International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Assessment of Free and Total Prostate Specific Antigen Levels among a Cross-section of Ghanaian Men with Prostate and Liver Diseases

H. Asare-Anane[1], Osman M. Yunusah[2], Emmanuel K. Ofori[3], Francis A. Yeboah[4]

[1, 3] Department of Chemical Pathology, University of Ghana Medical School, Korle-Bu, Ghana

[2] Central Laboratory Services, Korle-Bu Teaching Hospital, Korle-Bu, Ghana

[4] Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract: ***The primary site of Prostate Specific Antigen (PSA) metabolism is the liver. PSA is however not cancer specific and high levels can also be found in benign prostate hyperplasia (BPH). Again, diseases or disorders of liver metabolism could contribute to differences in the ratio of free to total PSA levels in serum. The aim of this study was to investigate the effect of liver disease on total and free PSA levels in a cross-section of Ghanaian men. Thirty-six (36) prostate cancer subjects, 28 benign prostate hyperplasia subjects, 23 cirrhosis of the liver subjects and 25 subjects with hepatitis B were age-matched with 131 control subjects. Liver function tests, free and total PSA were measured. Mean free PSA (fPSA) values were highest in cancer of the prostate subjects and least in hepatitis B subjects. Significant associations were observed for fPSA and tPSA values between the control group with CA of the prostate ($p < 0.05$), BPH ($p<0.05$), cirrhosis of the liver subjects ($p < 0.05$) and hepatitis B subjects ($p < 0.05$) respectively. PSA levels did not correlate with the aminotransferases. The findings suggest that composite measurement of tPSA and fPSA with tests for liver function may be necessary in patients presenting with liver diseases to give a better prognostic and clinical outcome in the management of such patients.***

Keywords: Prostate Specific Antigen, Hepatitis, Cirrhosis of liver, Benign Prostate Hyperplasia

1. Introduction

Prostate Specific Antigen (PSA) is transported in the blood either bound to blood proteins or circulates freely [1]. PSA levels of 4.0ng/ml or higher are strong indicators of the possibility of prostate cancer. However, elevated serum PSA levels have also been attributed to benign prostate hyperplasia (BPH), leading to a large percentage of false positive screening results [2]. A potential solution to this problem involves the determination of free PSA levels [3-12]. Preliminary studies have suggested that free PSA is lower in patients with prostate cancer than those with benign prostate hyperplasia [13-14]. Thus, the measurement of serum free PSA in conjunction with total PSA levels can improve the specificity of prostate cancer screening and subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates [11]. PSA level and fPSA/tPSA ratios have all been investigated in a bid to enhance the accuracy of PSA in diagnosing prostate cancer. The primary site of metabolism of prostate specific antigen is the liver [15]. Diseases or disorders of the liver could contribute to differences in the ratio of total to free PSA levels in serum. It has been suggested that in the presence of liver disease, tPSA and fPSA are specific and reliable markers in the clinical management of prostate diseases [16]. In an earlier study, serum levels of total PSA in liver cirrhosis as well as in chronic hepatitis were significantly lower than those observed in controls, whiles free PSA concentration remained unchanged [16]. Another study reported that despite severe liver dysfunction, tPSA, fPSA as well as the ratio of fPSA/ tPSA were not elevated [17]. Thus, this study aimed at investigating the effect of liver disease on total and free PSA levels in a cross-section of Ghanaian men.

2. Research Design and Methods

This was a hospital based cross-sectional study. Patients were recruited from the Department of Medicine and Therapeutics of the Korle-Bu Teaching Hospital (KBTH) in Accra. Age-matched controls were randomly recruited from voluntary blood donors at the Blood bank of KBTH. Digital rectal examination (DRE) was performed on all patients. Patients with elevated PSA levels and or abnormal DRE were recommended to undergo further assessment including transrectal ultrasonography (TRUS) and a biopsy performed by an urologist. The case participants were made up of 36 prostate cancer subjects, 28 benign prostate hyperplasia subjects, 23 cirrhosis of the liver subjects and 25 subjects with hepatitis B. One hundred and thirty one apparently healthy individuals were recruited as controls. Anthropometric measurements such as weight and height were taken to obtain body mass index (BMI) of participants.

