

ROLE OF FREE / TOTAL PSA RATIO TO DIFFERENTIATE BPH AND PROSTATE CANCER

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ABSTRACT

This study was undertaken to determine Free to Total PSA ratio to differentiate between Benign Prostate Hyperplasia (BPH) and Prostate Cancer (PCa) and to improve the accuracy of the PSA test and the specificity of PCa detection particularly when PSA levels fall between 4-10ng/ml levels. The study group consisted of a total of 124 subjects. Free PSA and Total PSA were determined using assay, which is based on Chemiluminescent Microparticle Immunoassay (CMIA) technology. The Statistical test used were Students' t test for difference of Mean and Pearson Correlation test, using SPSS and MS Excel Statistical packages. Free/Total PSA ratios in the prostate cancer group were significantly lower than those in the BPH group. A cut-off of F/T PSA ratio% at 18% improves diagnostic sensitivity and specificity for prostate cancer.

KEYWORDS: Benign Prostate Hyperplasia (BPH), Prostate cancer (Pca), Free PSA, Total PSA, Chemiluminescent Microparticle Immunoassay (CMIA)

PCa is the third most common cause of death from cancer in men of all ages. It is the most prevalent cancer found in men above the age of fifty year and is frequently diagnosed in men (Malati and Rajanikumar, 2006) between 45 and 90 years of age with a median age of 72 years. The age of Indian patients of PCa varies from 35-86, which is much lower when compared with average age of patients in western countries. The actual incidence of prostate cancer in India and in nonresident Indians is lower than that in the Western populations (Krishnamoorthy and Padmanabha, 2016). According to a recent study, PCa ranks fifth in incidence and fourth in cancer mortality for men in Mumbai and the incidence is increasing by 1% every year (Yeole, 2008). Fifty to sixty percent of men over the age of 60 in India have Benign Prostate hyperplasia (BPH). 30-40% show related symptoms and 10% out of this group may have PCa. BPH affects 40 percent of men in their 50s and 90 percent of men in their 80s. BPH is a noncancerous enlargement of the prostate gland that may restrict the flow of urine from the bladder. The possibility of having PCa varies with age, being 13.7% for the age group 60-79 years, 2.2% for those aged 40-59, and 0.005% in those younger than 39 years. Men who have a brother or father with PCa have twice the usual risk of developing PCa (Steinberg *et al.*, 1990). Gene alterations on chromosome 1, 17, and the X chromosome have been found in some patients with a family history of PCa. African American men have a higher prevalence and more aggressive prostate cancer than white men, who, in turn, have a higher prevalence than men of Asian origin (Hoffman *et al.*, 2001). Men with higher serum

levels of the short-chain omega-6 fatty acid linoleic acid have higher rates of PCa. However, the same series of studies showed that men with elevated levels of long-chain omega-3 fatty acids (EPA and DHA), found in fatty fishes like salmon had lowered incidence (Gann and Giovannucci, 2005). Other dietary factors that may increase PCa risk include low intake of vitamin E and the mineral selenium. Decreased levels of vitamin A may be a risk factor because this can promote cell differentiation and stimulate the immune system. Vitamin D deficiency was suggested as a risk factor, and studies show an inverse relationship between ultraviolet exposure and mortality rates for PCa (Peters *et al.*, 2007).

PCa can also be attributed to hormonal reasons. For instance, androgen ablation causes a regression of PCa. Obesity and elevated blood levels of testosterone might increase the risk for PCa. Daily use of anti-inflammatory medicines such as aspirin, ibuprofen, or naproxen may decrease PCa risk. Use of the cholesterol-lowering drugs known as the statins may also decrease PCa risk (Hsing and Comstock, 2009) (Jacobs *et al.*, 2005) (Shannon *et al.*, 2005) (Platz *et al.*, 2005).

More frequent ejaculation also may decrease a man's risk of PCa (Giles *et al.*, 2003). The researchers hypothesize that this could be because regular ejaculation reduces the buildup of carcinogenic deposits such as 3-methylcholanthrene, produced from the breakdown of cholesterol, which could damage the cells lining the prostate.

