Original article

Assessment of the risk of prostate cancer in adult smokers in Nnewi, Nigeria using prostate specific antigen as a biomarker

Patrick O. Manafa,[1] AIMLT, (Med. Lab. Science)MSc (Biochem.), PhD (Biochem.);**Augustine C. Ihim**,[1] B.MLS (Med. Lab. Science), MSc (Med.Lab.Science Option in Chem.Path.); **Chinaza .I. Ekwueme[1] B.MLS** (Med. Lab. Science); **Adeola O. Oluboyo**,[1] AIMLT, (Med. Lab. Science) MSc (Chem. Path), PhD (Immunology);**George Chukwuma**,[1] B.MLS (Med. Lab. Science), MSc (Haematology);PhD Immunology; **Chukwuanukwu Beccky** AIMLT(Med. Lab. Science), MSc (Haematology);PhD Immunology; **Onyemelukwe Anuli** [1] B.MLS (Med. Lab. Science), MSc (Med.Lab.Science Option in Histopathology)

[1] *Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus.*

Corresponding author: Augustine C. Ihim (chihimaustin@yahoo.com, phone: +2348035985883)

Abstract

Introduction: Prostate cancer is one of the most prevalent types of cancer in men, when detected early, it is potentially curable by prostatectomy . Therefore early detection is important**.**

Objective: In this study the relationship between cigarette smoking and the risk of prostate carcinoma using the prostate specific antigen (PSA) as a marker was evaluated.

Materials and Method: One hundred adult male subjects participated in this study. Fifty of these subjects were smokers while the other fifty were non-smokers. Subjects were grouped into four categories of varying ages. About 5mls of blood were collected. Prostate Specific Antigen Levels were determined by the Enzyme-linked Immunosorbent Assay (ELISA).

Result: The mean total Prostatic Specific Antigen (PSA) value for smokers was 1.93±1.26ng/ml and for non-smokers, it was 2.77±1.40ng/ml, while the mean free PSA value for smokers was 1.06±0.79ng/ml and 1.45±0.80ng/ml for non-smokers. There was a significant decrease in the mean total PSA value of smokers compared with non- smokers ($P<0.05$). There was also a significant decrease in the mean value of free PSA of smokers compared with the control group ($p<0.05$). Furthermore, the total PSA levels and the ages of test subjects showed a significant positive correlation value of $r = 0.91$, while a positive correlation value of 0.75 was obtained between the Free PSA levels and the ages of the test group

Conclusion: Although the above findings suggest a reduction in prostate cancer risks in cigarette smokers, low levels of prostate specific antigen does not completely exclude the possibility of prostate cancer risk

Keywords: Prostate Cancer, Adult Smokers, PSA

Introduction

The prostate gland is a part of the male reproductive organs that helps make seminal fluid in adult men. A typical prostate gland is about three centimeters. It is located in the pelvis under the urinary bladder and in the front of the rectum [1]. The prostate surrounds parts of the urethra, the tube that carries urine from the bladder during urination and semen during ejaculation [2]. Due to its location, prostate diseases often affect urination, ejaculation, and rarely defecation [3]. The prostate contains many small glands which make about twenty percent (20%) of the fluid constituting semen [4].

Prostate-specific antigen (PSA), also known as gamma-seminoprotein, is a single-chain glycopr-oteinenzyme encoded in humans by the kallikrein-

3(*KLK3)* genehaving carbohydrate side chains with linkages at amino acid 45 (asparagines), 69 (serine), 70 (threonine) and 71 (serine) [5]. Prostate-specific antigen is a member of the kallikrein-related peptidase family and is secreted by the epithelial cells of the prostate gland. Prostate-specific antigen is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely. It is also believed to be instrumental in dissolving cervical mucus, allowing the entry of sperm into the uterus. Prostate-specific antigen was discovered by Hara and colleagues [6]. Prostate-specific antigen is present in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer or other prostate disorders [7].

Prostate cancer is a slow growing cancer and the most commonly diagnosed malignancy in males in the developing world and ranks second among causes of cancer death in men [8][9].

