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Research Article

Combined use of t-PSA and other PSA related parameters in differential diagnosis of BPH and Prostate Carcinoma

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ABSTRACT

Objectives: Cancer is the second common cause of deaths after CHD, all over the world. The rate of prostate cancer has increased in the last decades. Prostate cancer is usually discovered in advanced metastasizing state, which in many cases difficult to treat.

Aim of work: Is to clarify the clinical significance of combined use of TPA and PSA related parameters with t-PSA, in the early and differential diagnosis carcinoma and BPH.

Patients and Methods: The study included 88 subjects; BPH, carcinoma and controls. TPA, t-PSA and f- PSA were measures using ELIZA technique and % f-PSA was estimated.

Results: mean age statistically correlated with CaP and BPH. t-PSA and f-PSA showed significant variation between CaP and BPH. Precision of t-PSA is enhanced by age-specific reference ranges. Sensitivity of t-PSA was increased when combined to TPA or PSA ratio but on the expense of specificity. t-PSA was the most sensitive, f-PSA was the most specific, and PSA ratio at 0.1 cut-off was the most accurate among all.

Conclusion: combination of any of the PSA related parameters (PSA-age specific reference ranges; f-PSA; % f-PSA) or TPA to t-PSA will enhance the later discriminative ability and PPV, but on the expense of specificity. Moreover, Age specific-PSA reflex ranges could be useful in differential diagnosis after standardizing and validating in large-scale prospective clinical studies.

Key Words: CaP, BPH, t-PSA, % f-PSA, TPA.

1. INTRODUCTION

Cancer constitutes an enormous burden on society in more and less economically developed countries alike. The occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, physical inactivity, and changing reproductive patterns associated with urbanization and economic development. About 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide [1,2]. Prostate Carcinoma "CaP" is the second cause of cancer deaths in men all over the world [3]. Also Benign Prostatic Hyperplasia "BPH' is the most common benign tumor in men, resulting in annoying urinary problems in the majority of men older than 50 years. BPH would be of no importance if it were not for the consequent bladder outlet obstruction [4]. On the other hand patients with prostatic enlargement may constitute a high risk for CaP [5].

The most frequent cancers in Egypt estimated using the results of the National Population-Based Registry

Program of Egypt 2008–2011; found that CaP ranking was the 6th (4.27%) in frequency among male tumors, and as the elderly population continues to expand, it is likely that scope of this problem will continue to increase, with an estimated increased up to 3398 cases by year 2020 [6].

Prostate-specific Antigen "PSA" is a kallikrien-like serine protease [7], involved in the liquefaction of seminal coagulum upon ejaculation, first discovered by WANG et al. [8]. It is an organ specific marker, secreted primarily by prostatic epithelial cells [9, 10]. PSA exists in circulation as 70-90% complexed to antichymotrypsin "one of the extracellular protease inhibitors" [11], the uncomplexed form "f-PSA" is less in carcinoma than that of BPH, which gives a clue to the importance of free to total PSA ratio in the enhancement of PSA sensitivity and specificity [12].

In 1993, the American Cancer Society recommended the use of PSA for early diagnosis of prostatic carcinoma. In fact, PSA has become one of the major tumor markers as it provides a very sensitive index, being increased in over 90% of cases when first diagnosed, and now it has well established monitoring values.

In 1980, Björklund isolated tissue polypeptide antigen "TPA" from epithelia of human placenta and cancer tissue, identified as unique polydispersed heterogeneous unconjugated polypeptide protein, intermediate filaments, identified by antibodies that react with Cytokeratins [13], released into circulation with normal and malignant epithelial turn over or tumor necrosis, Therefore, considered as cell proliferation marker [14]. Serum TPA is elevated in pregnancy, some inflammatory diseases but with much lesser levels than cancers and usually return to normal levels much quicker [15]. Until now TPA is used with other specific markers for differentiation between tumors [16].

Aim of this study was to clarify the role of % f-PSA in the improvement of both sensitivity and specificity of t-PSA, and effect of combined with TPA in the differential diagnosis of prostate carcinoma and BPH.

2. SUBJECTS AND METHODS

2.1. Subjects:

This study included 132 consecutive patients who presented themselves to the in/ out patient urology clinics of El-Hussein and Saied Galal educational hospitals, for the evaluation of prostatic diseases or complaining from urinary dysfunction not attributed to other causes. All subjects gave an informed written consent for participation, and the study was approved by the ethics committee of clinical oncology department, Al-Azhar university hospitals.