2.1 Laboratory Testing and Analysis

Fasting venous blood (5mls) was collected into serum separator tubes and processed. The resulting serum samples were aliquoted in 1ml portions and stored at -20^{o}C until assayed. The University of Ghana Medical School Ethical and Protocol Review Committee approved the consenting process. Total and free PSA were estimated using an enzyme-linked immunosorbent assay (Human Diagnostics, Germany) and the multiscan-plate reader (Germany). All reactions took place in a coated well specific for one type of assay. Liver function markers were estimated using ATAC 8000 chemistry analyzer (ELAN Diagnostics, U.S.A).

3. Data analysis

Data was entered unto a spreadsheet and analyzed using Microsoft Office Excel 2007(Louisville, Kentucky) and the values were expressed as mean plus/minus standard deviations (mean ± SD). GraphPad Prism 3.02 (San Diego, Califonia) was the statistical software used in this study with a level of statistical significance set at $p<0.05$ for all tests. Student's t-tests, analysis of variances and Mann-Whitney U-test were performed to assess significant differences of variables between case and control subjects.

4. Results

4.1 General demographics and clinical characteristics

Two hundred and forty-two subjects comprising 36 prostate cancer subjects between the ages of 40 and 78 years, 28 benign prostate hyperplasia subjects between the ages of 40 and 76 years, 23 cirrhosis of the liver subjects between the ages 40 and 76 years, 25 subjects with hepatitis B between 40 and 76 years and 131 one apparently healthy individuals between 40 and 77 years respectively were recruited for the study. Subjects were all age-matched. Mean age (years) was highest in BPH subjects and least in the hepatitis B subjects (Table 1). Differences observed in age (years) between controls and case subjects was not statistically significant ($p > 0.05$).

Table 1: General characteristics of the study population

Subjects	*N*	*Mean age (years) ± SD*	*minimum*	*maximum*
Control	131	53.24 ± 10.36	40	77
CA of prostate	36	55.56 ± 10.21	40	78
BPH	28	56.39 ± 11.60	41	76
Cirrhosis of the liver	23	54.26 ± 8.97	40	76
Hepatitis B	25	52.68 ± 10.35	40	76

Table 1 shows the general characteristics of the study population. Values are given as mean ± standard deviation (SD), N= number of subjects. CA=cancer BPH= benign prostate hyperplasia *mean difference was significant at ($p<0.05$**4.2 Biochemical markers of the study population**

Biochemical tests of liver function were assessed in the study population as shown in table 2. The mean aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transaminase (GGT), alkaline phosphatase (ALP) and total bilirubin (TB) were higher in case subjects and was statistically significant ($p<0.05$) compared to controls. ALT was highest in subjects with hepatitis B whiles AST, TB, DB, GGT and ALP were highest in liver cirrhotic subjects. Total proteins (TP) and albumin (ALB) levels were lowest in liver cirrhosis. Differences in TP and ALB values between the control and CA of the prostate, BPH, cirrhosis of the liver and hepatitis B subjects were significant ($p < 0.05$). Mean total PSA (tPSA) for controls, CA of prostate, BPH, cirrhosis of liver and hepatitis B were (1.61 ± 1.98) ng/ml, (40.16 ± 17.31) ng/ml, (12.94 ± 3.95) ng/ml, (7.88 ± 5.05) ng/ml and (4.76± 2.65) ng/ml respectively. Total PSA (tPSA) value was highest in cancer of prostate subjects and least in the controls. There were significant differences in tPSA values between the control subjects and CA of the prostate, BPH, cirrhotic liver and hepatitis B subjects respectively ($p < 0.05$)(Table 2). Mean free PSA (fPSA) for control, CA of prostate, BPH, cirrhotic liver and hepatitis B subjects were (0.22 ± 0.37) ng/ml, (2.89 ± 2.96) ng/ml, (1.61 ± .28) ng/ml, (1.04 ± 1.45) ng/ml and (0.60 ± 0.27) ng/ml respectively. Mean fPSA value was highest in cancer of prostate subjects and least in control subjects (Table 2). There was significant difference in fPSA values between the control group with CA of the prostate and cirrhosis of liver respectively ($p < 0.05$) (Table 2).

