European Urology

Eur Urol 2000;38:550–554

Accepted after revision: February 9, 2000

Free and Total Prostate-Specific Antigen Levels in Saliva and the Comparison with Serum Levels in Men

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Key Words

Prostate-specific antigen · Saliva · Salivary gland

Abstract

Objective: We investigated free and total prostate-specific antigen (PSA) levels and free/total (f/t) ratio in the fasting saliva and compared them with the serum levels in normal individuals, in patients with benign prostatic hyperplasia (BPH) and prostate cancer. Our aim was to determine free and total PSA and f/t ratio in saliva and to improve and simplify the differentiation between BPH and prostate cancer by using saliva as an alternative to serum.

Methods: Serum and fasting saliva concentrations of free and total PSA were measured in 35 men with BPH, 16 men with stage D prostate cancer, and 25 healthy men. Serum and fasting saliva samples were collected at the same time and were analyzed on the same day at our laboratory with microparticle enzyme immunoassay technology.

Results: For the total of 76 men, there was a significant correlation between free and total PSA levels in each sample ($r = 0.97$ for serum and $r = 0.44$ for saliva, $p < 0.001$). Although there was a significant difference between three groups for serum-free and total PSA levels and serum f/t ratios, no significant difference was determined between groups for salivary free and total PSA levels and salivary f/t ratios. No correlations were found between patient age and salivary PSA levels.

Conclusions: Fasting salivary free and total PSA levels are not effected by high serum levels of prostatic origin. Although there was a significant difference between mean serum and salivary levels of free and total PSA in each group, the f/t ratio of saliva was very close to the serum ratio of normal subjects. Determination of free and total PSA in saliva to improve and simplify the differentiation between prostate cancer and BPH is not suitable for use as alternative measurement of serum.

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Introduction

Prostat-specific antigen (PSA) is recognized as a very useful tumor marker for prostate cancer because of its high tissue specificity. The major site of PSA production is the glandular epithelium of the prostate. Serum PSA levels are routinely used to facilitate the diagnosis and to monitor patients with prostate cancer. However, PSA was shown to be present in embryologically prostate-related glands [1–5] and several recent reports have described elevation in serum PSA levels in a variety of nonprostatic malignancies [6–9]. Many groups have also reported immunoreactivity for PSA in a variety of normal and neoplastic tissue types [10–16]. These findings raised a debate concerning the specificity of prostatic acini and ducts as an exclusive source of PSA.

Prostate-specific antigen has previously been detected in patients with salivary gland neoplasms and in normal salivary gland by using immunohistochemistry. Van Krieken [11] found immunoreactivity in a variety of both benign and malignant salivary gland tumors by using polyclonal antibodies. In this study, PSA immunoreactivity was also demonstrated along the luminal aspect of the duct epithelial cells in the normal salivary gland.

James et al. [17] reported a case of salivary duct carcinoma associated with elevated serum levels, and both the primary tumor and metastases were stained positively with anti-PSA monoclonal antibodies. In another immunohistochemical study, Elgamal et al. [18] demonstrated a consistently positive reaction for PSA and prostatic acid phosphatase, independent of patient sex, in ductal cells of normal salivary glands and salivary gland tumors using monoclonal antibody assay.

These investigations concluded that PSA may also be secreted by salivary glands, so it is important to report on extraprostatic sources of PSA by biochemical detection to avoid errors in diagnosis and management. Our aim was also to determine free (fPSA) and total PSA (tPSA) and free/total (f/t) ratio in saliva and to improve and simplify the differentiation between benign prostatic hyperplasia (BPH) and prostate cancer by using saliva as an alternative to serum.

Based on these findings, we investigated fPSA and tPSA levels and f/t ratio in the fasting saliva and correlated them with the serum levels in normal individuals, in patients with BPH and prostate cancer by using microparticle enzyme immunoassay technology.

To our knowledge, our investigation is also the first report in the literature including both fPSA and tPSA levels in the saliva.

