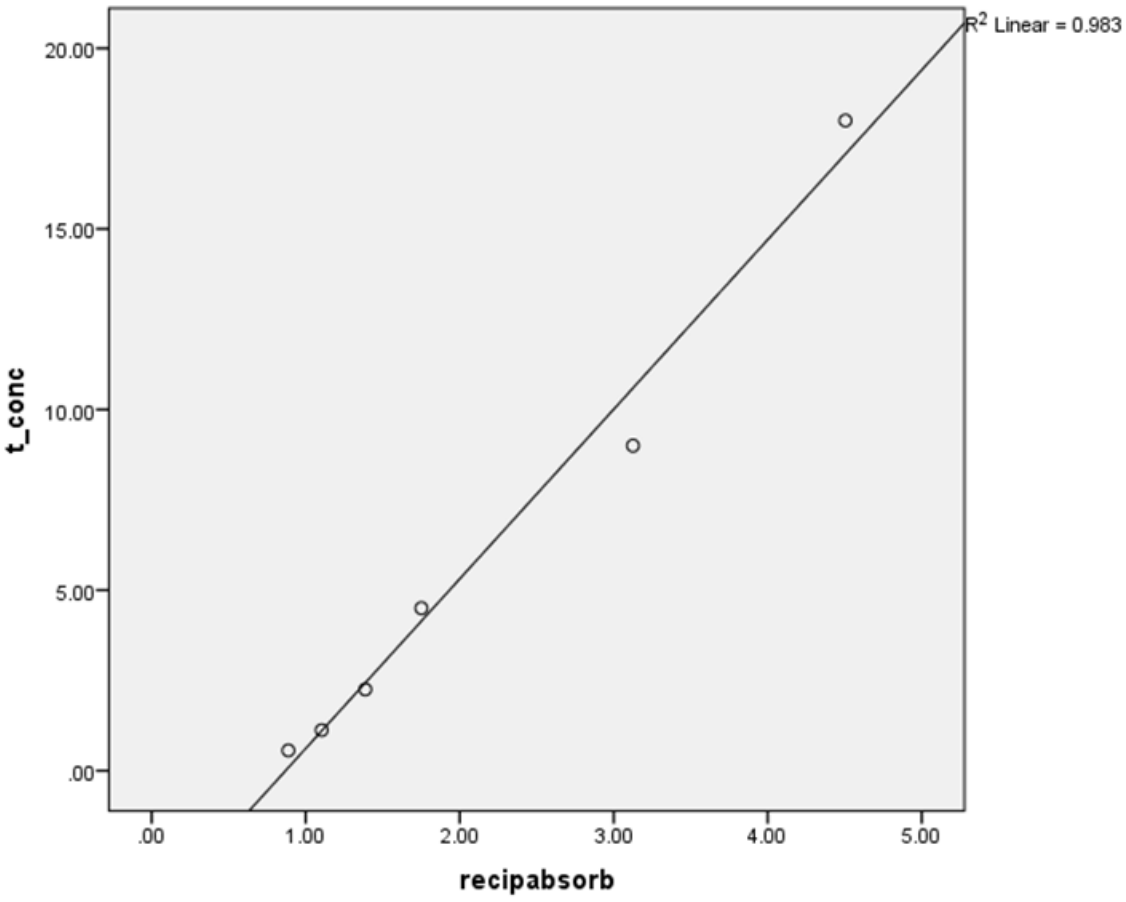


Figure 2. Prostate Specific Antigen Linearity

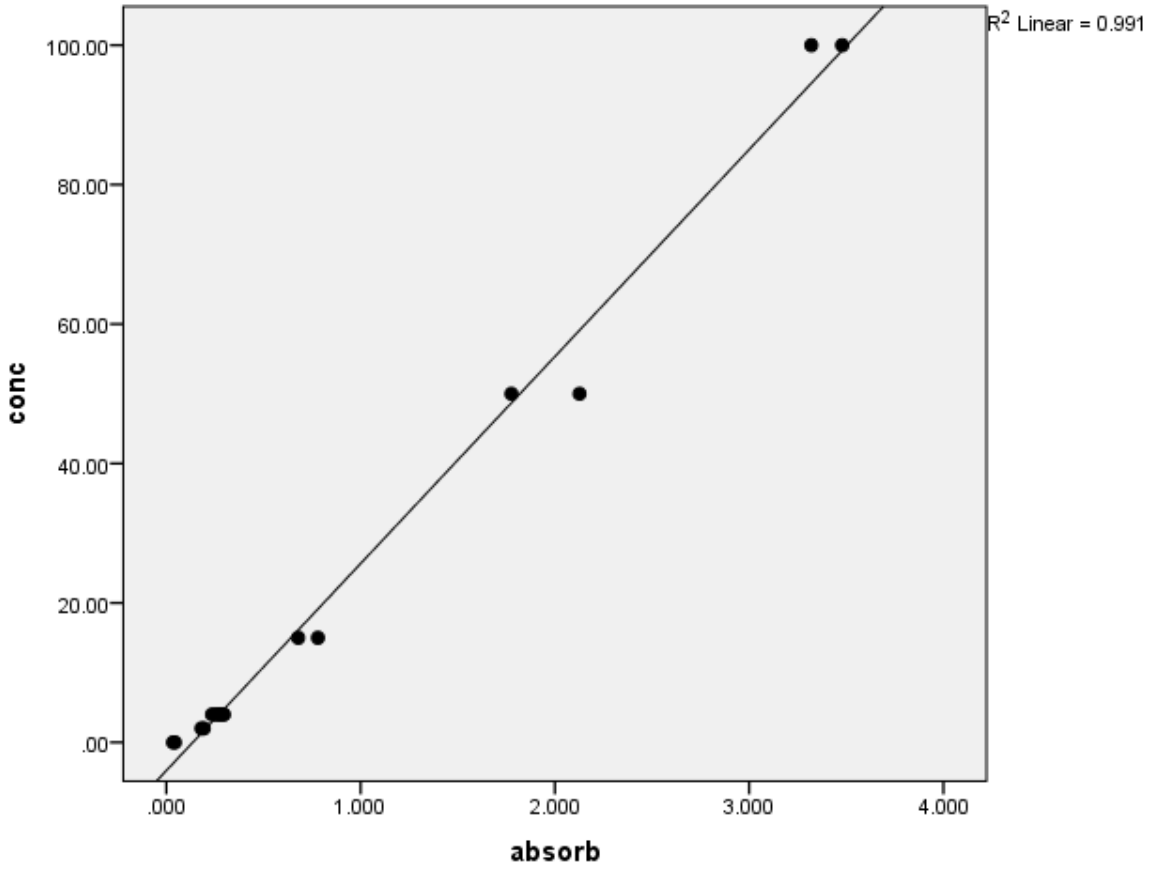