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Symptoms for PCa

Early diagnosis of PCa is hindered by lack of symptoms in men with localized tumors. Some men, however, will experience symptoms such as frequent, hesitant, or burning urination, difficulty in having an erection, or pain or stiffness in the back, hips or upper thighs. Some advanced PCa can slow or weaken the urinary stream or increase the need to urinate more often. But noncancerous diseases of the prostate, such as BPH are a more common cause of these symptoms. If the PCa is advanced, one might develop blood in the urine (hematuria) or might find it difficult to get an erection (impotence). Advanced PCa commonly spreads to the bones, which can cause pain in the hips, spine, ribs, or other areas (Springhouse, 2005). Cancer that has spread to the spine can also cause it to press on the spinal nerves, which can result in weakness or numbness in the legs or feet, or even loss of bladder or bowel control.

Diagnosis of Prostate Cancer

The following screening tests may be used initially.

1. Digital rectal examination (DRE): Data from community-based studies suggest that the positive predictive value of digital rectal examination for prostate cancer is 15% to 30% and varies relatively little with age (Chodak *et al.*, 1989).
2. Prostate Specific Antigen (PSA) Test: If higher than normal levels of PSA are detected or if levels rise over time, it could indicate prostate inflammation, prostate enlargement or prostate cancer (Brawer *et al.*, 1992).
3. Ultrasound (Transrectal Ultrasonography): A transrectal ultrasonography (TRUS) provides a visual image of the prostate and is used if the DRE indicates the presence of cancer, the procedure may help to confirm an uncertain preliminary diagnosis and is useful as a guide for needle biopsies (Gupta *et al.*, 2005).
4. Biopsy: This is more of a confirmation diagnosis.

These tests are also used to determine which tumors are localized within the prostate and are, thus, potentially treatable. Observational studies suggest that DRE and PSA, combined with TRUS and biopsy, can identify localized prostate cancer in 3-5% of men, although

the tests do result in a number of false positives and negatives.

Treatment for Prostate Cancer

Three major treatment options are available for localized prostate cancer: radical prostatectomy, radical radiotherapy and conservative management (involving monitoring and treatment of symptoms) (Milin, 1947).

PSA

PSA has been characterized as the most useful serum marker for the detection and management of PCa, however, Screening with PSA is problematic because it is really an organ specific marker for the prostate rather than a specific marker for cancer. There is considerable overlap in PSA concentration in men with benign prostate disease. In this regard, the recognition of the molecular forms of PSA, Free PSA has shown the most promise. Although PSA is considered to be the most effective tumor marker in human oncology, its role in screening strategies for PCa has not been completely established. It is a glycoprotein produced exclusively by the epithelial cells lining the prostatic ducts and acini. Normally, it is secreted into the prostatic ducts and is present only in prostate tissue, prostatic fluid, and seminal plasma. PSA is produced by normal, hyperplastic, and cancerous prostatic tissue (Emil and Jack). A major function of PSA is proteolytic cleavage of gel forming proteins in the seminal fluid, resulting in the liquification of the seminal gel and increased sperm mobility.

PSA and Protease Inhibitors

PSA circulates in serum in complex forms (bound to protease inhibitors) or in non-complex (free or unbound) forms. Approximately 60% to 70% of PSA occurs in an active free form (Zhang *et al.*, 1998) with less than 5% in complex with protease inhibitors. The major protease inhibitor, to which active single-chain PSA is covalently linked in a 1:1 molar ratio, is PCI where by the PSA-PCI complex could only be detected in seminal plasma and not in serum.