Patients were not pre-selected for this study and referral to the clinic was solely on suspicion of CaP or BPH, as indicated. The investigator was unaware of the previous clinical history until after sampling to prevent bias. Prostate was assessed in all cases by DRE, TRUS, and/or histologically either by TRUS guided needle biopsy, radical prostatectomy, transuretheral resection or open prostatectomy in order to build a complete clinical picture for each patient. Histopathology was carried out according to the WHO classification, grading and TNM system was used for staging [17].

Only patients who had no previous prostatic manipulation or catheter indwelling for at least one month were included. Cases that matched the specification for this study including the control group were 88 patients. Patients were subdivided into BPH and CaP groups. Volunteers, according to their recent clinical data available; suffering no prostatic diseases and away from any factor that influence the studied markers, were included as control subjects.

2.2. Specimens' collection, handling and storage:

Before any prostatic manipulation blood samples from all the studied groups were freshly withdrawn by venipuncture, incubated in decline tubes at room temperature (using Centrifuge Type: Z 200 A, SN: 44970371, 6000 rpm, 50– 60 Hz., 1997 – Germany). Serum was aspirated then divided into four aliquots. Samples were obtained and processed within one hour and immediately frozen [18] at –70 ºC until time for analysis. These storage conditions were proven to be sufficient to prevent deterioration of investigated parameters [19].

2.3. Investigated parameters:

After one cycle of slowly thawing, serum was left to reach room temperature, thoroughly mixed (using Vortex Cat. No.: SA 6, SN: 6004, 50 Hz., Great Britain.), then used for:

1. Quantitative determination of TPA using IdeaLTM Monoclonal TPAcyk ELISA kit (Cat. No. 30, Sweden) [20].
2. Quantitative determination of t-PSA using CanAg PSA EIA kit (CanAg Diagnostics Prod. No. 300-10, Sweden) according to the manufacturer's instructions.
3. Quantitative determination of f-PSA using CanAg Free PSA EIA kit (CanAg Diagnostics Prod. No. 330-10, Sweden) according to the manufacturer's instructions.
4. Calculation of f/ t-PSA ratio.

2.4. Statistical analysis

Statistics were calculated for the entire study cohort, using GraphPad Instat tm V2.04. Appropriate graphs were plotted when needed using Prism V4.03. Diagnostic accuracy was calculated according to Reed et al. [21].

3. RESULTS

3.1. Clinical and demographic profile of the studied groups

As shown in table-1: The study included 88 patients with age range 45-88 years. 55 were BPH patients: 12.73% had a history of TURP, 3.6% have had prostatectomy long ago. 26 were CaP patients: 15.38%

were had distant metastasis, 7.69% had a history of TURP, 3.85% were combined with BPH. The two groups were compared with 7 healthy controls.
In the present study mean age statistically correlated with high significance to incidence of CaP (70.02±1.6) and BPH (63.13±1.2) compared to control (56.00±2.2) at $p < 0.01$, using Tukey-Kramer Multiple), which indicate higher prevalence of cancer with age.

3.2. Descriptive analyses

3.2.1. Investigated serum markers:

As shown in table-2: TPA was elevated in all patients compared to controls despite it was lower than its established cutoff value (70 U/L). t-PSA and f-PSA was elevated in all patients with statisticaly significant difference between BPH and CaP at $p< 0.01$ and $p< 0.05$ respectively (using Kruskal-Wallis non-parametric ANOVA). TPA and f/t-PSA ratio of CaP showed no significant variation neither from control nor BPH.

3.2.2. Age-specific reference range of t-PSA:

Distribution of t-PSA according to age-specific reference ranges established by Oesterling et al.[21] is demonstrated in table-3. It was clear that precision of t-PSA is enhanced by age-specific reference ranges, which is statistically significant at $p< 0.01$, using Chi-square test.

3.3. Correlation studies

Linear regression is shown in table-4 and Fig-1: There was no significant correlation between age and any of the investigated parameters. TPA showed a direct but weak correlation with both t- and f-PSA ($r= 0.2383$ and 0.2231 respectively at $p< 0.05$). f-PSA showed directly moderate correlation with f/t-PSA ratio, and directly strong correlation with t-PSA ($r= 0.3916$ and 0.8921 at $p< 0.001$ and $p< 0.0001$ respectively).

3.4. Diagnostic accuracy

A comparison of the effectiveness of the investigated markers and f/t-PSA (at different cutoffs) in differential diagnosis between BPH and CaP was carried out by calculating the five diagnostic accuracy indices: Accuracy, sensitivity, specificity, positive predictive value, and negative predictive value. Best accuracy as attained by f/t-PSA ratio at 0.1 cutoff value then f-PSA, as shown in table-5.