Table 2: Biochemical parameters of the study population

Variables	*Controls*	*CA Prostate*	*BPH*	*Liver Cirrhosis*	*Hepatitis B*
AST (U/L)	26.9±8.9	46.4±35.8*	51.2±47.4*	199.8±12.7**	26.32±10.7
ALT (U/L)	24.9±8.1	32.6±19.3*	28.5±13.8*	108.1±57.5**	178.8±89.9**
GGT (U/L)	29.8±10.0	63.4±59.1*	47.0±26.5*	187.9±156.2**	37.2±13.5
ALP (U/L)	58.6±15.4	97.1±68.9*	88.0±32.8*	137.6±77.6**	64.5±15.8*
TB(µmol/L)	13.8±5.2	24.5±22.0*	18.4±8.1	108.8±89.1**	17.1±8.5
DB(µmol/L)	3.1±2.7	9.6±13.4*	7.2±5.9	64.3±59.5 **	5.8±4.7
TP(g/L)	70.8±6.6	77.9±8.3*	77.6±9.6*	57.9±10.9*	73.7±8.0
tPSA(ng/ml)	1.61±1.98	40.16±17.31**	12.94±3.95**	7.88±5.05*	4.76±2.65*
fPSA(ng/ml)	0.22±0.37	2.89±2.96**	1.61±1.28*	1.04±1.45*	0.60±0.27
f/tPSA(%)	13.7	7.2	12.4	13.2	12.6

Values are given as mean ± standard deviation. AST = aspartate transaminase, ALT =alanine transaminase, GGT = gamma glutamyl transaminase. ALP = alkaline phosphatase. TP = total protein. ALB= albumin,
*TB=total bilirubin, DB= direct bilirubin. fPSA=free Prostate Specific Antigen, tPSA= total Prostate Specific Antigen. *mean difference is significant at (p<0.05). **mean difference is highly significant at (p<0.0001)*

5. Discussions

Prostate-specific antigen (PSA) is a serine protease produced by both benign and malignant prostatic epithelium [1-2], [14]. PSA is the most useful marker currently available for the detection, management and follow-ups of patients with prostate cancer [18]. However, PSA is also found to be elevated in many benign prostatic diseases, such as Benign Prostatic Hyperplasia [18-19]. This study showed increased tPSA and fPSA levels in subjects with CA of prostate and BPH than apparently healthy controls (Table 2). This is consistent with earlier reports [2], [7], [20-22]. Age differences observed between case and control subjects were found not to be statistically significant. However, Oesterling and friends hypothesized that age-specific reference ranges would increase the sensitivity of PSA in the detection of prostate cancer in younger men at a stage when the disease is

potentially amenable to cure with surgery [21]. Free PSA (fPSA) was lower in apparently healthy men and in patients with benign prostate hyperplasia than subjects with CA of prostate. This was consistent with an earlier report [14]. Total PSA (tPSA) in this study was higher in CA of prostate subjects than BPH subjects and was statistically significant. This may be as a result of stronger expression of alpha-1-antichymotrypsin (ACT) in prostate cancer tissue than in BHP tissue [14]. The higher proportion of PSA-ACT in cancer patient could be explained by the release from tumor tissue of PSA that is enzymatically more active. Indeed, it had been shown that the contribution of prostate cancer tissue to the serum concentrations of PSA is ten-fold than that of BPH tissue [23]. The percentage (%) fPSA/tPSA ratio in this study was found to be significantly lower in CA of prostate subjects compared to those with BPH. This supported earlier reports that fPSA/tPSA ratio could discriminate benign from malignant prostatic diseases [19], [22], [24]. Free to total PSA (fPSA/tPSA) ratio in this study also proved a better indicator for CA of prostate than tPSA. This was in agreement with a prior study [25] that documented that fPSA/ tPSA ratio is most useful when the tPSA level is between 4-10 ng/ml. The potential usefulness of fPSA/tPSA to replace biopsy was not considered in this study. Liver is the primary site for prostate specific antigen metabolism and in a diseased hepatic state; serum PSA concentration may be affected [26]. In this study, activities of liver enzymes such as AST, ALT, ALP and GGT as well as other parameters (total bilirubin, direct bilirubin, total protein and albumin) were investigated. Results revealed marked elevations in liver enzymes for liver cirrhotic and hepatitis B subjects compared to controls (Table 2). This was in agreement with prior studies [17]. Increase in enzyme activities suggests either hepato-cellular damage or cholestasis [27-28]. There was also a significant reduction in albumin concentration in all the subject groups compared with controls suggesting a chronic state rather than acute. This finding was consistent with other studies [17], [27]. It was also observed in this study that cirrhosis of the liver subjects and hepatitis B had significantly higher tPSA values than the controls (Table 2). This observation agrees with earlier finding, who reported that serum PSA is influenced by the severity of liver disease [13], [29]. The report however disagrees with other findings who reported that severe hepatic dysfunction does not alter serum tPSA levels [30-31]. It is however not clear enough, and relevant investigations with larger number of subjects are required to make conclusive findings. Differences in these finding could be due to the different methodology used in both studies. In this study total protein, bilirubin and the hepatic enzymes were found not to have any effect on tPSA, fPSA or fPSA/tPSA ratios. This finding agreed with Kilic and friends [16]. A limitation of this study was with sample size. Larger sample size needs to be used in future research to enable draw definite conclusions. Additional PSA measures such as PSA velocity and density was not investigated. The hypothalamic-Pituitary-gonadal axis should also be investigated for irregularities.

