Journal of
Medical Sciences

Research Paper

For further information about this article or if you need reprints, please contact:

M.T. Agyei-Frempong
Department of Molecular Medicine,
School of Medical Sciences,
College of Health Sciences,
Kwame Nkrumah University of Science and Technology,
Kumasi, Ghana

Tel: + 233 20 818 6136
Fax: +233 51 60 137

Correlation of Serum Free/Total Prostate Specific Antigen Ratio with Histological Features for Differential Diagnosis of Prostate Cancer

[1]M.T. Agyei-Frempong, [1]N.Y.F. Frempong, [2]K. Aboah and [3]K.A. Boateng

This study sought to find the correlation between Free to TPSA (F/T PSA) ratio and the histological grades of prostate cancer. Among 120 selected from both in-patients and out-patient clinic of the urology unit, 51 underwent tissue biopsies within six months of their PSA measurements using enzyme immunoassay. The F/T PSA ratios of patients with prostate cancer were compared to those with BPH, using the students' t-test. Correlation between histology grades and F/T PSA ratios was calculated by the Pearson test and linear regression. Receiver Operating Characteristic (ROC) curves were generated from sensitivities and specificities of various F/T PSA ratios, FPSA and TPSA levels. The F/T PSA ratio was significantly lower in the group of patients with prostate cancer (PCa) (0.118±0.0043) compared to BPH (0.302±0.0179, p<0.0001). There was also an inverse correlation between F/T PSA ratios and aggressiveness of histology grades (r^2 = 0.76, p<0.0001), that is, the higher the histology grade, the lower the F/T PSA ratio. The best accuracy was obtained with F/T PSA ratio cut-off at 0.15, with specificity of 87.50% and sensitivity of 100%. The use of the F/T PSA ratio improves PSA-based differential diagnoses of prostate cancer in patients and can also lead to more specific identification of potentially curable prostate cancer and reduce unnecessary prostate biopsies.

Key words: Prostate tumour marker, Gleason score, prostate biopsies, benign prostatic hyperplasia, prostatitis, malignancy

[1]Department of Molecular Medicine,
[2]Department of Surgery,
[3]Department of Pathology, School of Medical Sciences,
College of Health Sciences, Kwame Nkrumah University of Science and Technology, Private Mail Bag, Kumasi, Ghana

INTRODUCTION

Prostate cancer is the most common malignancy in men over 50 years old. The chance of any man being diagnosed with prostate cancer is one in ten. The incidence of prostate cancer rises with age, 30% in men 30 to 40 years of age, 65% are diagnosed from 60 to 70 years and 80% at the age of 80 years (Badoe *et al.*, 2000). Geographically, there is a high incidence in the United States particularly, among the Blacks. In Ghana, there has been a steady increase in the number of deaths from prostate cancer since 1991 and it is now the leading cause of deaths in men (Nelson *et al.*, 2003; Arthur *et al.*, 2006). However, the prevalence rate has not been ascertained.

Prostate Specific Antigen (PSA) has been characterized as the most useful serum marker for the detection and management of prostate cancer. However, it is well recognized that serum PSA is also found to be elevated in many benign prostatic diseases, as in Benign Prostatic Hyperplasia (BPH), in prostatitis and after manipulations of the prostate (Oesterling, 1991, Leers *et al.*, 2008; Mcneill and Hargreave, 2000). Between 25 and 50% of patients with BPH show PSA concentrations above the upper limit of the normal range (4 ng mL^{-1}). As a consequence, the specificity of an elevated PSA is often considered as being too low for prostate cancer diagnosis. Thus men reporting to the Hospital with prostate disorders are most likely to have a biopsy taken for histological investigation since only a biopsy can confirm or rule out cancer when PSA level is high. However, 70% of men that undergo biopsy do not have cancer, yet they go through the anxiety and trauma of the procedure, its complications afterwards including haematuria, a dull ache in the perineum and the cost involved. These factors actually deny men with prostate conditions access to healthcare leading to their eventual death.

During the last decade several efforts have been made to increase PSA's diagnostic specificity using methods such as PSA-density, PSA-velocity and age specific PSA (Benson *et al.*, 1992).