3.5. Combined sensitivity

Table-6 shows the t-PSA sensitivity when combined to the investigated markers. Sensitivity of t-PSA was increased when combined to TPA or PSA ratio (cut off 0.1, 0.15, 0.22, and 0.25) but on the expense of specificity (using chi-square test). Sensitivity of t-PSA was not enhanced when combined to f-PSA.

4. DISCUSION

Although BPH would be of no importance if it were not for the consequent bladder outlet obstruction [4], it may constitute a high risk for CaP [5]. According to the last National Population-Based Registry Program of Egypt 2008–2011; CaP ranked the 6th (4.27%) in frequency among male tumor, and as the elderly population continues to expand, it is likely that scope of this problem will continue to increase, with an estimated increased up to 3398 cases by year 2020 [5].
Unfortunately, the majority of CaP has spread beyond the gland when first diagnosed using the conventional detection method, DRE. Prognosis is poor and treatment options are limited to palliative therapy with late stage disease. The most promising alternative for improving the prognosis of patients with CaP is to enhance early detection of organ confined CaP in younger men, and to enhance the differential diagnosis of CaP from BPH [23].
In this study we sought to clarify the impact of combined use of f/t-PSA ratio and TPA on the improvement of t-PSA accuracy, in addition to their role in the differential diagnosis between CaP and BPH.

4.1. Investigated markers

4.1.1. PSA

PSA has become one of the famous tumor markers, being increased in over 90% of cases when first diagnosed, although it typically lacks sensitivity and specificity desired of a diagnostic marker [24], but still clinically the most useful marker available for diagnosis and management of CaP. PSA level is the best diagnostic method for the early detection of CaP when compared to DRE at a detection rate of 2.2-2.5% versus 1.3-1.7% [25].
PSA not only a diagnostic tool but helps in the prediction of pathological and histology of the tumor when combined with other staging systems and also used in monitoring and evaluation of therapy. Follow up of patients after radical prostatectomy can consist solely of monitoring for the reappearance of detectable serum PSA due to its unique organ specificity [26].
The American Urological Association and the American Cancer Society recommend PSA and DRE from age 50 years in general population, also high-risk populations (mainly African-Americans and men with a positive family history) start screening at the age 40, in contrast the U.S NCI didn't.
In the present study t-PSA of control didn't exceed the well established cut-off, in agreement with previous studies [27]. It was significantly elevated in both CaP and BPH, without a clear discriminative borderline. That is attributed to that PSA is an epithelial cell marker rather than a CaP marker, therefore, other proliferative processes, *i.e.*, inflammatory and benign transformations, are also able to induce such cell alterations and affect PSA levels [28]. In addition 38% to 48% of patients (with organ confined CaP) have normal PSA values. Molecular forms of PSA have demonstrated potential benefits in distinguishing BPH from CaP [29].

4.1.2. f-PSA and % f/t-PSA ratio

Since there is a substantial overlap in total PSA levels between men with BPH and those with CaP, recently

the measurement of the %f-PSA ratio has been introduced as a useful clinical tool for early detection of clinically localized CaP [30].

It is presently unclear why f-PSA is less in CaP than BPH. Partin and Carter have speculated that this difference might be due to the mechanisms that prostatic cells use to prevent the escape of PSA from the ductal system into the blood stream [31].

In the contrary, our results showed that serum level of f-PSA was clearly elevated in CaP with a discriminative ability from that of BPH, taking in consideration that the mean value of BPH was within the established cut-off.

The existing study demonstrated f/t-PSA ratio had no significant difference from that of control, contradicting Stamey et al. who reported clinical significance of % f-PSA in the t-PSA values between 4.0-10.0 ng/ml, where the differential diagnosis of CaP is most difficult [32]. These findings were confirmed by Christensson et al. (1993), who demonstrated that %f-PSA, is lower in patients with CaP than BPH, and is a more sensitive means of discriminating between these two conditions [33]. In line, Partin et al. (1996) demonstrated that a patient with a low %f-PSA (less than 10%) had a higher probability of cancer (63±9%) than patients with a high %f-PSA less than 26% (2±3%) [34].

Discussing the suitable cut-off of PSA ratio; Luderer et al. reported that ≤ 0.15 could differentiate between BPH and CaP at t-PSA within 4.1-10 ng/ml ($p < 0.0001$) [35]. Partin and Carter demonstrated that using a cutoff of 20% f/t-PSA, maintained a sensitivity greater than 95% for detecting cancer while eliminating 29% of the unnecessary biopsies for men with serum PSA levels between 4-10 ng/ml [31].