Prostate cancer tends to develop in men over fifty years old and although it is one of the most prevalent types of cancer in men, many never undergo therapy, and eventually die. When detected early, it is potentially curable by prostatectomy. Therefore early detection is important [10].Prostate cancer may cause pains, difficulty in urination, problems during sexual or erectile dysfunction [11]. Prostate cancer does cause symptoms, often similar to those of diseases such as benign prostate hyperplasia related to the problem of an enlarged prostate in most male adults. The few well-established risk factors for prostate cancer incidence include increasing age, race/ethnicity (being African American or Jamaican), having a positive family history and certain lifestyle habits which may include smoking [12]. The most common system for evaluating prostate cancer is the four stage TNM system (abbreviated from Tumor-/Nodes/Metases), its complements includes the size of the tumor, the number of involved lymph nodes, and the presence of any other metastases [13].

Smoking is a practice in which a substance, most commonly tobacco, is burned and the smoke is tasted or inhaled. This is primarily practiced as a route of administration for recreational drug use, as combustion releases the active substances in drugs such as nicotine and makes them available for absorption through the lungs [14]. Smoking is one of the leading causes of preventable death globally.

Constituents of cigarette smoke: Cigarette or tobacco smoke contains more than 4,000 different chemical compounds which are present in the solid phase, the gas phase or the liquid phase. Solid phase chemicals are phenols, nicotine and naphthalene. The major gases include carbon monoxide, nitrogen oxides and hydrogen cyanide and the liquid vapors include formaldehyde, methane, benzene, ammonia and acetone [15]. Tar, carbon monoxide and nicotine are the mainstream components of the smoke but they are not alone responsible for the deleterious effects associated with smoking and passive smoking [15]. There are at least 60 chemicals in smoke which have been identified as carcinogens. Some of these are Acetaldehyde, 4-Aminobiphenyl, Arsenic, Benzene, Beryllium, 1,3-Butadiene, Cadmium, 1,1-Dimethylhydrazine, Ethylene oxide, Formaldehyde, Heterocyclic amines, Hydrazine, Isoprene, Lead, 2-Naphthylamine, Nitromethane, Polycyclic aromatic hydrocarbons, o-Toluidine, Vinyl chloride [16].

A 2007 report states that about 4.9 million people worldwide each year die as a result of smoking [17]. Among the diseases that can be caused by smoking are vascular stenosis, lung cancer [18] chronic obstructive pulmonary disease [19] and erectile

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dysfunction [20]. Smoking is also a risk factor in Alzheimer's disease [21], while smoking more than 15 cigarettes per day has been shown to worsen the symptoms of Crohn's disease [22].Smoking one cigarette a day results in a risk of heart disease that is halfway between that of a smoker and a non-smoker [23].

Although a relationship between both cigarette smoking and environmental cadmium (Cd) contamination with prostate cancer exist, the mechanisms are unclear [24].

The aim of this study is to evaluate the relationship between cigarette smoking and the risk of prostate carcinoma using the prostate specific antigen (PSA) as a marker.

Justification of the study

Prostate specific antigen (PSA) level is affected by many factors, of which lifestyle habits which may include smoking. Smoking has been associated with different cancer especially lung cancer. It is probably that this association may be represented in the carcinoma of the prostate. This study is aimed at investigating the association between cigarette smoking and PSA levels. Any "negative risk factor" or effect of smoking on PSA levels can be identified This was done using the Enzyme-linked Immunosorbent Assay (ELISA) procedure as described by [25]

Principle

The PSA ELISA test is based on a solid phase enzyme linked immunosorbent assay. The assay system utilizes a goat anti-PSA antibody directed against PSA for solid phase immobilization (on the microtiter wells). A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution.

and used in the diagnosis of clinical benign prostatic hypertrophy (BPH). PSA is preferred for the purpose of diagnosis of prostate cancer an d also used for monitoring prostate disease progression and the effect of therapy.

Materials and methods

Materials

ELISA Machine (MR-96A).

Method

Study area/colle ction of samples

This study was carried out in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria. This area was chosen because the male participants were readily available. A total of 100 subjects participated in this research work. This comprised two main groups: 50 adult male smokers (test group) and 50 adult Non-smokers (control group). Subjects were grouped in varying age categories.