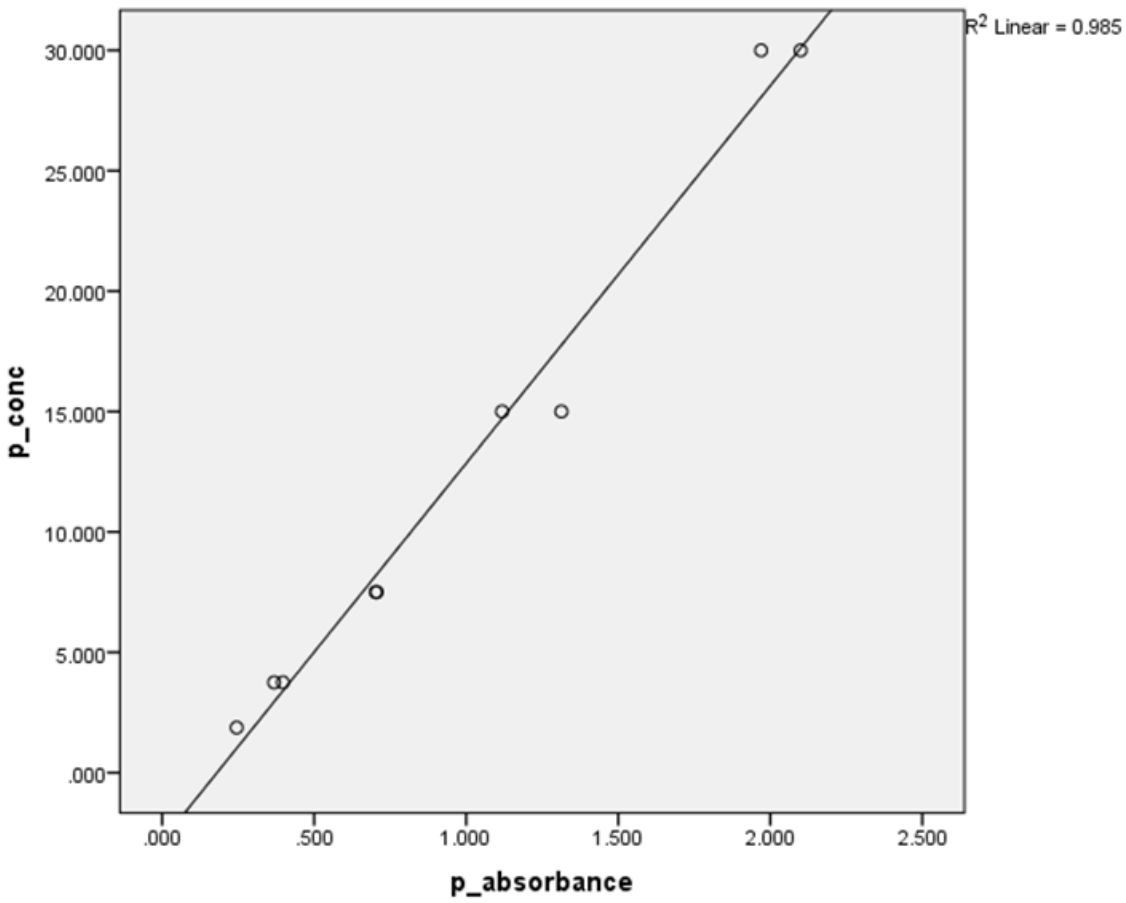


Figure 3. Prostatic Acid Phosphatase Linearity



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