In the intermediate t-PSA range of 4.0-10.0 ng/ml the f/t-PSA ratio improves the specificity of total serum PSA significantly. A cutoff level of 0.20 or less combined with a positive DRE as indicators for biopsy decreases the number of biopsies in that range by 38%, while maintaining the level of sensitivity at 88% [36].

Patients with a %f-PSA cut point of 0.25 could detect at least 95% of CaP and decrease 26.9% of negative biopsies in the grey zone [37].

%f-PSA may be used for diagnosis and staging of prostate cancer. When used for diagnosis, patients with greater than 25% f-PSA need not undergo biopsy unless family or medical history suggests otherwise. This approach would detect 95% of cancers and spare 20% of men with benign disease from biopsy. The missed cancers with high %f-PSA are more likely to be in older men and are primarily organ confined, small tumors with low Gleason sums. With annual screening it would be possible to monitor patients, with increasing PSA levels or decreasing %f-PSA, to detect what tend to be less advanced tumors [38].

Chronic prostatitis is not characterized by elevated t-PSA concentrations alone but also by a decreased %f/t-PSA, a tendency similar to that in CaP. This unspecific change in percentage of free PSA must be considered to interpret the f/t-PSA correctly [39].

4.1.2. TPA

Patients with malignancies and normal TPA serum levels fare better than those for whom TPA is elevated. It has been shown to be the most reliable prognostic marker in single-test estimates as well as in a multivariate life analysis ($p<0.01$) in men with CaP when compared with PAP, ESR, patient age, tumor grade, and presence or absence of skeletal metastases [40].

The present study investigated TPA role in differential diagnosis of prostate tumors. Serum levels of TPA, was elevated in both of BPH and CaP with no discriminative ability, proportional only to tumor size.

4.2. Impact of Age

Age is one of the risk factors for cancer [41]. A positive family history in addition to race and age are among the strongest known risk factors for CaP [42]. In the present study age was significantly correlated to incidence of CaP and BPH, in accordance with Wang and Shen [43].

4.2.1. Age-specific reference range of t-PSA:

To improve the diagnostic usefulness of serum PSA, attempts have been made to streamline its ranges by adjusting with dependable variables such as: prostate size (*i.e.*, PSA-density), increase over time (*i.e.* PSA-velocity) and patient age [22, 29].

The concept of age-specific reference ranges was introduced by Oesterling and coworkers in 1993 as a modality by which the sensitivity and specificity of PSA test could be improved [22]; based on the fact that serum PSA concentration positively correlates with age, with a higher proportion of men found to have PSA level above the standard reference range (0-4 ng/ml) as their ages increase [43]. This increase is due to gland enlargement as well as other factors intrinsic to aging of prostate, such as leaky physiologic barriers [24], clinical or subclinical prostatitis, prostatic ischemia, etc [45].

In the present study, distribution of t-PSA according to age-specific reference ranges established by Oesterling et al. [22] had statistically significant discriminative ability between BPH and CaP. In line with Arcangeli et al. who reported that these ranges will increase cancer detection in younger men (in whom early detection and cure are most desirable) and minimize detection of possibly insignificant tumors in older men who are less likely to benefit from treatment [46]. On the contrary, Babaian et al. reported that clinically volume referenced PSA is comparable to PSA, and both are superior to age referenced PSA and PSA density in the detection of prostate cancer [47]. In line, many other researches opposed according to age-specific reference ranges of t-PSA [22, 48].

In men with total PSA values between 2.5-20.0 ng/ml, the f/t-PSA significantly differentiated between benign and malignant histologic states. Log linear modeling indicated distinct differences in the risk for cancer as a function of f/t-PSA, t-PSA, and age. The highest probability for cancer was observed in men over 70 years old who had f/t-PSA less than 7% and t-PSA >10.0 ng/ml [49].

4.3. Correlation studies

In the present study, t-PSA showed a statistically significant correlation with TPA and f-PSA. While f-PSA correlated with TPA and PSA ratio. None of the used markers showed significant correlation with age in the contrary to Oesterling et al. [22].

In the contrary, Partin et al. demonstrated that %f-PSA increase with increase age and decrease in t-PSA. Although the correlation was weak (r = - 0.21, p = 0.01), it resulted in a 95% sensitivity (± 5%, 95% confidence interval) cut-point of 22% in men with PSA between 4 and 6 ng/ml and 20% in men with PSA between 6 and 10 ng/ml. Caution must be used in interpreting data <4 ng/ml and more than 10 ng/ml [34].

In contrast, a direct correlation of PSA ratio with patient age was reported. Conversely when including younger men in the study cohort they also demonstrated a direct, negative correlation [23].