At the same time several groups have been investigating the molecular forms of circulating PSA (Lilja *et al.*, 1991; Stenman *et al.*, 1991). It has been found that most of serum PSA is in complex with two serine protease inhibitors, alpha-1 antichymotrypsin (ACT) and alpha-2 macroglobulin (AMG). A smaller portion of serum PSA is found to be unbound to any serine protease inhibitor and this has been characterized as free PSA. With the current immunoassays in clinical use, free PSA and PSA-ACT can be detected in serum (Bjork *et al.*, 1994). The sum of PSA-ACT and free PSA fractions contribute to the total PSA.

Subsequently, it has been demonstrated that a higher proportion of PSA-ACT and lower one of free PSA exist in patients with prostate cancer than in normal men or men with BPH (Bjork *et al.*, 1994). As a result, the use of free PSA/total PSA ratio in the differential diagnosis between prostate cancer and BPH has been proposed.

The aim of this study therefore was to compare the ratio of free PSA to total PSA with histopathology of prostate biopsies for the differential diagnosis of prostate cancer.

MATERIALS AND METHODS

The study was conducted from July 2006 to March 2007. All study protocols were approved by the Committee for Human Research and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital, Kumasi, Ghana.

Patients' examination, specimen collection (both biopsy and blood specimens) and analysis were performed with patients' consent and in accordance with the Helsinki Declaration.

One hundred and twenty patients, who attended the out-patient clinic and in-patients of the urology unit at the Komfo Anokye Teaching Hospital (KATH) Kumasi were selected for the study. These consisted of confirmed prostate cancer patients and those who came for routine checks for prostate diseases. The criteria for patient selection included; 50 years and older, the duration between blood sampling and tissue biopsy being less than 6 months and those who consented to the study. Fifty people from Our Lady of the Holy Rosary Catholic Church, Kumasi who had not been diagnosed of any prostate disease and who consented to the study were used as controls.

Four milliliters of venous blood were collected from each of the 120 consented patients as well as the 50 controls and all samples were processed to obtain sera. Samples were kept at -80°C until assayed and were brought to room temperature before assay.

Free Prostate Specific Antigen Immunoassay (fPSA EIA) and total Prostate Specific Antigen Immunoassay (tPSA EIA) kits respectively from HySkill Diagnostics GmbH were used to analyse the samples; both are quantitative enzyme immunoassays for the determination of free PSA and tPSA concentrations respectively in human serum. Both assays had detection limits of 0.1 ng mL^{-1}.

Tissue biopsies obtained from 51 of the 120 selected patients within 6 months of their PSA measurements were processed and stained with haematoxylin and eosin (HE). The stained sections were examined under the light microscope and diagnosis made by a specialist pathologist who also determined the Gleason grades and score of tumour in those biopsies with prostate cancer.

Statistics: Data were analyzed using the statistical software packages, GraphPad Prism 4.00 for windows and Microsoft Excel. Comparison was done using the student's t-test, correlation was calculated by the Pearson test, followed by linear regression, and the Receiver Operating Characteristic (ROC) curves were generated from sensitivities and specificities of methods.

Receiver Operating characteristic (ROC) curve analysis was used to evaluate the reciprocal relationship between sensitivity and specificity and to compare total PSA with free PSA and free/total PSA with the aim of distinguishing between prostate cancer and BPH.

RESULTS AND DISCUSSION

One hundred and twenty male patients including those who attended the Out-patient Clinic, and In-patients, of the urology unit of KATH from July 2006 to January 2007 were used for the study.

From the histopathology examinations, 35 were diagnosed with BPH and 16 with prostate cancer (PCa). Those with PCa had their Gleason grades and scores determined.

The highest mean TPSA was observed in the 80-89 age group (45.22 ng mL^{-1}), followed by the $\geq$90 age group (37.9 ng mL^{-1}), 70-79 age group (35.99 ng mL^{-1}), 60-69 age group (21.0 ng mL^{-1}) and the lowest mean TPSA was found in the 50-59 age group (8.81 ng mL^{-1}). The same trend was found with the mean FPSA (Table 1).

Conversely, the mean F/T PSA ratio was lowest in the $\geq$90 age group (0.12) and increased gradually down the age groups, 80-89 (0.14), 70-79 (0.15), 60-69 (0.17), and 50-59 (0.25), respectively with a weak significance (p = 0.0463) (Table 1).

