Research Article

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Serum proPSA as a marker for reducing repeated prostate biopsy numbers
Tekrarlanan prostat biyopsi sayısı azaltılma belirteci olarak serum proPSA

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Abstract

Introduction: Prostate specific antigen (PSA) has a lower sensitivity and specificity range of 4–10 ng/mL. We aimed to investigate the effectiveness of pPSA in reducing number of prostate biopsies.

Methods: This study enrolled 80 patients aged 50 years or older whom had serum total PSA levels between 4 ng/dL and 10 ng/dL. Age, prostate volume, tPSA, fPSA, pPSA, PSA%, pPSA%, t/pPSA, f/pPSA, p/tPSA, p/f/tPSA, PSAD, sPSA/PSAD, pPSA/PSAD, (Prostate Health Index) PHI, (t/f/pPSA)/tPSA, and PHI2 (New Prostate Health Index) biopsy results were compared between subjects BPH and PCa.

Results: Out of 80 subjects, 23 (29%) had PCa and 57 (71%) had BPH. Prostate volume was 51.65 mL in PCa and 64.85 mL in non-PCa group (p > 0.05). The rate of PCa increased as prostate volume was reduced and age increased. fPSA, PSA%, p/f/tPSA, pPSA/PSAD values were significant in favor of respectively; BPH, BPH, PCa and BPH (p < 0.05).

Discussion: Using prostate health index (PHI) was beneficial for predicting PCa. In addition, using pPSA in formulas such as PHI2, pPSA/(sPSA*√tPSA), p/f/tPSA, (t/f/pPSA)/tPSA may also be useful. This study suggests that the use of pPSA may have a role in reducing the number of prostate biopsies in differentiating PCa and BPH.

Keywords: Prostate cancer; Prostate biopsy; PSA; proPSA, Prostate health index.

Özet

Amaç: Prostat spesifik antijen (PSA) 4–10 ng/mL aralığında düşük sensitivite ve spesifiteye sahiptir. Biz prostat biyopsilerinin sayısını azaltmak için proPSA (pPSA) ve türevlerinin etkinliğini göstermek amaçladık.

Yöntem: Elli yaş ve üzeri, PSA serum değeri 4–10 ng/mL arasında olan 80 hasta çalışmaya dahil edildi. Yaş, prostat hacmi, tPSA, sPSA, pPSA, PSA%, pPSA%, t/pPSA, s/pPSA, p/tPSA, s/p/tPSA, sPSA/PSAD, pPSA/PSAD, (Prostat Sağlık İndeksi) PHI, (t/f/pPSA)/tPSA, ve Yeni Prostat Sağlık İndeksi (PHI2) değerleri benign prostat hiperplazisi (BPH) ve prostat kanseri (PCa) arasında t-test, MANOVA ile karşılaştırıldı.

Bulgular: Seksen hastanın 23’ü (29%) PCa ve 57’si (71%) BPH idi. Prostat hacmi PCa'da 51.65 mL, PCa olmayan grupta 64.85 mL olarak bulundu (p > 0.05). PCa oranları prostat hacmi azaldıkça ve yaş arttıkça arttırdığındaydı. fPSA, PSA%, p/f/tPSA, pPSA/PSAD değerleri sırasıyla BPH, BPH, PCa ve BPH (p < 0.05).

Sonuç: PHI kullanımları PCa’nın öngörülmesinde faydalıydı. Buna ilaveten, PHI2, pPSA/(sPSA*√tPSA), p/f/tPSA, (t/f/pPSA)/tPSA benzeri farklı formüller içinde pPSA kullanımda PCa ve BPH’nin ayırtedilmesinde prostat biyopsi sayısının azaltılmasında bir rol oynamabilir.

Anahtar Kelimeler: Prostat Kanseri; Prostat Biyopsisi; PSA; proPSA; Prostat Sağlık İndeksi.
**Introduction**

Prostate-specific antigen (PSA) is the most widely used marker in the early diagnosis, treatment and monitoring of prostate cancer (PCa). However, despite being an organ-specific marker, PSA is not perfect because of the uncertainties it creates. It has low sensitivity and specificity for the diagnosis of PCa, particularly in conditions when total PSA (tPSA) in serum is in the range of 4–10 ng/mL [1]. Despite the development of various formulations in which PSA kinetics and PSA derivatives such as tPSA, free PSA (fPSA) and proPSA (pPSA) are used in order to increase the sensitivity and specificity of PSA, no significant progress has yet been achieved. No markers or formulae are currently available to permit diagnosis of PCa solely on the basis of serum markers without biopsy.

The probability of PCa being detected in the first biopsies of patients with serum tPSA values of 4–10 ng/dL is 20%–25%. Biopsy is therefore repeated after a specific interval. The probability of identifying PCa from repeat biopsy results is low. The search for serum and urine markers for the purpose of preventing repeated prostate biopsies is therefore continuing. Persisting PSA elevations in patients undergoing biopsy therefore necessitate a second biopsy or saturation biopsy, and in the event of small acinar proliferation (ASAP), prostatic intraepithelial neoplasia (PIN) benign prostate hyperplasia (BPH) or prostatitis at repeat biopsies a third biopsy or even saturation biopsy may be required [2]. Biopsy is an invasive procedure. Although it has few complications, symptoms such as sepsis, bleeding or bloody ejaculation can be troubling to patients [3]. It is therefore desirable to reduce the need for biopsy, and biopsy should be performed on cases with a high probability of diagnosis of cancer. However, it is also important that diagnosis of patients with PCa should not be delayed.

While research concerning PSA is continuing, the higher specificity of pPSA in particular in the sera of patients with PCa and increased pPSA in histopathological terms at pathological examination of tissues with PCa in some recent studies have directed to the attention of researchers toward this marker. pPSA and its derivatives and the prostate health index (PHI) have been increasingly reported in the literature [4]. ProPSA is a precursor of PSA consisting of 244 amino acids. It is largely secreted from cancerous tissue in the peripheral zone. It is frequently described as [-7]pPSA, although another important form is [-2]pPSA, which consists of 239 amino acids. Although subsequent research has also identified [-1], [-5], [-6] and [-4]pPSAs, the most stable form has been defined as [-2] pPSA (p2PSA) [5].

This study used sera from 80 consecutive patients presenting to the urology clinic, with normal prostate at rectal finger examination and serum tPSA levels of 4–10 ng/dL. The purpose of this study was to compare pPSA and its derivatives with prostate biopsy results and to investigate, in a Turkish population, whether the PHI, which has recently become popular and approved for routine use in various countries, can play an effective role in reducing biopsy numbers. This study also describes comparative studies of various formulations added by ourselves.

**Materials and methods**

**Patients and characteristics**

Permission for the study was granted by the Ordu University Non-Interventional Research Ethical Committee (No. 58293865-50 dated 20/02/2013). Eighty consecutive patients aged 50 or more presenting to our hospital’s urology clinic in 2013–2014, with tPSA values of 4–10 ng/dL and electing to undergo biopsy were included in the study. Patients were informed about the pre- and post-biopsy procedures and potential complications, and informed consent forms were obtained. Patients with a doubtful condition at rectal finger examination (such as nodule or areas of hardness) and with urinary tract infection were excluded.

Age, prostate volume, tPSA, fPSA, pPSA, PSA%, pPSA%, t/pPSA, f/pPSA, p/tPSA, p/f/tPSA, f/p/tPSA, fPSA/PSAD, pPSA/PSAD, [(pPSA/fPSA)*√tPSA] (PHI) and (t/f/pPSA)/tPSA [pPSA/(fPSA*√tPSA)] (PHI2) defined by ourselves were compared among patients with PCa and BPH diagnosed at biopsy.

**Specimen collection and preparation**

Sera separated from blood collected for the investigation of tPSA and fPSA were stored at – 80°C until pPSA assay. Total PSA and fPSA tests were performed using Abbott brand kits on an Abbott Architect i2000 immunoanalyzer. ProPSA assay (proPSA kit Cusabio Human prostate specific antigen Catalog No: CSB-ETO27786HU) (CUSABIO, China) was performed on an Quantitative Sandwich Enzyme Immuno Assay (ELISA) method (BioTek Instruments, Inc., Winooski, VT05404 USA) using the quantitative sandwich enzyme immunoassay technique.
Statistical analysis

Statistical analyses were performed on SPSS® 16 software using Multivariate analysis (MANOVA) and the t-test. Significance was set at \( p < 0.05 \).

Results

PCa was present in 23 (29%) of the 80 patients and BPH was present in 57 (71%) of the 80 patients (Table 1). Mean age of the patients with PCa was 69.17, and 65.16 in the non-PCa patients (\( p > 0.05 \)). Prostate volumes could not be determined in six cases among the patients with PCa presenting to the urology clinic and in nine cases among the non-PCa patients. Prostate volume was 51.65 mL in patients with PCa (\( n = 17 \)) and 64.85 mL in non-PCa subjects (\( p > 0.05 \)). In terms of general picture, fPSA, PSA%, fPSA/PSAD was statistically significant in favor of BPH (\( p < 0.05 \)), while p/f/tPSA, and (t/f/pPSA)/tPSA were significantly in favor of PCa (\( p < 0.05 \)) according to the MANOVA analysis.