Partin and Carter concluded from their study that age correlated directly with f-PSA (r = 0.45) and t-PSA (r = 0.45). The correlation between age and PSA ratio was linear and no age-specific cut-off ranges was demonstrated [31].

4.4. Diagnostic accuracy of investigated markers

A comparison of the effectiveness of TPA, t-PSA, f-PSA, PSA ratio for detecting CaP was carried out by calculating the four diagnostic accuracy indices, revealing that; t-PSA is the most sensitive, f-PSA is the most specific, and PSA ratio at 0.1cut-off is the most accurate among all. It was clear that as the cut-off increases the discriminative property of ratio PSA decreases.

Recent studies by Catalona et al. have confirmed the earlier observations suggesting that % cut-off values ranging between 0.17-0.25 (0.23) could maintain a sensitivity greater than 90% while decreasing the number of unnecessary biopsies 25- 40% among men with serum PSA levels between 4-10 ng/ml with a more than 40cm^3 prostate volume [11, 33, 50].

Wolff et al. reported that a threshold value for f/t-PSA of 14% was chosen, as it showed the highest sum of sensitivity and specificity, this gave a sensitivity of 84%, a specificity of 80%, a PPV of 78%, and NPV of 85% and wt. accuracy of 82% [51]. Recker et al. reported that by the use of different cut-off values for %f-PSA the following detection rate (sensitivity) for both of CaP/BPH respectively was: at cut-off value of 0.15 was 67.4/ 20%, at 0.1 was 38.78/ 4.4%, at 0.2 was 81.07/ 38.9%, and at 0.25 was 89.27/ 58.9%. Taking in consideration, although the sensitivity is decreased by decreasing the cut-off, the specificity is increased [52].

4.5. Combined sensitivity

The different PSA molecular forms have withdrawn a global attention, in attempt to increase the specificity and the sensitivity of PSA testing [23, 29]. In our study the combined use of f-PSA with t-PSA, didn't affect sensitivity or specificity, while combination with TPA or PSA ratio increased sensitivity of t-PSA on the expense of specificity.

In the contrary, it was reported that sing the %f-PSA an increase in specificity from 55% to 73% without compromising sensitivity, however, these studies were limited by small numbers of patients who had a wide range of PSA values [33]. Partin et al. demonstrated that the use of %f-PSA increased PSA specificity and resulted in a 95% sensitivity (± 5%, 95% confidence interval) cut-point of 0.22 in men with PSA between 4 and 6 ng/ml and 20% in men with PSA between 6 and 10 ng/ml [34].

The use of f/t-PSA ratio enhances the specificity of PSA in distinguishing benign from malignant prostatic lesions. However, that ratio provides no additional diagnostic information with respect to pathological tumor stage, volume or grade than t-PSA only [53].

5. CONCLUSION

PSA is by no means the best, but until now it is the most reliable tumor marker available for the detection of CaP. Still as a single marker, is the most effective in detecting CaP, and the least was TPA. Despite that a combination of any of the PSA related parameters (PSA-age specific reference ranges; f-PSA; % f-PSA) or TPA to t-PSA will enhance the later discriminative ability and PPV, but on the expense of specificity. Moreover, Age specific-PSA reflex ranges could be useful in differential diagnosis after standardizing and validating in large-scale prospective clinical studies.

6. RECOMMENDATIONS

This study recommends the use of a combination of f/t-PSA ratio for the differential diagnosis of CaP from BPH, after adjusting the cutoff values of PSA ratio.

Age specific-PSA reflex ranges could be useful in differential diagnosis after standardization and validation in large-scale prospective clinical studies.

7. ACKNOWLEDGMENT

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8. DECLARATION

Work of this study was a part of master thesis of Dr. Amel Hashim, under supervision of the rest of authors. Our results were orally presented in 4th National Conference "Drug Handling in the 21st Century", Egypt.

9. CONFLICT OF INTEREST AND FUNDING

Authors declare no conflicts of interest that might bias the study.

Table 1
Clinical and Demographic Profile of the Studied Population

Parameters	Control	CaP	BPH
№	7	26	55
Age: (years)			
Mean ± SE	56.00 ± 2.2	70.02 ± 1.6 ***	63.13 ± 1.2 ##
Range	50 – 63	57 – 88	45 – 83
Clinical history: (№)			
TURP		2	7
History of open prostatectomy		1	1
Distant metastases		4	1
Subcapsular orchiectomy		6	
TURP then radical prostatectomy		1	
History of other cancers			
Bladder cancer		2	1
Testicular cancer			
Renal cancer			1
Combined with BPH		1	
Pathological Grade: (№)			
I		1	
II		5	1
III		1	2
Combined Gleason Score: (№)			
(4+5)		2	
6 (3+3)		1	
5 (3+2)		2	
(3+4)		3	
(5+4)		1	
(5+6)		1	
Stage: (№)			
T_{4a}		1	
T_{2+3}		1	
T_{3a}		1	
T_3		1	

№: Total number in each group, BPH: Benign prostatic hyperplasia, CaP: Carcinoma of prostate. C: Control volunteers.***: p< 0.001 when compared to control group, ##: p< 0.01 when compared to CaP using Tukey-Kramer Multiple Comparisons Test. Data were approximated to the second decimal.