The mean TPSA was lower in the 50-59 age group (1.50$\pm$0.15) than in the >60 age group (2.09$\pm$0.29) but the difference was weak (p = 0.09). However, the mean FPSA was higher in the = 60 age group (0.74$\pm$0.13) as compared to that of the 50-59 age group (0.51$\pm$0.06) with a weak significance (p = 0.18) (Table 2).

Most importantly the mean F/T PSA ratio was higher in the 50-59 age group (0.34$\pm$0.02) than the = 60 age group (0.33$\pm$0.02) but it was of little significance (p = 0.94) (Table 2).

Mean TPSA was lower in the control group (1.68$\pm$0.14) than in the test group (28.56$\pm$3.07) and the difference was significant (p<0.0001). Also, mean FPSA was significantly higher in the test group (4.59$\pm$0.36) than in the control group (0.58$\pm$0.06, p<0.0001). The mean F/T PSA ratio was higher in control group (0.34$\pm$0.01) than in the test group (0.16$\pm$0.01), but the difference was weak (p = 0.0463) (Table 3).

Table 1: Distribution of TPSA, FPSA and F/T PSA ratio among age groups of test group

Age group	Mean total PSA (ng mL^{-1})	Mean free PSA (ng mL^{-1})	Mean F/T PSA ratio	Total No.	Frequency (%)
50-59	8.81	2.16	0.25	14	11.67
60-69	21.00	3.63	0.17	41	34.17
70-79	35.99	5.38	0.15	43	35.83
80-89	45.22	6.22	0.14	16	13.33
$\geq$90	37.90	4.52	0.12	6	5.00

Table 2: Distribution of TPSA, FPSA and F/T PSA ratio among age groups of the control group

Age group	Total PSA (ng mL^{-1})		FREE PSA (ng mL^{-1})		F/T PSA ratio	
	Mean$\pm$SEM	p-value	Mean$\pm$SEM	p-value	Mean$\pm$SEM	p-value
50-59	1.50$\pm$0.15	0.09	0.51$\pm$0.06	0.12	0.34$\pm$0.02	0.94
$\geq$60	2.09$\pm$0.29		0.74$\pm$0.13		0.35$\pm$0.02	

Table 3: Distribution of TPSA, FPSA and F/T PSA ratio in the control, test, BPH and PCa groups

Groups	No. of cases	TPSA (ng mL^{-1})		FPSA (ng mL^{-1})		F/T PSA ratio	
		Mean$\pm$SEM	p-value	Mean$\pm$SEM	p-value	Mean$\pm$SEM	p-value
Control group	50	1.68$\pm$0.14	<0.0001	0.58$\pm$0.060	<0.0001	0.34$\pm$0.010	0.0463
Test group	120	28.56$\pm$3.07	<0.000	4.59$\pm$0.360	0.0276	0.16$\pm$0.010	<0.0001
BPH	35	17.95$\pm$2.54		7.21$\pm$0.920		0.40$\pm$0.020	
PCa	16	65.49$\pm$9.33		14.68$\pm$0.57		0.07$\pm$0.004	

SEM = Standard Error of the Mean; p-value, Significance value

The mean total PSA was found to be significantly higher in the group of patients with prostate cancer (65.49±9.33) compared to BPH group (17.95±2.54, p<0.0001). However, mean free PSA was significantly higher in the BPH group (7.21±0.92) than the PCa group (4.68±0.57, p<0.0001) (Table 3).

The mean F/T PSA ratio was significantly lower in the PCa group (0.07±0.004) than BPH group (0.40±0.02, p<0.0001) (Table 3).

Out of 16 patients with PCa, nine (56.25%) had a Gleason score of eight; four (25%) had seven; two (12.5%) had three; and one (6.25%) had a Gleason score of two.

There was a weak correlation between the histological grades (Gleason score) and TPSA concentration ($r^2 = 0.35$, p = 0.0157) (Fig. 1a), and a similar weak correlation between histological grades and FPSA concentration ($r^2 = 0.28$, p = 0.0360) (Fig. 1b). However, there was a significant inverse correlation between the histological grades and F/T PSA concentration ($r^2 = 0.76$, p= 0.0001) (Fig. 1c).

The best accuracy was obtained with a cut-off F/T PSA ratio at 0.15, with specificity of 87.50% and sensitivity of 100%. Furthermore, the area under the curve (AUC) of F/T PSA ratio (z = 1.00, p<0.0001) was higher than AUC of TPSA (z = 0.88, p<0.0001) or FPSA (z = 0.69, p = 0.0269) (Fig. 2a-c).