PSA and Protease Inhibitors in Blood

PSA occurs in three major forms in the blood. The major immunodetectable form is PSA complexed with the serine protease inhibitor and alpha-1-antichymotripsin (PSA-ACT). In contrast to seminal fluid, 55% to 95% of immunodetectable PSA forms covalent 1:1 molar

complexes with ACT. Uncomplexed, or FREE PSA, is the other immunodetectable form of PSA in serum between 5% to 45% of the immunodetectable PSA is found in a free noncomplexed form, which is most likely catalytically inactive as it remains virtually nonreactive with the large excess of protease inhibitors (ACT or AMG) in blood. A third form of PSA, a complex with alpha-2-macroglobulin (AMG) is not detectable with current immunoassays for PSA due to the engulfment and subsequent masking of PSA epitopes by the alpha-2-macroglobulin molecule (Stephan et al., 1981). The serum concentration of the PSAAMG complex is difficult to assess, likely due to the conformation of the AMG molecule that engulfs the PSA molecule, hence blocking antibody access to the PSA epitopes.

PSA level in the blood is found if the barrier between the epithelium and the blood stream is damaged. Typical sources for damage are cancer, bacterial infection, and prostate infection or destruction of part of the prostate by damage to its blood supply. PSA is also present in urine and serum. Low levels of PSA are found in the blood as a result of leakage of PSA from the prostate gland. Increasing levels of PSA are associated with prostatic pathology; including prostatitis, BPH, and PCa (Lija *et al.*, 1999). Numerous studies have shown that for groups of men with PCa, serum PSA correlates directly with pathologic stage. However, in most cases, PSA level alone does not provide accurate information because of overlap in PSA level between stages. False positive elevations in the PSA increases in the PSA that are caused by conditions other than PCa for example BPH and infection or prostates can elevate the PSA (Ornstein *et al.*, 1998).

Role of PSA in Pca

In general, normal PSA levels are below 4ng/ml. A reading between 4 and 10ng/ml indicates about 20% chance that cancer is present and a measurement above 10ng/ml is considered a strong indication of PCa (Catalona and Scoott, 1986). But because various factors can cause PSA levels to fluctuate, one abnormal PSA test does not necessarily indicate that cancer is present. Once cancer is diagnosed, PSA level is also measured to determine its extent. If PSA level is less than 20ng/ml then it is likely the cancer has not spread. Metastasis is strongly indicated as the level rises over 40ng/ml. PSA level is also used for monitoring the

course of the disease after initial treatment of prostatic cancer. Rising level indicates recurrence of tumour, although the exact location cannot be determined just by using PSA. In contrast to this, if the PSA falls significantly after treatment and remains persistently low, then it will indicate the disease is under control (Lodding *et al.*, 1998).

The present study is undertaken with a view to determine Free to Total PSA ratio to differentiate between BPH and PCa and to improve the accuracy of the PSA test and the specificity of PCa detection particularly when PSA levels fall between 4-10ng/ml levels.

MATERIALS AND METHODS

- Target population was male referred to genitourinary unit of our Institute between the age group 50–90 yrs. In the duration of course the medical records of histological proven patients were studied. The blood sample were collected from these patients. Normal control blood were taken from 30 healthy ambulant donors. Serum were extracted by centrifugation of samples at 4000rpm for 10 min, and were stored at –20 ° C until use.

Methods

- Serum levels of Free PSA and Total PSA were determined using assay, which is based on Chemiluminescent Microparticle Immunoassay (CMIA, Marketed) technology. The Architect i system and reagent kit from Abbott were used.
- The Statistical test used were Students' t test for difference of Mean and Pearson Correlation test, using SPSS and MS Excel Statistical packages.

RESULTS AND DISCUSSION

The study group consisted of 104 male patients comprising of 54 (57%) PCa patients and 40 (43%) patients with BPH. The mean age for these groups was 65.90 ± 8.11 and 67.32 ± 7.54 respectively. 30 age matched healthy male formed the control group with the mean age of 60.90 ± 7.17 . For the three groups viz. Normal cases, BPH patients and PCa patients, the data was analyzed for FPSA, TPSA and F/T PSA%. Following is the tabulation for the data.

Table 1: Normal cases, BPH patients and PCa patients

Normal Healthy controls			
	FPSA ng/ml	TPSA ng/ml	(FPSA)/(TPSA) %
Range	0.040 to 1.162	0.147 to 4.00	25.98 - 58.04
Mean	0.38	1.12	37.17
Standard Deviation	0.26	0.68	10.66
Benign Prostate Hyperplasia			
	FPSA ng/ml	TPSA ng/ml	(FPSA)/(TPSA) %
Range	0.386 to 3.396	2.075 to 16.816	10.47 -54.6
Mean	1.75	7.54	24.68
Standard Deviation	1.02	4.64	10.56
Prostate Cancer			
	FPSA ng/ml	TPSA ng/ml	(FPSA)/(TPSA) %
Range	0.052 to 2.937	2.078 to 30.0	4.06 - 20.85
Mean	1.02	9.29	10.91
Standard Deviation	0.94	7.82	6.92

We note that the mean TPSA levels are the least for the healthy patients and it increases in diseased condition being higher in prostate cancer patient. We also note that the F/T PSA % ratio were significantly different amongst the three groups that we have at a significance level ($p < 0.001$). Percentage F/T PSA levels ranged from 10.47 to 54.6 % (mean \pm SE, 24.68 ± 10.56) and from 4.06 to 20.85% (mean \pm SE, 10.9 ± 6.92) in patients with BPH and PCa, respectively. The mean F/T PSA ratio was found to be significantly lower in the group of patients with prostate cancer. This finding is consistent with the findings of Filella *et al.*, (1997).

Our findings are consistent with the study done by V Thakur *et al.* wherein they discussed the utility of F/T PSA ratio in diagnosis of prostate carcinoma. They found that mean total PSA was the least in normal healthy subjects and was the highest in the cancer patients. Further they also found that the F/TPSA ratio was significantly different between the three groups. Recently numerous studies have demonstrated a lower ratio of FPSA/TPSA in Ca P patients compared with patients with BPH (Brawer *et al.*, 1992) (Gupta *et al.*, 2005) (Milin, 1947)

If we look at the frequency distribution of no. of cases of Normal, BPH and Cancer across various values of Total PSA, as shown in the graph below:

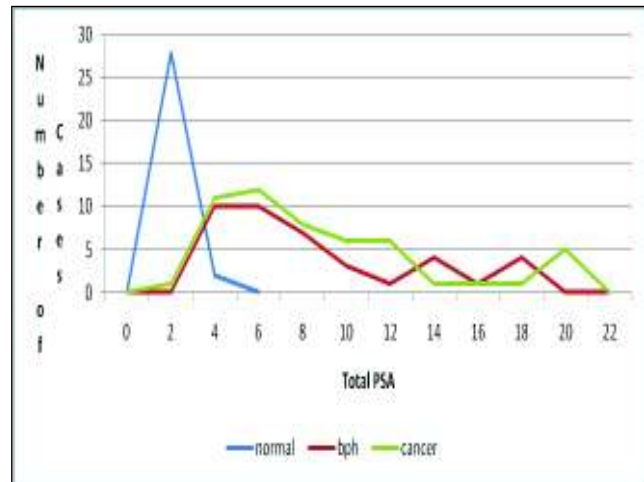


Figure 1: BPH and Cancer across various values of Total PSA

We find that there is an area of overlap between BPH and Cancer between the Total PSA values of 4 to 10; *the grey zone*. Theoretically, the occurrence of the various PSA forms in serum allows for a better discrimination between prostate cancer and BPH (Malati and Rajanikumar, 2006).

If we do the two tailed t-test for difference in mean between Cancer, BPH and control (normal) we find that there is significant difference between BPH (24.68 ± 10.56) and control (normal) (37.17 ± 10.66 , $p = 0.00$) and between Cancer (10.91 ± 6.92) and control (normal) (37.17 ± 10.66 ,

p= 0.00) (significance level $\alpha=0.05$, using student’s t test for difference of means)

Now if we replicate a similar test on the Cancer and BPH groups to find if there is a significant difference between the mean of two groups we find that they are significantly different with the value of p =0.000 at a significance level of $\alpha=0.05$. This establishes that the ratio (FPSA/TPSA) % can be used to identify cases of Cancer from BPH in the grey zone (TPSA from 4 to 10ng/ml)

The study was performed on 94 consecutive male patients consisting of 40 patients with BPH and 54 PCa patients. The sensitivity and specificity was calculated at different %FPSA/TPSA ratio cutoff. From cutoff value 16 to 22, sensitivity increased from 86% to 96%, but specificity decreased from 98% to 80%. Increasing the cutoff value thereafter showed no change in sensitivity, but specificity further reduced to 65%. Hence in this patient’s series we find that at 18% (%FPSA/TPSA) we get the maximum sensitivity with a stable level of specificity.

Also if we look at plot of positive predictive and negative predictive value vs %FPSA/TPSA we find that at 18% point we have a very high level of “positive predictive value” and a relatively high and stable level of “negative predictive value”. The trend is evident even in the plot of “False negative” and “False positive” vs %FPSA/TPSA plot. Here again we see that at 18% point, the “False negative” is the lowest with a relatively very low and stable level of “False positive” cases

Hence we can see that 18% ratio FPSA/TPSA can be taken as a cutoff value to differentiate between BPH and PCa cases; any value of the % ratio FPSA/TPSA lower than 18% is indicative of a PCa case and any value higher than 18% is indicative of BPH.

The cutoff found here is similar to the findings of a study conducted in Shaikh Zayed hospital & Mayo hospital by Saima Naz, Sarah Ahmad *et al.*, wherein they found that at the 18% cutoff point, the sensitivity was 86% and specificity was 94%. Using a cutoff of 18%, the free-PSA test significantly improved the differential diagnosis of BPH and Ca P in the ‘gray zone’ as compared to the use of total PSA alone in the study group.

In a case controlled study of 200 patients conducted by Iqbal *et al.*, at Jinnah hospital, Lahore, found that at 18% cutoff, the test gives a specificity and sensitivity

of 91.9% and 92.1% respectively with 87.2% PPV. Further concurrence on 18% cutoff for the test was found with a study done by Gaspar and Arribas in 2000, Madrid hospital.

Numerous retrospective studies demonstrated that ~ 19–64%of unnecessary, negative biopsies could be eliminated (Jacobs *et al.*, 2005) (Brawer *et al.*, 1992) (Stephan *et al.*, 1981) Reasons for these substantial differences could be the use of a wide range of fPSA% cutoffs (14–28%) with different assay.

Currently there are many different recommendations and different cutoffs for the use of fPSA% depending on the various assays used and on the targeted sensitivity or specificity, the reported rates of sensitivity, specificity, and predictive values are widely scattered (Chodak *et al.*, 1989) (Brawer *et al.*, 1992). The analytical variability of assay methods could be the cause of discrepancies among the results of different studies (Milin, 1947). But, let us evaluate the strength of the test based on the %FPSA/TPSA with cut off 18% on the patient data that we have. (Table 2)

Table 2: Percentage of FPSA/TPSA

TPSA>=4	BPH	Cancer	Total
Actual	30	41	71
TPSA	0	71	71
%FPSA/TPSA (18%)	28	43	71
	False negative	False positive	
TPSA (>=4)	0	30	
%FPSA/TPSA (18%)	0	2	

If we first apply the TPSA test, then all the cases where TPSA>=4, 71 out of 124, will be indicative for biopsy. From the actual data, we know that only 41 out of these 71 are actual positive cancer cases. This means that 30 cases of those sent for biopsy are only BPH and negative for PCa.

Now, if we apply our %FPSA/TPSA ratio test with a cutoff of 18%, then only those cases (amongst those with TPSA>=4) where the ratio<=18% will be sent for biopsy i.e. 43. Comparing it with actual data we find that of the 43 cases sent for biopsy only 2 cases are those which are only BPH and negative for prostate cancer.

Comparing 30 false positive cases versus only 2 false positive cases, we can easily conclude that employing the ratio test along with the TPSA test increases the effectiveness of differentiating cases of BPH from positive cancer cases. We further try the ratio test with 18% cutoff on the “Grey zone” or overlap between BPH and Cancer cases which is between $4 \leq \text{TPSA} < 10$ and we have the following results (Table 3)

Now, if we apply our %FPSA/TPSA ratio test with a cutoff of 18% to the grey zone (TPSA 4- 10ng/ml)

Table 3: TPSA Test

$4 \leq \text{TPSA} < 10$	BPH	Cancer	Total
Actual	20	26	46
TPSA		46	46
%FPSA/TPSA (18%)	19	27	46
	False negative	False positive	
TPSA (≥ 4)	0	20	
%FPSA/TPSA (18%)	0	1	

In the above tables we can easily see only a TPSA test is unable to differentiate between cases of BPH and Cancer. But if we apply the ratio test with cutoff of 18% we find that, it detects 19 BPH cases compared to 20 as per actual data and 27 cancer cases compared to 26 as per actual data. So we can conclude that the %ratio FPSA/TPSA test, with a cutoff of 18%, is highly effective at differentiating BPH cases from cancer cases in the “grey zone”.

We found that the percent free PSA was not associated with patientage, in agreement with Oesterling *et al.*, Filella *et al.*, Morgan *et al.*, and Jung *et al.* A recent, prospective study by Catalona *et al.*, showed a statistically significant and clinically relevant direct association between percent free PSA and age. (Table 4)

Now, if we apply our %FPSA/TPSA ratio test with a cutoff of 18% to the cases where $\text{TPSA} \leq 4 \text{ng/ml}$

Table 4: Percentage FPSA/TPSA ratio test

TPSA < 4	Non Cancer	Cancer	Total
Actual	40	12	52
TPSA	52		52
%FPSA/TPSA (18%)	40	12	52
	False negative	False positive	
TPSA (≥ 4)	12	0	
%FPSA/TPSA (18%)	0	0	

For the cases where $\text{TPSA} < 4$, if we use only TPSA as a test, all the cases would be diagnosed as negative for cancer and none would be sent for biopsy as a result. From the actual data we find that this would be a wrong diagnosis for 12 of the cases, which should have been sent for Biopsy. However, if we use %FPSA/TPSA test with a cut off of 18%, as found earlier, we would be correctly identifying all the 12 cases as positive for cancer and would be rightly sending them for biopsy.

Hence we see that for cases where $\text{TPSA} < 4$, the test %FPSA/TPSA enhances the sensitivity of detecting the cancer patients. Needless to say it will help in early detection for cancer.

CONCLUSION

In conclusion, F/T PSA ratios in the PCa group were significantly lower than those in the BPH. A cut-off of F/T PSA ratio% at 18% improves diagnostic sensitivity and specificity for PCa.

The results from this study show that the use of the percentage FPSA value ratio improves PSA-based differential diagnosis of PCa in patients and help differentiate PCa from BPH and reduce unnecessary prostate biopsies. Selective measurement of percent free PSA in cases of uncertain diagnosis can improve the specificity of PCa detection especially when the TPSA falls between 4-10ng/ml thus making F/T PSA ratio an excellent adjuvant to TPSA for diagnosis of PCa, increasing the specificity and predictive value for positive test which in turn will assist the physician in determining the probability of PCa, managing the patient and taking informed care

decision. In the near future, the FPSA% value will be used more frequently with additional clinical and laboratory values to calculate the risk of having cancer.

In conclusion, we predict that better insight in regard to PSA molecular forms will lead not only to better diagnostic markers, but also to an understanding of PCa development and possible new therapeutic options.

FPSA measurements and the use of its ratio to TPSA are well established in numerous, large-scale, multi-institutional clinical trials. A forthcoming standardization of the FPSA and TPSA measurements will provide a better comparability of different PSA assays. In the near future, the FPSA% value will be used more frequently with additional clinical and laboratory values to calculate the risk of having cancer.

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