Table 2
Serum level of investigated markers in the studied population

Markers (cut-off)	Control	CaP	BPH
TPA: (70 U/L)			
№	7	25	54
Mean ± SE	35.12±7.23	64.34±10.02	56.71±7.63
Range	6.72 - 65.73	5.74 -173.11	0.00 -192.78
t-PSA: (4 ng/ml)			
№	7	26	54
Mean ± SE	1.19 ± 0.47	72.09± 31.55	4.87± 0.90 ##
Range	0.25 - 3.610	0.52 - 820.0	0.13 - 32.81
f-PSA: (1 ng/ml)			
№	7	26	55
Mean ± SE	0.10 ± 0.02	16.70 ± 8.51	0.83 ± 0.17 #
Range	0.03 - 0.19	0.00 -192.5	0.00 - 6.99
f/t-PSA Ratio:			
№	7	26	54
Mean ± SE	0.10 ± 0.02	0.16 ± 0.04	0.17 ± 0.02
Range	0.03 - 0.19	0.00 - 0.90	0.00 - 0.62

№: Total number in each group, BPH: Benign prostatic hyperplasia, CaP: Carcinoma of prostate, TPA: Tissue polypeptide antigen, t-PSA: total prostate specific antigen, f-PSA: free prostate specific antigen, f/ t-PSA ratio: ratio of free to total prostate specific antigen. #: p< 0.05, ##: p< 0.01 when compared to CaP using Tukey-Kramer Multiple Comparisons Test. Data were approximated to the second decimal.

Table 3
Distribution of t-PSA (ng/ml) according to Age-Specific Reference Range

Age Range (years)	**t-PSA Reflex Range** (ng/ml)	**BPH**	**CaP**	**C**
40 – 49	0.0 - 2.5	0.0	0.0	0.0
50 – 59	0.0 - 3.5	66.67	0.0	100
60 – 69	0.0 - 4.5	61.9	30.77	100
> 70	0.0 - 6.5	64.3	25	0.0

t-PSA: Total prostate specific antigen, BPH: Benign prostatic hyperplasia, CaP: Carcinoma of prostate. C: Control volunteers. All data are expressed in percentage and approximated to the second decimal. Age-specific reference ranges were calculated according to Oesterling et al. (1993). Chi-square= 8.20513 at p< 0.01

Table 4
Linear regression in-between investigated markers.

Tested correlation	***№***	***r***	***p***
Age vs. TPA	79	0.09023	> 0.05
Age vs. t-PSA	80	0.1801	> 0.05
Age vs. f-PSA	81	0.1553	> 0.05
Age vs. Ratio	80	0.02371	> 0.05
t-PSA vs. TPA	79	0.2383	< 0.05
t-PSA vs. f-PSA	80	0.8921	< 0.001
t-PSA vs. Ratio	79	0.1116	> 0.05
f-PSA vs. Ratio	79	0.3916	< 0.001
f-PSA vs. TPA	79	0.2231	< 0.05
TPA vs. Ratio	79	0.03756	> 0.05

№: Total number of patients (BPH and CaP), r: linear regression coefficient, TPA: Tissue polypeptide antigen, t-PSA: Total prostate specific antigen, f-PSA: Free prostate specific antigen, Ratio: Free to total prostate specific antigen ratio.

Table 5
Diagnostic accuracy indices of the studied markers at their reference cut-off values in serum

Marker (cut-off)	**Sn.**	**Sp.**	**PPV**	**NPV**	**A**
TPA (70 U/L)	40	70.4	38.46	71.7	60.76
t-PSA (4 ng/ml)	73.08	62.96	50	85	67.5
f-PSA (1 ng/ml)	65.4	94.6	56.7	82.35	72.84
PSA Ratio					
≤ 0.1	46.2	74.1	46.15	74.1	77.5
≤ 0.15	57.7	53.7	37.5	72.5	55
≤ 0.22	80.8	31.5	36.21	77.27	47.5
≤ 0.25	84.62	22.2	34.38	75	42.5

TPA: Tissue polypeptide antigen, t-PSA: Total prostate specific antigen, f-PSA: Free prostate specific antigen, Ratio: Free to total prostate specific antigen ratio, Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, A: Accuracy. All data are expressed in percentage.