5ml of blood sample was collected by veni puncture from each of the subjects. The samples were allowed to clot and separation performed by centrifugation at 3,000 rmp.

Determination of prostate specific antigen levels (psa) of the subjects

The test sample was allowed to react first with the immobilized goat antibody at room temperature for 60 minutes. The wells were washed to remove any unbound antigen. The monoclonal anti PSA-HRP conjugate was then added and allowed to react with the immobilized antigen for 60minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The wells were washed with water to remove unbound-labeled antibodies. A solution of TMB reagent was added and incubated at room temperature

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for 20minutes, resulting in the development of a blue color. The color development was stopped with the addition of stop solution changing the color to yellow.

The concentration of PSA is directly proportional to the color intensity of the test sample. Absorbance was measured spectrophotometrically at 450nm.

Statistical analysis

Data were subjected to statistical analysis using the Students' test and the Analysis of Variance (ANOVA).

Values were considered significant if $P < 0.05$

Ethical approval: Ethical approval was obtained from the Ethical Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Informed consent of the participants was obtained prior to sample collection.

Result

TABLE 1.1 Determination of Total and Free PSA levels in Adult smokers and Non-smokers (control group)

TEST	N	Mean	Std. Deviation	Std. Error Mean
Smokers (Test) TPSA	50	1.93	1.26	0.17
Non-smokers (Control)	50	2.77	1.40	0.19
Smokers (Test) FPSA	50	1.06	0.79	0.112
Non-smokers (Control)	50	1.44	0.80	0.113

The mean Total PSA level of smokers was 1.93±1.26ng/ml while it was 2.77±1.40ng/ml for Non-smokers.

The mean Free PSA value for smokers was 1.06±0.79ng/ml and 1.45±0.80ng/mlfor Non-smokers.

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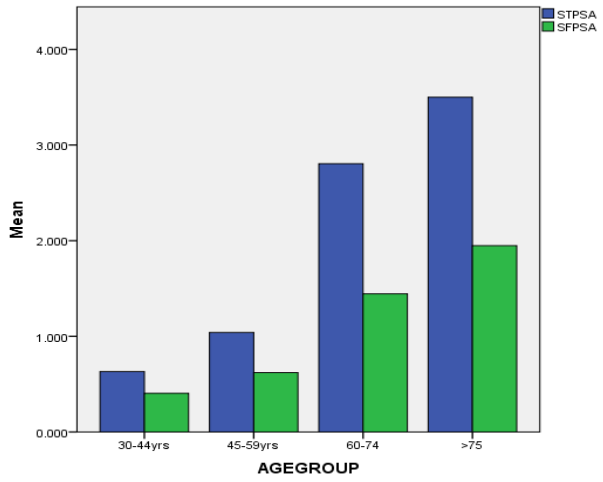
TABLE 1.2 Comparison of Total and Free PSA levels between Test group (smokers) and Control group (Non-smokers)

TEST	N	Mean	Std. Deviation	P- value
Smokers (Test)	50	1.93	1.26	
TPSA				
0.002				
Non-smokers				
(Control)	50	2.77	1.40	
Smokers (Test)	50	1.06	0.79	
FPSA				
0.019				
Non-smokers				
(Control)	50	1.44	0.80	

[There was a significant decrease in the mean total PSA value of smokers (test subjects) when compared with that of the mean total PSA Levels of Non- smokers (control subjects) (P<0.05).

There was also a significant decrease in the mean Free PSA value of smokers (test subjects) when compared with that of the control group (P<0.05).]

FIGURE 1.1 Total and free psa levels in the various age categories in the test group



[There was a positive correlation of r = 0.91between the age groups and Total PSA levels of smokers. This pattern was also observed between the age groups and Free PSA levels of smokers (r = 0.75).]

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Discussion

Smoking is one of the leading causes of preventable death globally with about 4.9 million people worldwide dying each year as a result of smoking [17]. Cigarette smoking poses a health risk on various organs thereby causing diseases such as lung cancer [18] and chronic obstructive pulmonary disease [19].

Prostate-specific antigen (PSA) is secreted by the epithelial cells of the prostate gland in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer or other prostate disorders [26].