6. Conclusion

Elevated tPSA and fPSA levels in subjects per se are not conclusive for the confirmation of prostate cancer as the liver efficiently handles PSA metabolism. A confirmation of prostate cancer may be done after a differential diagnosis of hepatocellular damage had been ruled out. Ratio of free PSA to total PSA proved a better indicator for CA of prostate than tPSA. Further study should be exploited to establish clinical relevance of tPSA/fPSA in liver disease patients. The molecular mechanisms between ACT synthesis and PSA levels should also be further elucidated.

7. Acknowledgement

The authors thank the Central Laboratory, Department of Medicine and the National Blood Bank of the Korle-Bu Teaching Hospital, for technical, material and financial support. They also thank the Department of Chemical Pathology of the University of Ghana Medical School, for institutional support.

8. Conflict of interest

None

References

[1] Catalona W., Smith, D, Ornstein, D. (2000). Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination: Enhancement of specificity with free PSA measurements. *JAMA* 277 (18): 1452–1455.

[2] Christenson A. Bjork T, Nilsson O (1993). Serum Prostate Specific Antigen Complexed to alpha-1-antichymotrypsin: As an indicator of Prostate Cancer. *J Urol;* **150**: 100-105.

[3] Carter H.B, Pearson J.D, Metter J (1992). Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA*; **267**: 2215-2220.

[4] Benson M.C, Whang I.S, Pantuck A (1992). Prostate specific antigen density: A means of distinguishing benign prostatic hypertrophy and prostate cancer. *J Urol*. **147**:815-816.

[5] Catalona W.J, Hudson, M.A, Scardino P.T. (1994). Selection of optimal prostate specific antigen cut-offs for early detection of prostate cancer: receiver operating characteristic curves. *J Urol*; **152**: 2037-2042.

[6] Smith D.S, Catalona W.J. (1994).Rate of changes in serum prostate-specific antigen levels as a method of prostate cancer detection. *J Urol*; **152**: 1163-1167.

[7] Luderer A.A, Chen Y-T, Soriano T.F. (1995). Measurements of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total PSA. *Urology*; **46**:187-194.

[8] Catalona W.J., Partin A.W, Slawin K.M. (1995). Evaluation of Percentage of Free Serum Prostate Specific Antigen improves specificity of prostate cancer screening. *JAMA***; 274**:1214-1220.

[9] Bangma, C.H, Kranse R, Blijenberg B (1995). The value of screening tests for detection of prostate cancer. *J Urol*; **46**: 773-785.

[10] Yemoto C.M, Nolly R, Prestigiacomo A.F. (1996).Free and total PSA density in patients with prostate cancer