Table 6
Combined sensitivity of the studied serum markers at their reference cut-off values

A. TPA and t-PSA:

Marker (cut-off)	**True Positive**	**False Negative**	**True Negative**	**False Positive**
TPA (70 U/L)	40	60	70.37	29.63
t-PSA (4 ng/ml)	73.08	26.92	62.96	37.04
Combined	80	24	57.41	42.59

TPA: Tissue polypeptide antigen, t-PSA: Total prostate specific antigen. №: 25 patients. All data are expressed in percentage and approximated to the second decimal. Combined Sensitivity= 80 %, Chi-square = 10.58 at p< 0.01

B. t-PSA and f-PSA:

Marker (cut-off)	**True Positive**	**False Negative**	**True Negative**	**False Positive**
t-PSA (4 ng/ml)	73.08%	26.92%	62.96%	37.04%
f-PSA (1 ng/ml)	65.39%	34.62%	76.36%	23.64%
Combined	73.08%	26.92%	62.96%	37.04%

t-PSA: Total prostate specific antigen, f-PSA: Free prostate specific antigen. №: 26 patients. All data are expressed in percentage and approximated to the second decimal. Combined Sensitivity= 73.08 %, Chi-square = 14.37 at p< 0.0001

C. t-PSA and f/t-PSA Ratio.

Marker	**True Positive**	**False Negative**	**True Negative**	**False Positive**	**X^2**	**p**
t-PSA	73.08%	26.92%	62.96%	37.04%	-	-
Ratio ≤0.1	46.15%	53.85%	74.07%	25.93%	-	-
Combined	84.62%	15.38%	46.30%	53.70%	9.31	< 0.01
Ratio ≤0.15	57.69%	42.31%	53.7%	46.3%		
Combined	88.46%	11.54%	38.89%	61.11%	4.31	< 0.05
Ratio ≤0.22	84.62%	15.39%	31.48%	68.52%		
Combined	100%	0.0%	20.37%	79.63%	6.14	< 0.05
Ratio ≤0.25	84.62%	15.39%	22.22%	77.78%		
Combined	100%	0.0%	16.67%	83.33%	4.28	< 0.05

t-PSA: Total prostate specific antigen, Ratio: Free to total prostate specific antigen ratio, Combined: combined sensitivity, x^2: Chi-square. №: 26 patients. Cut-off of t-PSA= 4 ng/ml. All data are expressed in percentage and approximated to the second decimal.

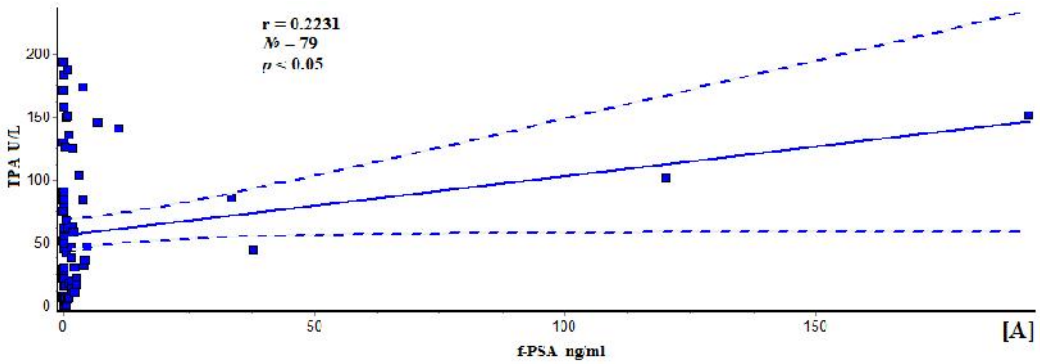

Fig. 1A

Linear regression between TPA and f-PSA

TPA: Tissue polypeptide antigen, f-PSA: Free prostate specific antigen, linear regression coefficient (r), total number of cancer patients (№).