Patients and Methods

Serum and fasting saliva concentrations of fPSA and tPSA were measured in 35 men with BPH, 16 men with stage D prostate cancer and 25 healthy men. The mean age ± SD was 40.28 ± 5.51 years in normals, and 63.05 ± 5.21 and 70.06 ± 5.54 years in patients with BPH and prostate cancer, respectively.

Patients presenting with an enlarged prostate with normal PSA levels and no evidence of prostate carcinoma at digital rectal examination and transrectal ultrasonography, and patients with an enlarged prostate but negative prostate biopsy for elevated serum PSA levels were classified as BPH group. Both the BPH and prostate cancer group had no medical, hormonal and surgical therapy before our study. The control group (healthy men) included men who were admitted for a check-up investigation including routine urological investigation and in whom no disease was found. None of the 76 men had renal functional impairment, urinary tract infection, and none of them had clinical evidence of salivary gland disease according to the routine outpatient examination. Serum and fasting saliva samples were collected at the same time and obtained before urethral or rectal manipulation and at least 72 h after ejaculation.

For the detection of salivary PSA levels, 3 ml saliva was collected after having chewed gum for 10 min. The samples were centrifuged at 3,000 *g* for 10 min. A manual dilution of 1:10 was performed on supernatant. All samples were inspected for bubbles and bubbles were removed prior to analysis. The pH of the saliva samples were 7.5–8 in all men.

Serum and saliva samples were analyzed on the same day at our laboratory with microparticle enzyme immunoassay technology that uses monoclonal/monoclonal antibodies (Abbott AxSYM PSA assay). The PSA levels were expressed as ng/ml and the lowest measurable concentration that can be distinguished from zero was 0.01 ng/ml for fPSA and 0.02 ng/ml for tPSA. The precision of our method for saliva was determined according to the following protocol: ten samples of saliva were assayed in replicates of two at two separate times per day for 20 days. Data from this study are summarized below: mean (ng/ml) = 0.54, within-run (%) CV = 5.47, total (%) CV = 7.1.

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) for Windows 6.1 software. Mean fPSA and tPSA concentrations and f/t ratios for each group were calculated with standard deviations (SD). The Kruskal-Wallis-one-way ANOVA test was used as a nonparametric test for independent samples to investigate whether there were significant differences between groups. The Wilcoxon signed ranks test was used to compare the serum and saliva levels and ratios of fPSA and tPSA in groups. Pearson's correlation test was used for correlation analysis. $p < 0.05$ was considered to be statistically significant.

Results

The values of fPSA and tPSA levels and f/t ratios of the serum and saliva samples for the normal subjects and patients with BPH and prostatic cancer are given in table 1. Although there was a significant difference between the three groups for serum fPSA and tPSA levels and serum f/t ratios, no significant difference was determined between the

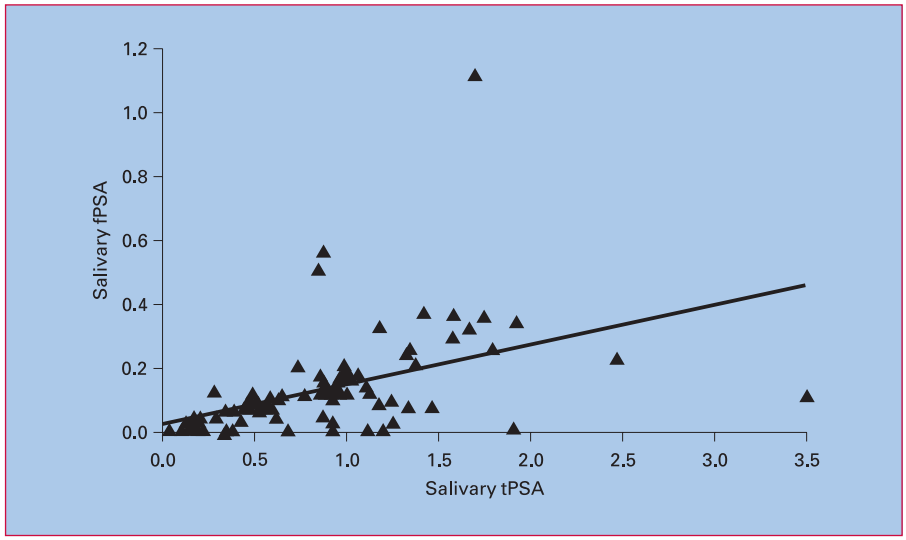

Fig. 1. Correlation between fPSA and tPSA levels (ng/ml) in saliva for all men (r = 0.44, $p < 0.001$).