Prostate cancer is a form of cancer that develops in the prostate which tends to occur more prevalently in men over the age of fifty [27]. Globally, it is the sixth leading cause of cancer-related death in men [28]. Prostate cancer causes symptoms similar to benign prostate hyperplasia (BPH) but it is characterized by metastasis of cells in the prostate gland and thus may cause pain, difficulty in urinating and even problems during sexual intercourse [11]. Although smoking is an important risk factor for many cancers, the role of smoking as a risk factor in prostate cancer remains unclear.

In Table 1.1, the mean Total PSA value for smokers was 1.93±1.26ng/ml and 2.77±1.40ng/ml for non-smokers, while the mean Free PSA level for smokers was 1.06±0.79ng/ml and 1.45±0.80ng/ml for non-smoker.

This implies that Smokers had a significantly lower total and free PSA level than Non-smokers [29] had earlier reported no difference in baseline PSA levels between current smokers and non-smokers, but statistically significant lower PSA velocity for current smokers than non-smokers.

[30] Suggested that the PSA and %free PSA levels of current smokers and former smokers may be statistically significantly different when compared with those of men who rarely or never smoked. They observed that approximately a third of the men that participated in their study had a percent free prostate specific antigen less than 25% and therefore current smokers had a significantly lower percent free prostate specific antigen than former smokers.

Table 1.2 showed a significant decrease in the mean total PSA value of smokers (test subjects) when compared with that of the mean total PSA Levels of Non- smokers ($P<0.05$) and a significant decrease in the mean Free PSA value of smokers (test subjects) when compared with that of the control group ($P<0.05$).

In the work of [56], smokers had a significantly lower free prostate specific antigen than Non-smokers. They also suggested that smokers may be at decreased risk of being diagnosed with non-advanced prostate cancer but they are at an increased risk of dying from prostate cancer.

This supports the study by [31] that used a multivariable Cox regression approach with data from the health professional follow up survey and reported that smoking was not associated with incident of prostate cancer.

[32] suggested that smoking appeared to be associated with a 10% lower risk of less aggressive prostate cancer, whilst heavy smoking was associated with an increased risk of prostate cancer death. Therefore, current smokers had a significantly lower risk of prostate cancer than never smokers.

Few studies have investigated how smoking may play a protective role in prostate cancer; however, several biological pathways could be involved including insulin-like growth factors (IGFs) and sex hormone-binding globulin (SHBG). Higher IGF-I and IGF binding protein-3 (IGFBF-3) have been associated

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with increased risk of prostate cancer, with a stronger association noted for IGF-I and low-grade cancers [33]. Current smokers had lower IGFBP-3 levels and non-significantly decreased IGF-I levels as compared to never-smokers in one cross-sectional study [34] therefore, smoking has a protective effect with non-advanced (i.e., low-grade) prostate cancers observed in their study. [35] also observed lower IGF-1 and IGF-3 serum concentrations in cigarette smokers, factors that have been protectively associated with prostate cancer risk. Male smokers also have higher circulating levels of SHBG [36], which have been associated with decreased prostate cancer risk [37].

A protective effect of smoking also has been noted for benign prostatic hyperplasia [38][39], which may be affected through similar pathways. Furthermore, PSA levels were approximately 10% lower in ever smokers compared to never smoker in 1,319 men in the 2001–2002 National Health and Nutrition Examination Survey [40] and PSA velocity was 33% lower in smokers than nonsmokers in the placebo arm of the Prostate Cancer Prevention Trial [29].

In a recent study, current smoking was not associated with risk of prostate cancer, but there was an increased risk among heavy smokers [41]. However, in the study, current smoking was associated with increased prostate cancer mortality.

[42] had earlier reported that smoking at the time of diagnosis was related to a higher risk of prostate cancer-specific mortality. [43] observed that smoking was associated with a moderately increased relative risk of prostate cancer and suggested that prostate cancer should be added to the list of tumors for which cigarette smoking is a risk factor. They also observed a decline in the relative risk within a decade of smoking cessation, thus supporting the theory that cigarette smoking induced hormonal changes which may have a promotional effect on prostate tumor growth that diminishes fairly soon after exposure ceases.

There are several potential mechanisms whereby cigarette smoking may increase risk of prostate cancer. One is the ability of cigarette smoking to increase bioavailable testosterone and decrease bioavailable estradiol, which may alter the hormonal milieu favoring higher androgenic exposure to the prostate [44][36]. Several lines of evidence suggest that hormones are involved in the etiology of benign and malignant prostate disease[45][46][47][48].

In vitro animal models have been used to demonstrate that prolonged testosterone administration can induce and promote prostate tumors [49][50] showed that lower dihydrotestosterone (DHT) levels and higher testosterone to DHT ratios were found in Asian men who subsequently developed prostate cancer during a 14-year follow-up. [37] reported that higher levels of circulating testosterone and lower levels of sex hormone binding-globulin (SHBG) were associated with significantly increased relative risks of prostate cancer. Another possible mechanism for an association between smoking and prostate cancer is exposure to carcinogenic substances found in cigarettes. For example, cadmium is an inorganic toxicant that is widely used in industry and is also found in cigarettes [51][52][53]. Although not directly mutagenic in the prostate, cadmium has been shown to indirectly induce prostate carcinogenesis through interaction with the androgen receptor[54][55]. [18] have reported that cadmium has the property of activating the androgen receptor response in human prostate cancer cell lines in the absence of androgen but in the presence of the androgen receptor. Furthermore, when applied in combination with androgen,

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cadmium enhances androgen-mediated transc-riptional activity in the prostat[54].

Chronic cadmium exposure in rats has been shown to induce prostate tumors in the presence of normal testicular function [55]. Chronic smoking in men with otherwise normal testicular function and androgen levels may effectively increase their androgen exposure through the interaction of cadmium with the androgen receptor and over the long term increase their risk of developing prostate cancer [43].

[24] investigated the interaction between zinc and cadmium and the potential risk of prostate cancer in smokers. Serum cadmium level was significantly higher in smokers compared with non-smokers, the level in smokers was three-fold that in non-smokers. In contrast zinc was significantly reduced in smokers compared with non-smokers. The researchers suggested that a high Cd: Zn ratio could be useful as the critical determinant of the risk of prostate cancer in smokers and possibly a biomarker of susceptibility to this environmental disease.

In Figure 1.1, there was a positive correlation between the Total PSA concentrations and the ages of the test subjects (r= 0.910). Similarly, the Free PSA levels and the ages of the test group gave a positive correlation value of r= 0.755.

The independent works of [57][58] supported our findings. Both individual studies report a significant increase in PSA velocity of smokers as the subjects' ages increased. Similarly, study results from [59] suggested a statistically significant positive association of smoking with baseline PSA in relation to age. They observed that smoking remained independently associated with PSA velocity in a model.

The works of [60] also agreed with our study. They showed that in men younger than 60, a baseline PSA value between the age-specific median and 2.5ng/mL was a significant predictor of later prostate cancer and was associated with a significantly greater PSA velocity.

Other researchers have explored the relationship between smoking and prostate specific antigen (PSA) levels in relation to prostate cancer using a variety of research designs. As reviewed by[61], different studies reported conflicting results, depending upon the research design used and how well the study controlled for possible confounding factors, thus the role of smoking as a modifiable risk factor for prostate cancer remains unclear. [61] reviewed 65 prior studies of smoking and prostate cancer and found that most of the prospective studies and all of the nested case-control studies showed no association between current smoking and prostate cancer, thus suggesting that smoking was not a significant risk factor for prostate cancer incidence in majority of the prospective cohort studies.

Conclusion

Prostate specific antigen (PSA) levels are elevated in prostate cancer and the risk of cancer increases if the free to total ratio is less than 25%. The lower the ratio, the greater the probability of prostate cancer. Our work has been able to establish that PSA levels are diminished in cigarette smokers. We have also been able to show that a positive correlation exists between the ages of the test subjects and PSA levels.

Recommendation

Although the findings suggest a reduction in prostate cancer risks in cigarette smokers, the effect of quantity and duration of smoking in long term risk evaluation of prostate cancer is uncertain. We however recommend frequent assessment of PSA levels in adult males.

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