Moreover, the ROC analysis showed that a specificity of 85.70 and 81.25% were obtained for F/T PSA = 0.15 and TPSA, 18 ng mL^{-1} but for this TPSA value, the sensitivity was very low (62.86%).

PSA is a tumour marker for diagnosis and management of patients with prostate cancer. However, low specificity makes TPSA assay clinically less useful. As an organ-specific antigen, the increase in serum TPSA level is not cancer-specific. A better test is needed to distinguish benign conditions such as BPH and prostatitis from malignant conditions (Stamey *et al.*, 1987). Abnormal results of TPSA assay often give patients unnecessary concerns; the investigation procedures and treatments can affect their quality of life. Previous reports proved that PSA ratio improved specificity without compromising sensitivity (Christensson *et al.*, 1993).

In this study it was found that there was a gradual increase in the TPSA and FPSA concentrations with increasing age with the peak occurring in the 80-89 year group for patients in the test group (Table 1) and also for the normal individuals in the control group (Table 2). This goes to prove that PSA concentration increases with advancing age as suggested by Malarkey and McMorrow (2000).

The mean FPSA concentration was significantly lower in PCa group (4.683±0.567) than that of the BPH group (7.213±0.917, p<0.0001), as confirmed by Bjork *et al.* (1994) and Van Iersel *et al.* (1996) (Table 3).
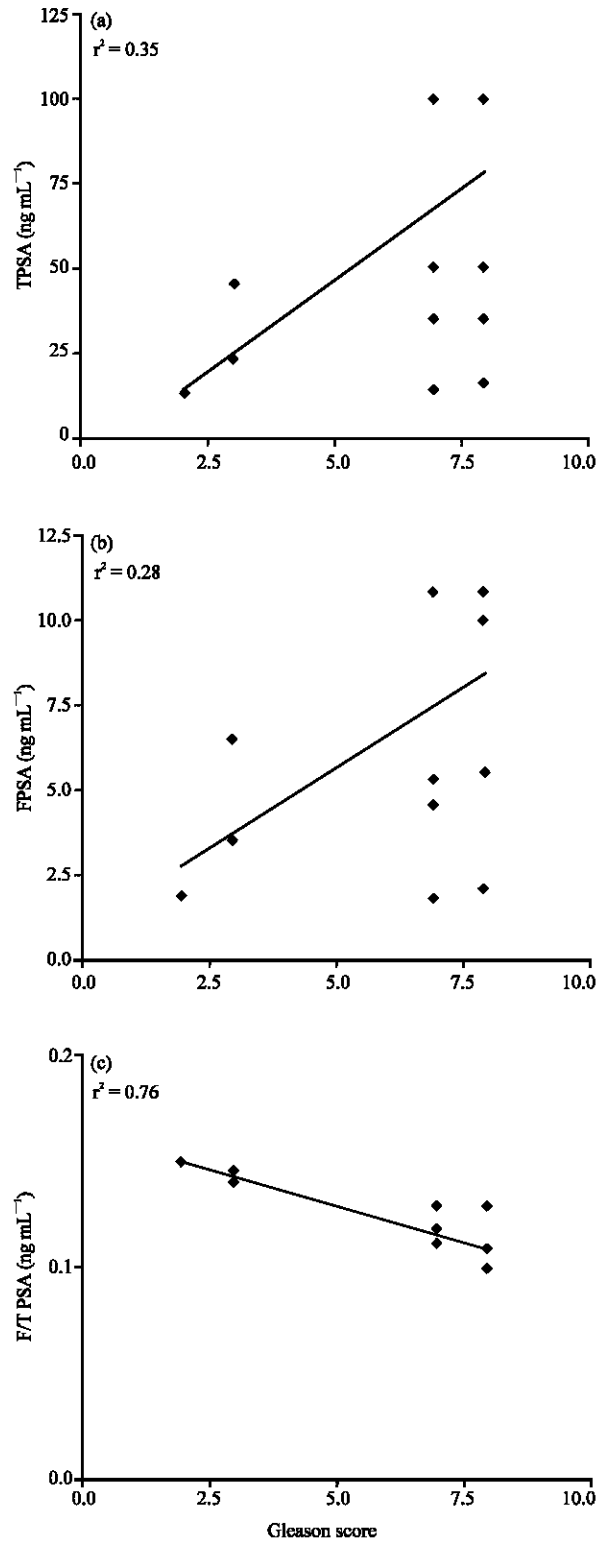