Using MANOVA, examination of the results for proPSA itself and the formulae in which it was used revealed insignificance in terms of its presence in pPSA%, t/pPSA, f/pPSA, p/tPSA, p/tPSA, pPSA/PSAD (\( p > 0.05 \)). When PHI and PHI2 results were analyzed p values were found too close to the 0.05 significance values (respectively; \( p = 0.052 \), \( p = 0.051 \)) (Table 1).

Discussion

PSA is routinely used in the diagnosis of PCa and is one of the most widely used tumor markers. However, although this marker is a sensitive test for the prostate, it is not a specific test for prostate cancer. Definite diagnosis is made with prostate biopsy. The range between 4 and 10 ng/mL is regarded as the gray zone. Cancer is observed at a mean level of 20%–25% in the first biopsy in this gray zone. Second, third and even saturation biopsies are therefore performed in this patient group [6].

Although ProPSA is not significant by itself in studies in the literature, it has been shown to be of considerable significance within the PHI [7]. In other words, when pPSA is used in various formulations it has been shown to elicit more significant results than PSA and pPSA by itself in the diagnosis of PCa. It therefore seems appropriate for pPSA to be used in varying formulations as a marker to reduce biopsy numbers in current practice. However, studies of proPSA have addressed very diverse groups. It has been used at all ranges of tPSA in the literature [5]. We considered only patients in the gray zone, those with tPSA levels of 4–10 ng/mL, and their results, and compared these with information from the literature.

In a multicenter European study of patients with tPSA levels of 2–20 ng/mL, Lazzeri et al. reported a mean pPSA value of 14.7 pg/mL in 382 patients with PCa and a mean value of 15.0 pg/mL in 264 patients without cancer. The
difference was not statistically significant. However, when
the PHI was evaluated, the index was higher in patients
without cancer, and high significance was reported
[PCa = 38.0; non-PCa = 48.2] [4]. In a multicenter study of
892 patients with normal rectal examination findings
and tPSA levels of 2–10 ng/mL, Catalona et al. [7] reported
PHI scores of 49 in patients with PCa and 34 in those
without. However, the US Food and Drug Administration
has only approved the PHI test in subjects with tPSA of
4–10 ng/mL [8]. A multicenter study from Japan evaluated
pPSA% values and PHI values between classified
and non-classified groups; median pPSA% was 2.44
and PHI 60.3 in the classified group, compared to pPSA% of
1.88 and PHI of 47.8 in the non-classified group, the dif-
fences between the groups being statistically significant
[9]. In our study, pPSA values were 0.18 ng/mL in cases
with PCa and 0.14 ng/mL in non-PCa cases. The difference
was not statistically significant. PHI values were 0.34 in
non-PCa cases and 1.74 in patients with PCa. In agreement
with the literature, we didn't identify a statistically sig-
nificant difference by using MANOVA. But, in multivariete
analysis PHI results were similar with 0.05 significance
values. Although pPSA% was 3.03 in PCa cases and 2.19 in
non-PCa cases, the difference was not significant.

Khan et al. [10] reported 90% sensitivity and 44%
specificity for pPSA investigated in order to avoid unnec-
essary biopsy in subjects with tPSA values between 4 and
10 ng/mL, while Miyakubo et al. reported 33% sensitiv-
ity for f/tPSA and 75% sensitivity for t/pPSA/mm. In our
study, t/pPSA values were 77.52 in cases with PCa and
81.72 in BPH cases. Despite the decrease in PCa cases,
the difference was not statistically significant. Patient
numbers need to be increased in order to assess sensitiv-
ity and specificity.

Miyakubo et al. [11] reported significant elevation in
p/fPSA and p/f/tPSA values in subjects with cancer
among patients with PSA levels between 4.1 and 10 ng/
ml. In our study, p/fPSA was quite high in PCa patients,
at 0.67. Our p/f/tPSA values in patients with cancer were
0.11, significantly higher compared to the non-PCa group.
Catalona et al. [12] revealed in pathological studies that
these values were useful for PCa, that the best marker in
this context was p2PSA, and that aggressive cancers could
be identified by this means. pPSA has been reported to be
potentially highly useful in young patients, with a long
life expectancy, before the development of complications
[11, 12]. pPSA and p/f/tPSA ratios may be important in
risk determination together with the Gleason score com-
ponent before proceeding to definitive treatment, particu-
larly in young males with a long life expectancy [11]. In
our study, however, although p/fPSA was higher in PCa
patients than in BPH patients, (0.67 and 0.14, respec-
tively), the difference was not statistically significant.