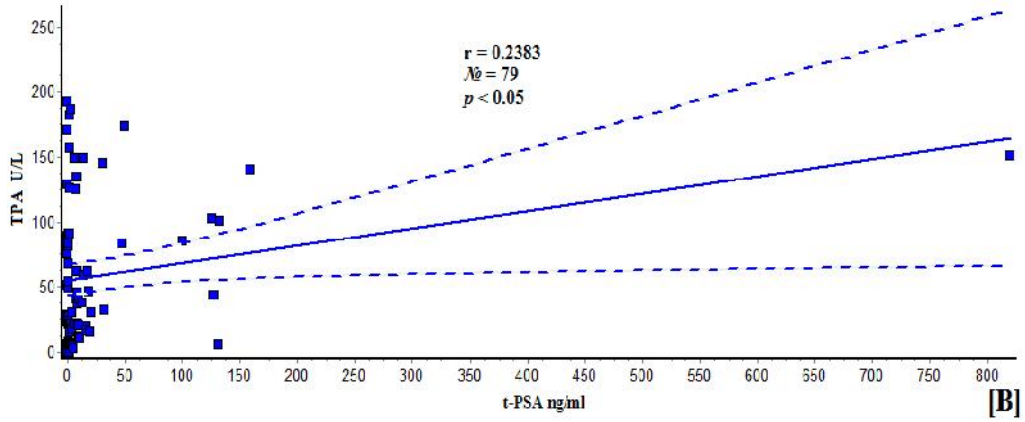

Fig. 1B

Linear regression between t-PSA and TPA

TPA: Tissue polypeptide antigen, t-PSA: Total prostate specific antigen, linear regression coefficient (r), total number of cancer patients (№).

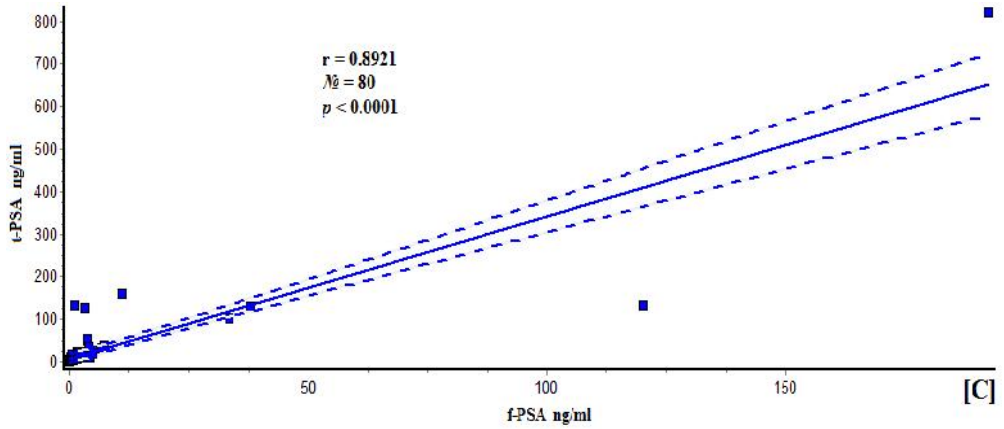

Fig. 1C

linear regression between t-PSA and f-PSA

t-PSA: Total prostate specific antigen, f-PSA: Free prostate specific antigen, linear regression coefficient (r), total number of cancer patients (№).

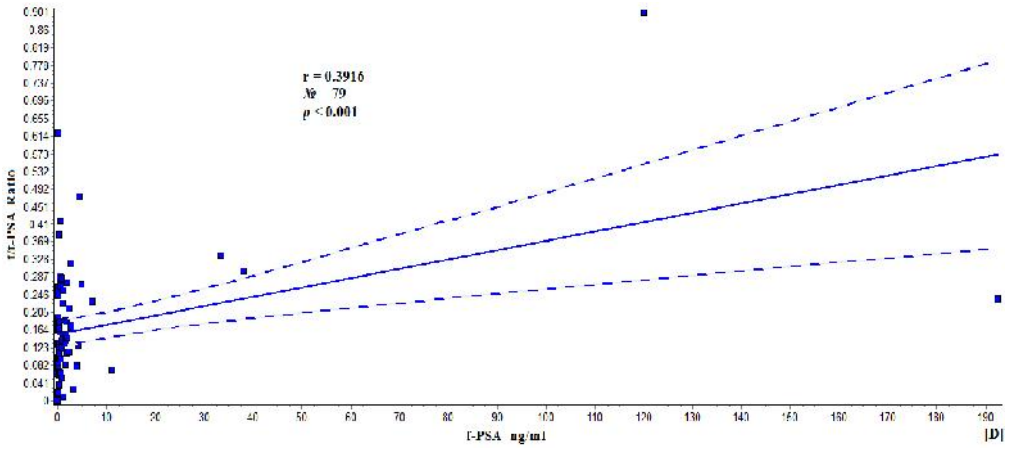

Fig. 1D

Linear regression between f-PSA and f/t-PSA ratio.

f-PSA: Free prostate specific antigen, f/t-PSA Ratio: Free to total prostate specific antigen ratio, linear regression coefficient (r), total number of cancer patients (№).

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