Fig. 1: Correlation between the histological grades (gleason score), (a) TPSA, (b) FPSA and (c) F/TPSA ratio
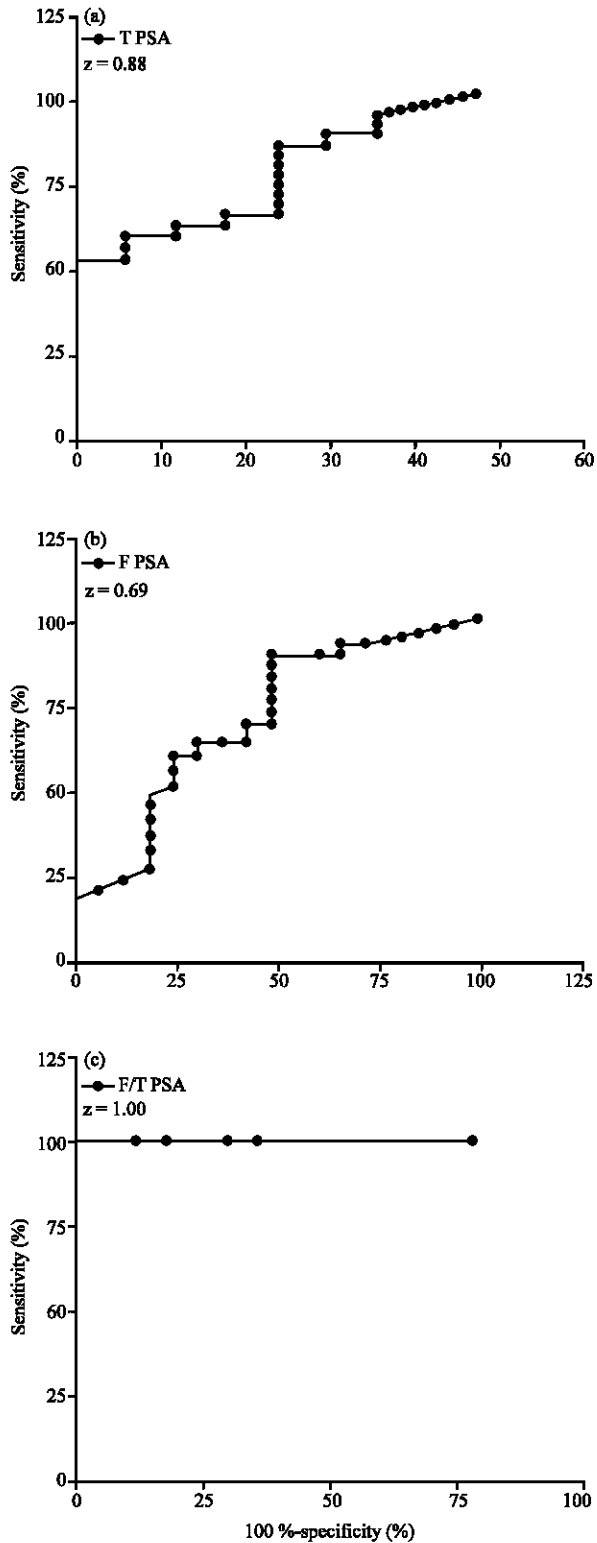
Fig. 2: ROC analysis of (a) TPSA, (b) FPSA and (c) F/T PSA ratio

The mean F/T PSA ratio was found to be significantly lower in the group of patients with prostate cancer (0.07 ± 0.004) compared to BPH (0.40 ± 0.02, $p<0.0001$). This finding is consistent with the findings of Li *et al.* (1999), Filella *et al.* (1997) and Toubert *et al.* (1996). However, distributions of individual values show an overlap in the two groups (Table 3). The distribution overlap of the F/T PSA ratio between prostate cancer and BPH groups may be due, at least in part, to coexisting hyperplasia and carcinoma areas within the same prostatic gland. More than 80% of patients with prostate cancer would also have histological BPH (Bostwick *et al.*, 1990). It may be hypothesized that carcinomas associated with BPH might not lead to a significant decrease in the F/T PSA ratio, compared to BPH alone.