(CaP) and benign prostatic hyperplasia (BPH). *J Urol.* **155**:347A.
[11] Junker R, Brandt B, Zechel C, Assmann, G (1997). Comparison of Prostate-specific antigen (PSA) measured by four combinations of free-PSA and total PSA assays. *Clinical Chemistry;* **43**: 1588-1594.
[12] Vashi A.R, Wojno K.J, Henricks W. (1997). Determination of the "reflex range" and appropriatecut-off pointsfor percent free-PSA in 413 men referred for prostatic evaluation using the AxSYM system. *Urology* **49**: 19-27.
[13] Kubota Y, Sasagawa I, Sinzawa H, Kunii T, Itoh K, Miura H Imai K, Nakada T (1999). Serum levels of free and total prostate-specific antigen in males with liver cirrhosis; *Eur Urol*; **36** (5): 409-12
[14] Lilja H, Christensson A, Dahlen U (1991). Prosate-specific antigen in serum occurs predominantly in complex with α-1-Antichymotrypsin *Clin. Chem*; **37**: 1618-1625.
[15] Tietz N. W. (1987). Fundamentals of clinical chemistry. Department of pathology College of medicine,university of Kentucky. Pp 335.
[16] Kilic S. Guntekin E, Danisman A, Sevuk M. (1998). Serum free and total prostate specific antigen levels in patients with liver disease. *Urology*; **52**: 825-828
[17] Mittal R.D, Singh M.K, Selvaraju C, Choudhuri G. (2003). Total PSA and free PSA in patients with severe liver dysfunction. *Indian J Urol;***19**: 117-119.
[18] Leers M.P., Nap, M, Herwig, R, Delaere, K, Nauwelaers, F (2008).Circulating psa-containing macrophages as a possible target for the detection of prostate cancer: a three-color/five-parameter flow cytometric study on peripheral blood samples.
[19] *Am J Clin Pathol;* **129**: 649-656.
[20] Mcneill, S.A, Hargreave, T.B. (2000).Efficacy of PSA in the detection of carcinoma of the prostate in patients presenting with acute urinary retention. *J. R. Coll. Surg. Edinb*; **45**: 227-230.
[21] Collins G.N, Lee R.J, McKelvie G.B, Rogers C.A, Hehir M (1993). Relationship between prostate specific antigen, prostate volume and age in the benign prostate *British Journal of Urology;* **7***:*1445–1450.
[22] Oesterling J.E, Jacobsen S.J, Klee G.G. (1993). Free, complex and total serum prostate specific antigen: The establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* **154**: 1090-1095.
[23] Stenman U.H, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A. (1991). Complex between prostate-specific antigen and α -1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostate cancer: *Cancer Res*; **51**: 222-226.
[24] Stanley T.A, Yang N, Hay A.R, McNeal J.E, Freiha F.S, Redwine E. (1987).Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *New Engl J Med*. **317**:909-916.
[25] Li W., Ren, Y, Mee, V, Wong, P.Y. (1999). Prostate specific antigen ratio correlates with aggressiveness of histology grades of prostate cancer. *Clin. Biochem*; **32**:31-37.
[26] Filella X., Alcover, J, Molina, R, Rodriguez A, Carretero P, Ballesta A.M. (1997). Clinical evaluation of free psa/total psa (prostate specific antigen) ratio in the diagnosis of prostate cancer. *Eur. J. Cancer*; **33**: 1226-1229.
[27] Agha A.H, Schechter E, Roy J.B, Culkin D.J. (1996).Prostate specific antigen is metabolized in the liver. *J Urol*; **155**: 1332-1335.
[28] Mayne P.D. (1994). Clinical chemistry in diagnosis and treatment The Children's Hospital, Temple Street and The Rotunda Hospital, Dublin; London.
[29] Rod R. Seeley, Trent D. Stephens, Philip Tate D.A (1995). *Anatomy & physiology:* p950-953.
[30] Akdogan M, Hassoun B.S, Gurakar A, El-Sahwi K, Jazzar A, Wright H, Sebastian A, Nour B (2002). Prostate-specific antigen levels among cirrhotic patients. *Int J Bio Markers*; **17**(3): 161-164.
[31] Williams P.B, Eastman J.A, Culkin D.J. (1997). Influence of hepatic function on serum levels of prostate specific antigen. *J Urol;* **158**: 1867-1869.
[32] Kadayifci A, BenekliM, Simsek H (1996). Prostatic acid phosphstase and prostate specific antigen in liver disease. *Int. Urol. Nephrol* **28**: 67-71.

Author Profile

Dr. Henry Asare-Anane ia a Researcher and Senior Lecturer with the Department of Chemical Pathology, University of Ghana Medical School, Korle-Bu, Ghana.

Emmanuel Kwaku Ofori is serving as Lecturer with the Department of Chemical Pathology, University of Ghana Medical School, Korle-Bu, Ghana.

Yunusah Osman Mohammed is chief Biomedical Scientist with the Central Laboratory of the Korle-Bu Teaching Hospital.

Professor Francis Agyemang Yeboah is in Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology.