

Assessment of Prostate Specific Antigen Level Among Elderly Men in Port-Harcourt City, Nigeria

AJILEYE, A. S.¹, AJILEYE, A. B.^{2*}, OLADELE, A. A.³, ADEWUNMI F.O²

¹State General Hospital, Ipe-Akoko, Ondo State, Nigeria
²Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Ekiti State
³Department of Histopathology and Morbid Anatomy, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State ***Corresponding Author**AJILEYE, Ayodeji Blessing
Department of Medical Laboratory Science
College of Medicine and Health Sciences
Afe Babalola University
Ado Ekiti
Ekiti State
Nigeria
Cell no. +2348030445624
Email: avobless05@gmail.com

Received: 13 October 2016; / Revised: 25 March 2017; / Accepted: 18 April 2017

Abstract

Prostate specific antigen is known to be a specific marker for the diagnosis of prostate cancer which is a serine protease enzyme, secreted almost exclusively from the prostate gland. The purpose of carrying out this research work is to assess the level of prostate specific antigen and to know the prevalence of prostate cancer among adult men living in Port-Harcourt City, Nigeria. Eighty (80) apparently healthy elderly men of age 50 years and above were considered appropriately suitable for this research work, after due consultation with each patient, a questionnaire form was given to each of the subjects to fill which help in categorizing the patients into their age groups, patients whose age were below age studied group (50 years and above) and subject who were not apparently healthy were exempted from this research work. Blood samples were collected from the subjects into plane bottles, spun at 3,000 rpm (revolution per minutes), serum was separated and then analyze with a spectrophotometer at 450 nm. Between ages 50 to 59 years, the mean protein specific antigen concentration was 13.12±5.24 ng/ml; between the ages 60 to 69 years, the mean protein specific antigen concentration is 11.39 ± 8.13 ng/ml. while in ages 70 years and above, the mean protein specific antigen concentration is 17.53±10.47 ng/ml. it was observed that about 47.5% of the subjects worked on, had protein specific antigen values within the normal range and about 25% of the subjects are at risk and about 27.5% of the subjects has prostatic cancer with values above 10 ng/ml. Base on the assessment of protein specific antigen in Port-Harcourt city, the prevalence of the disease prostate cancer among elderly men is relatively high (hyperplasia).

1. Introduction

Prostate specific antigen (PSA), also known as gamma-seminoprotein or kallikrein-3 (KLK3), it's a glycoprotein enzyme encoded in humans by the KLK3 gene [1]. Prostate specific antigen (PSA) is a protein produced by the cells of the prostate gland. It is present in small quantities in serum of normal men and it is often elevated in the presence of prostate cancer and in other prostate disorders [1]. Therefore rising levels of PSA overtime has been associated with prostate cancer both localized and metastatic. Prostate cancer is a disease in which cancer develops in the prostate, a gland in the male reproductive system. It occurs when the cells of the prostate mutate and begin to multiply out of control [2]. Prostate cancer occurs only in male as the prostate is exclusively of male reproductive system. In the year 2,000, the estimated number of new United States cancer cases was 1.2 million. Breast cancer is the leading cancer followed by cancer of the prostate, lungs and Bladder respectively [3]. Here in Nigeria today, it has been shown that prostate cancer has become the number one cancer in elderly men and constitutes 11% of all male cancers. Men at higher risk include African men older than 50 years [4], farmers, plant workers, painters and men exposed to cadmium [5]. Though specifically, the cause of prostate cancer is unknown, but the risk of men developing the disease is related to age, genetics, race, lifestyle, medications and some other factors. The primary risk factor is age. The disease is uncommon in men less than 45 years but becomes more common and increasingly with advancing age. More than 75% of all prostate cancer was diagnosed in men older than 65 years [6]. Prostate cancer is asymptomatic particularly at the early stage [7]; it is more likely to get symptoms if and when cancer grows in the prostate gland and narrows the urethra [8]. These symptoms are often similar to those of disease such as benign prostate hyperplasia (BPH) and prostatitis which include: painful urination, increased urination at night, painful ejaculation and a steady stream of blood in urine [9]. Prostate specific Antigen screening is an attempt to detect the cancer at its

earliest stages before any symptoms develops [10]. The screening test leads to more specific follow up test such as prostate Biopsy, where small pieces of the prostate are removed for close study [10]. The screening for prostate cancer can be performed either by using the prostate Specific Antigen blood test (PSA) or the Digital Rectal Examination (DRE) [11]. PSA is a serine protease enzyme, the gene of which is located on the 19 chromosome. PSA is normally present in blood at low level; increased PSA may suggest the risk of cancer [12]. The reference range of less than 4 ng/ml are usually considered normal result, over 10 ng/ml are considered high and results between 4 ng/ml and 10 ng/ml are considered intermediate. PSA can also be elevated if other prostate problems are present such prostatitis, prostate infection, and recent as ejaculation, hence producing a false positive result [12]. However some men with prostate cancer have low levels of PSA e.g obesity has been reported to reduce serum PSA levels. Delay early detection may partially explain worse outcome in obese men with early prostate cancer.

2. Materials and Methods

2.1 Study Area (Site)

This research study was carried out in Port Harcourt City, Rivers State, Nigeria.

2.2 Ethical Clearance.

Approval for this research study was obtained from the research ethical committee of the department of Medical Laboratory Science, Faculty of Sciences, Rivers State University of Science and Technology, Rivers State, Nigeria.

2.3 Subject Selection

Subjects consisting of 80 adult men of 50 years of age and above were considered suitable for this research study. After due consultations with each patient, a questionnaire was given to each patient to fill, which help in categorizing the subjects into their age groups. Subjects whose age were below the age studied group (50 years and above) were excluded, also those subjects whose age were not apparently healthy were excluded.

2.4 Specimen Collection and Preparation

A questionnaire was given to each subject to fill using acceptable medical techniques, blood samples were collected from each of the subjects and were introduced into a plane centrifuge tube without any additives (anticoagulants). Serum was prepared from the whole blood specimen after centrifuging at 3,000 rpm (revolution per minute) for 5 minutes and stored in a refrigerator.

2.5 Intended Use of Test

For the quantitative determination of cancer antigen (Prostate Specific Antigen) concentration in human serum.

2.6 Principle of Prostate Specific Antigen Test.

The prostate specific antigen enzyme linked immunosorbent assay (ELISA) test is based on the principle solid of phase enzyme linked immunosorbent assay. The assay system utilizes a goat anti PSA antibody directed against intact PSA for solid phase immobilisaion (on the microtitre wells). A monoclonal anti-PSA antibody conjugated to Horseradish peroxidase (HRP) is in the antibody enzyme conjugate solution. The test sample is allowed to react first with the immobilized rabbit antibody at room temperature for 60 minutes. The wells are washed to remove any unbound antigen. The monoclonal anti- PSA-HRP conjugate is then reacted with the immobilized antigen for 60 minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color, the color development is 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 is measured spectrophotometrically at 450 nm.

2.7 Assay Techniques

The desired number of coated wells in the holder was secured, 50 μ l of standard, specimen and

controls were dispensed into the appropriate wells, $50 \ \mu$ l of zero buffer was added into each of the well which was thoroughly mixed for 30 seconds and then incubated for 60 minutes at room temperature (25° C).

The incubated mixture was removed by emptying the plate contents into a waste container and the microtitre wells were rinsed and emptied for five (5) times with distilled water and then the wells were stroked unto paper towels to remove all residual water droplets followed by the addition of 100 µl of enzymes conjugates reagent dispensed into each well and was gently mixed for 10 seconds and then incubated at room temperature for 60 minutes and the plate contents were emptied after incubations at room temperature (25°C), with microtitre wells rinsed and emptied 5 times with distilled water and stroked sharply onto absorbent paper to remove residual water droplets.

100 µl TMB solution was dispensed into each well and was gently mixed for 10 seconds, this was incubated at room temperature for 20 minutes and the reaction was stopped by adding 100µl of stopped solution to each well which was gently mixed for 30 seconds to allow color to develop (blue to yellow) and the use of microtitre plate reader, the optical density was read at 450 nm within 15 minutes with the aid of а spectrophotometer.

3. Results

The protein specific antigen concentration of 80 apparently healthy men was estimated and the data collected were analyzed based on different age categories ranging from 50 -59 years, 60 - 69 years, 70 years and above.

Table 1. Age related concentration of prostate

 specific antigen among 80 apparently healthy adult

 men

S/No	Age Group	Number	Mean±2SD
1	50- 59	42	5.24 ± 13.12
2	60 - 69	24	8.13 ± 11.39
3	70 and above	14	10.47 ± 17.53

Reference range (ng/ml)	No of prevalence	No of prevalence	No of prevalence of subjects with PSA
	subjects with PSA	subjects with PSA	
	less than 4 ng/ml	between 4 and 10 ng/ml	more than 10ng/ml
0.0 - 3.5	25 (31.25%)	10 (12.5%)	7 (8.75%)
0.0 - 4.5	7 (8.75%)	8 (10%)	9 (11.25%)
0.0 - 6.5	6 (7.5%)	2 (2.5%)	6 (7.5%)
	38 (47.5%)	20 (25%)	22 (27.5)
	range (ng/ml) 0.0 - 3.5 0.0 - 4.5	Reference range (ng/ml)subjects with PSA less than 4 ng/ml $0.0 - 3.5$ $25 (31.25\%)$ $0.0 - 4.5$ $7 (8.75\%)$ $0.0 - 6.5$ $6 (7.5\%)$	Reference range (ng/ml)subjects with PSA less than 4 ng/mlsubjects with PSA between 4 and 10 ng/ml $0.0 - 3.5$ $25 (31.25\%)$ $10 (12.5\%)$ $0.0 - 4.5$ $7 (8.75\%)$ $8 (10\%)$ $0.0 - 6.5$ $6 (7.5\%)$ $2 (2.5\%)$

Table 2. Prevalence of prostatic cancer and risk of prostatic cancer for different age groups in Port-Harcourt

4. Discussion

At the end of this research study, it was discovered that 47.5% (38 subjects) of the population study were found normal with plasma protein specific antigen concentration between 0.0 – 4ng/ml, 25% (20 subjects) of the population study were screened to be at risk of having Prostatic cancer whose plasma protein specific antigen concentration value were within 4 - 10 mg/ml while 27.5% (22 subjects) of the 80 subjects had prostatic cancer with plasma prostate specific antigen concentration above 10ng/ml. The high percentage of the number of subjects with prostate cancer in Port Harcourt city could be because these subjects resides in an industrialized environment with exposure to factors that could predispose them to prostate cancer risk, like exposure to engine exhaust, fuel, dust, cadmium associated with occupational environments of mining and newspaper printing [5]. Another reason could be that since most of the subjects are factory workers and also because of several dangerous elements released into environment from the refineries and the drainage from the oil well could be the cause of the prevalence of high Prostate Specific Antigen in Port Harcourt city. Moreover, because of the civilization and high economy influence in the city, it can be judged that subjects may have the privilege of high intake of animal fats like milk, intake of red meat which is a known risk factor of causing prostate cancer. Age has much effect in causing the proliferation of prostatic cancer [13]. False positive result can arise due to other factors which are: bacterial infection of the prostate (prostatitis), inflammation of the prostate (Benign prostate hyperplasia) or due to recent ejaculation [14], in the absence of any of these, what happened next depends on whether or not there are any symptoms of prostate cancer, the size and shape of the prostate gland when examined using digital rectal examination (DRE) [12], how high the Prostate Specific Antigen level is, the individual's risk of prostate cancer and of course the age of the individual.

5. Conclusion

It can however be concluded base on the assessment of Prostate Specific Antigen level among adult men in Port Harcourt city that the prevalence of the disease 'prostate cancer' among elderly men is relatively high (hyperplasia) due to the predisposition to some factors inducing prostate specific cancer.

References

 Balk, S. P, Ko, Y. J. and Bubley, G. J (2003). "Biology of prostate-specific antigen". Journal of Clinical Oncology. 21(2): 383– 91. <u>doi:10.1200/JCO.2003.02.083</u>.

- Barry, M. J. (2001). Clinical practice. Prostatespecific-antigen testing for early diagnosis of prostate cancer. New England Journal of Medicine: 344 (18):1373-1377. DOI: <u>10.1056/NEJM200105033441806</u> PMid:11333995
- 3. Landis, S.H., Murray, T. and Bolden, S. (2000): Cancer Statistics Cancer, J. Clin. 50: 7-33.
- 4. Achebe, C. and Victoria, H. R. (1998): Prostate Cancer and Black men: A call to action. 93: 1139-1150.
- 5. John, W. and Hole, J.R., (1992): Essentials of Human Anatomy and Physiology. Pp 487-488
- American College of Physicians (1997): Screening for Prostate Cancer. Am. Itern Med. 126: 480-484.
- Moyer, V. A. (2012). on behalf of the U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Annals of Internal Medicine; 157(2):120–134. DOI: <u>10.7326/0003-4819-157-2-201207170-00459</u> PMid:22801674
- Carter, H. B., Albertsen, P. C. and Barry, M. J. (2013). Early detection of prostate cancer: AUA Guideline. J Urol: 190(2):419-426. DOI: <u>10.1016/j.juro.2013.04.119</u> PMid:23659877 PMCid:PMC4020420
- Barry, M. J. (2001). Clinical practice. Prostatespecific-antigen testing for early diagnosis of prostate cancer. New England Journal of Medicine; 344 (18):1373-1377. DOI: <u>10.1056/NEJM200105033441806</u> PMid:11333995

- Moyer, V. A. (2012). On behalf of the U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Annals of Internal Medicine; 157(2):120–134. DOI: <u>10.7326/0003-4819-157-2-201207170-00459</u> PMid:22801674
- 11. Andriole GL, Crawford ED, Grubb RL, et al.,(2012). Prostate cancer screening in the randomized Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial: mortality results after 13 years of follow-up. Journal of the National Cancer Institute 2012;104 (2):125-132. DOI: <u>10.1093/jnci/djr500</u> PMid:22228146 PMCid:PMC3260132
- Catalona, W.J., Richie, J.P., Ahmann, F.R., Hudson, M.A., Scardino, P.T., Flanigan, R. C., Dekernion, J. B., Ratliff, T.L., Kavoussi, L. R. and Dalkin, B. L. (May 1994). "Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men". The Journal of Urology. 151 (5): 1283–90.
- Herschman, J. D., Smith, D. S., Catalona, W.J., (1997). Effect of Ejaculation on Serum total and free Prostate Specific Antigen Concentrations. Urology. 50 (2): 239-243. DOI: <u>10.1016/S0090-</u> <u>4295(97)00209-4</u>
- 14. Thompson, I. M., Pauler, D. K., Goodman, P.J., et al,. (2004). Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. New England Journal of Medicine;350 (22):2239-2246. DOI: <u>10.1056/NEJMoa031918</u>

PMid:15163773