The first question for this study was: Is F/T PSA ratio a better indicator than TPSA level for diagnosis of prostate cancer?

Earlier studies showed that F/T PSA ratio could discriminate benign from malignant prostate diseases (Stenman *et al.*, 1991) and even predict aggressiveness of prostate cancer many years before the cancer was diagnosed (Christensson *et al.*, 1990). This study has confirmed that F/T PSA ratios in prostate cancer patients not only differed from those in patients with BPH, but also inversely correlated with histology aggressiveness of prostate cancer. It has demonstrated that the more aggressive the histology grade, the lower the F/T PSA ratio; and the better the diagnostic sensitivity of F/T PSA ratio for prostate cancer (Fig. 1a-c). Therefore, F/T PSA ratio was not only prostate cancer-specific, but also specific for histological aggressiveness. F/T PSA ratio was a better indicator method than TPSA for diagnosis or screening of prostate cancer. The result is consistent with that of Li *et al.* (1999).

The second question considered in the study was: Does F/T PSA ratios have a potential to replace tissue biopsy?

Although tissue biopsy has been considered a gold standard in diagnosis of prostate cancer, the procedure is invasive, expensive and time-consuming. No investigation procedures have been considered to replace tissue biopsy. We found an inverse correlation between histological grades and F/T PSA ratios. That is, the lower the F/T PSA ratio, the more the aggressiveness of the prostate cancer. Lower range of F/T PSA ratios had a high sensitivity for diagnosis of prostate cancer with more aggressive histology grades. In contrast, higher range of F/T PSA ratios had a high specificity for ruling out prostate cancer with more aggressive histology grades. Previous studies reported a discrepancy of histology grades of biopsy samples and Radial Prostatectomy (RP)

specimen. The histology grades of biopsy samples gave only 64% to 87% accuracy due to sampling errors (Steinberg *et al.*, 1997). Because of this discrepancy, it is difficult to know which result is closer to real histology grade of RP, F/T PSA ratio or histology grading of biopsy samples.

When comparing the three methods (TPSA alone, FPSA alone and F/T PSA ratio), TPSA measurement alone and with a cut-off of 18 ng mL^{-1} provides a sensitivity of 81.25%, but with a low specificity (62.86%), a FPSA with a cut-off of 10 ng mL^{-1} provides a sensitivity of 88.57% and a low specificity of 37.50%, the F/T PSA ratio with a cut-off of 0.15 gave the most accurate results, sensitivity (100%) with a high specificity (87.50%) (Fig. 2a-c).

Furthermore, the area under the F/T PSA ratio curve remained the biggest (z = 1.00, p< 0.0001) versus FPSA (z = 0.69, p = 0.0269) or versus TPSA (z = 0.88, p<0.0001) thus making it the method of choice.

The results are in agreement with Filella *et al.* (1997), who found similar results (cut-off of 0.113 with a sensitivity of 65%, specificity of 90%) and also Toubert *et al.* (1996) who found a F/T PSA ratio at a cut-off of = 0.15 showing a sensitivity of 79.5% and a high specificity of 88.9% in spite of the great variability of the TPSA and FPSA assays.

Catalona *et al.* (1995), however, proposed a higher cut-off (23.4%) which would eliminate more than one third of the useless prostatic biopsies when 90% of the cancers were diagnosed

In conclusion, F/T PSA ratios in the prostate cancer group were significantly lower than those in the BPH group and there was an inverse correlation between the F/T PSA ratio and the histology aggressiveness of prostate cancer. A cut-off of F/T PSA ratio at 0.15 improves diagnostic sensitivity and specificity for prostate cancer.

The results from this study show that the use of the F/T PSA ratio improves PSA-based differential diagnosis of prostate cancer in patients and can also lead to more specific identification of potentially curable prostate cancer and reduce unnecessary prostate biopsies.

ACKNOWLEDGMENTS

REFERENCES

Arthur, F.K.N., F.A. Yeboah, M. Adu-Frimpong, E.E. Sedudzi and K.A. Boateng, 2006. Prostate cancer screening in Ghana-any clinical benefit? J. Sci. Technol., 26: 1-7.

Badoe, E.A., E.Q. Archampong and J.T. da Rocha-Afodu, 2000. Principles and Practice of Surgery Including Pathology in the Tropics. 3rd Edn., Tema Publishing Company, Ghana, ISBN: 9988-0-0510-5 pp: 850-871.