Mikolajczyk et al. [13] reported that pPSA% had
greater specificity and a higher positive predictive value
for PCa than PSA% in patients with tPSA values between
4 and 10 ng/mL, while de Vires et al. [14] reported that
pPSA% was not correlated with prognosis of PCa at tPSA
values <15 ng/mL, but that in the gray zone, at tPSA
values of 4–10 ng/mL, a high pPSA level was associated
with poor prognosis. In our study, PSA% in PCa cases was
15.41, significantly lower in comparison to non-PCa cases,
while pPSA% values, at 3.03, were slightly higher com-
pared to the non-PCa cases, although this elevation was
not statistically significant.

PHI is described as significant when other formul-
ations of ProPSA are evaluated in the literature. PHI was also
evaluated in our study and found significantly different
between two groups by univariate analysis while the p value
was near 0.05 (0.052) by MANOVA. The similar conclusion
can be made for PHI2 since there is a significant difference
between PCa and non-PCa groups with univariate analy-
sis despite a borderline p-value (0.051) with multivariate
analysis. The PHI index represents a mathematical equa-
tion [(pPSA/fPSA)*√tPSA]. High PHI values are reported
to be associated with a high probability of cancer [5, 15].
One meta-analysis of 12 separate studies reported that
PHI is 81% specific when tPSA values of 4–10 ng/mL are
considered [15]. These findings show that both pPSA and
PHI increase specificity in reducing the number of unnec-
essary biopsies for determination of PCa, although there
is no analysis of cut-off values for these tests. No cut-off
value was specifically revealed in our study. However, PHI
elevation was observed in cases with PCa compared to
those without PCa (PCa = 1.74; non-PCa = 0.34). Catalona
et al. reported that PHI values do not change with age, for
which reason it can easily be applied to both young and
old patients in determination of prostate cancer [5, 7].

In one wide-ranging study, Miyakubo et al. inves-
tigated f/tPSA, p/tPSA, p/fPSA, p/f/tPSA and PSAD in
patients with tPSA values of 4–10 ng/mL. They concluded
that only f/tPSA ratios differed significantly at values
between 4.1 and 10 ng/mL in cases with cancer compared
to non-cancer cases, that the others also increase, and
rose still further as Gleason scores increased [11]. In our
study, the p/tPSA ratio was 0.03 in cases with PCa and
0.02 in non-PCa cases, but the difference was statistically
insignificant, despite rising in PCa.

In agreement with the literature, our analysis results
showed that pPSA is not significant in the differential diag-
nosis of PCa and BPH. This finding may have been affected
by the limited patient number. Additionally, the results
for pPSA, t/pPSA, f/pPSA, p/fPSA, p/tPSA, f/p/tPSA and pPSA/PSAD, other parameters associated with pPSA, also exhibited no significant differences between the two diseases. In contrast, the parameters p/f/tPSA and (t/f/pPSA)/tPSA in which pPSA was used were significantly in favor of PCa. When multivariate analysis was used only p-values in the PHI vs PHI2 were in significance borderline statistically. One recently published study of p2PSA% and PHI showed that both parameters were highly significant with pathologically findings in radical prostatectomy and expressed tumor aggressivity [16]. Yu et al. evaluated PHI results in 261 patients with normal rectal examination by finger and transrectal ultrasonography. They reported that PHI, particularly at tPSA values of 10.1–20 ng/mL, was the best marker of biopsy results [17]. A recent systematic review of 6912 patients assessing clinical use of [-2]proPSA and evidence reported pPSA sensitivity of 90% and sensitivity of 13% [18].

Conclusion

In conclusion, pPSA values differ among the studies in the literature. Examination of multicenter studies with larger patient numbers shows that pPSA is by itself of no value in determining PCa, but that it is significant within formulations, and particularly in the PHI. In our study, proPSA was not significant by itself in PCa. However, and in agreement with the literature, PHI may be a useful test in predicting PCa. In addition, it may be useful to study pPSA in some cases within formulations [(PHI2) pPSA/(PSA*+tPSA), p/f/tPSA, (t/f/pPSA)/tPSA]. On the basis of these findings, routine use of pPSA has a place in reducing biopsy numbers in the differentiation of PCa and BPH. However, case numbers need to be increased for these analyses, and subgroup studies are now also required.

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