Benson, M.C., I.S. Whang, A. Pantuck, K. Ring and S.A. Kaplan *et al.*, 1992. Prostate specific antigen density: A means of distinguishing benign prostatic hypertrophy and prostate cancer. J. Urol., 147: 815-816.

Bjork, T., A. Bjartell, P.A. Abrahamson, S. Hulkko and A. di Sant'Agnese *et al.*, 1994. Alpha-1 Antichymotrypsin production in psa-producing is common in prostate cancer but rare in benign prostatic hyperplasia. Urology, 43: 427-434.

Bostwick, D.G., W.H. Cooner, L. Denis, G.W. Jones and P.T. Scardino *et al.*, 1990. The association of benign prostatic hyperplasia and cancer of the prostate. Cancer, 70: 291-301.

Catalona, W.J., D.S. Smith, R.L. Wolfert, T.J. Wang and H.G. Rittenhouse *et al.*, 1995. Evaluation of free serum prostate specific antigen to improve specificity of prostate cancer screening. J. Am. Med. Assoc., 274: 1214-1220.

Christensson, A., C.B. Laurell and H. Lilja, 1990. Enzymatic activity of prostate specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur. J. Biochem., 194: 755-763.

Christensson, A., T. Bjork, O. Nilsson, U. Dahlén and M.T. Matikainen *et al.*, 1993. Serum prostate specific antigen complexed to alpha-1 antichymotrypsin as an indicator of prostate cancer. J. Urol., 150: 100-105.

Filella, X., J. Alcover, R. Molina, A. Rodriguez and P. Carretero *et al.*, 1997. Clinical evaluation of free psa/total psa (prostate specific antigen) ratio in the diagnosis of prostate cancer. Eur. J. Cancer, 33: 1226-1229.

Leers, M.P.G., M. Nap, R. Herwig, K. Delaere and F. Nauwelaers, 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. Am. J. Clin. Pathol., 129: 649-656.

Li, W., Y. Ren, V. Mee and P.Y. Wong, 1999. Prostate specific antigen ratio correlates with aggressiveness of histology grades of prostate cancer. Clin. Biochem., 32: 31-37.

Lilja, H., A. Christensson, U. Dahlen, M.T. Matikainen and O. Nilsson *et al.*, 1991. Prostate specific antigen in human serum occur predominantly in complex with alpha-1 antichymotrypsin. Clin. Chem., 37: 1618-1625.

Malarkey, M.L. and M.E. McMorrow, 2000. Nurses Manual of Laboratory Test and Diagnostic Procedure. 2nd Edn., WB Saunders Company, New York, ISBN: 13-978-0721678122, pp: 28.

Mcneill, S.A. and T.B. Hargreave, 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.

Nelson, W.G., A.M. DeMarzo and W.B. Isaacs, 2003. Prostate cancer. The N. Engl. J. Med., 349: 366-381.

Oesterling, J.E., 1991. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J. Urol., 145: 907-923.

Stamey, T.A., N. Yang, A.R. Hay, J.E. McNeal and F.S. Freiha *et al.*, 1987. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. N. Engl. J. Med., 317: 909-916.

Steinberg, D.M., J. Sauvageot, S. Piantadosi and J.I. Epstein, 1997. Correlation of prostate needle biopsy and radial prostatectomy gleason grade in academic and community setting. Am. J. Surg. Pathol., 21: 566-576.

Stenman, U.H., J. Leinonen, H. Alfthan, S. Rannikko and K. Tuhkanen *et al.*, 1991. Complex between prostate specific antigen and alpha-1 antichymotrypsin is the major form of prostate specific antigen in the serum of patients with prostate cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res., 51: 222-226.

Toubert, M.E., J. Guillet, M. Chiron, P. Meria and C. Role *et al.*, 1996. Percentage of free serum prostate specific antigen: A new tool in the early diagnosis of prostate cancer. Eur. J. Cancer, 32A: 2088-2093.

Van Iersel, M.P., W.P.J. Witjes, C.M.G. Thomas, M.F.G. Segers and G.O.N. Oosterhof *et al.*, 1996. Review of the simultaneous determination of total prostate-specific antigen and free prostate specific-antigen. Prostate Suppl., 7: 48-57.