Table 1. Values of fPSA and tPSA levels (ng/ml) and f/t ratios of the serum and saliva samples according to the groups

	Control (n = 25)	BPH (n = 35)	Prostate cancer (n = 16)	p value[a]
Serum				
fPSA				
Range	0.01–0.03	0.05–4.91	2.39–23.18	
Median	0.29	0.49	4.21	<0.001
IQR	0.21	0.79	3.49	
tPSA				
Range	0.05–2.99	0.19–9.58	11.28–241.36	
Median	1.49	1.19	42.57	<0.001
IQR	1.08	3.39	28.36	
f/t ratio				
Range	0.04–0.26	0.06–0.9	0.07–0.24	
Median	0.18	0.37	0.12	<0.001
IQR	0.07	0.29	0.03	
Saliva				
fPSA				
Range	0.01–0.36	0.01–0.11	0.01–0.26	
Median	0.11	0.08	0.11	=0.731
IQR	0.15	0.20	0.07	
tPSA				
Range	0.13–1.74	0.04–2.47	0.17–3.5	
Median	0.86	0.88	0.73	<0.833
IQR	0.69	0.95	0.59	
f/t ratio				
Range	0.01–0.36	0.01–0.34	0.01–0.29	
Median	0.18	0.17	0.15	=0.442
IQR	0.09	0.19	0.03	

IQR = Interquartile range.
[a] Kruskal-Wallis-one-way ANOVA test.

groups for salivary fPSA and tPSA levels and salivary f/t ratios.

Comparisons of the mean serum and salivary levels of fPSA and tPSA and f/t ratios in each group are also given in table 2. Only the difference between mean serum and salivary f/t ratios in normal subjects was not statistically significant (p = 0.434). For the total of 76 men a significant correlation was found between fPSA and tPSA levels in each sample (r = 0.97 for serum and r = 0.44 for saliva, $p < 0.001$) (fig. 1). In the BPH group, there was a significant correlation between patient age and serum PSA levels (r = 0.41 for fPSA and r = 0.43 for tPSA, $p < 0.05$), and between fPSA and tPSA levels in each sample (r = 0.69, $p < 0.001$ for serum and r = 0.44, $p < 0.05$ for saliva). A significant correlation was also found between fPSA and tPSA levels in each sample in controls (r = 0.83 for serum and r = 0.77 for saliva, $p < 0.001$) and in patients with prostate cancer (r = 0.98, $p < 0.001$ for serum and r = 0.48, $p < 0.05$ for saliva). In controls there was a correlation between patient age and serum PSA levels (r = 0.43 for fPSA and r = 0.44 for tPSA, $p < 0.05$), and in the prostate cancer group we found a correlation between patient age and serum fPSA levels (r = 0.40, $p < 0.05$).

No correlation was found between serum and salivary PSA levels, and between patient age and salivary PSA levels in normal subjects or in patients with BPH and prostate cancer ($p > 0.05$).

Table 2. Statistical analysis comparing the means ± SD of serum and salivary PSA levels (ng/ml) and f/t ratios in each group

Group	fPSA			tPSA			f/t ratio		
	serum	saliva	p[a]	serum	saliva	p[a]	serum	saliva	p[a]
Control (n = 25)	0.28±0.21	0.13±0.11	<0.01	1.49±0.83	0.84±0.44	<0.01	0.18±0.06	0.16±0.08	0.434
BPH (n = 35)	0.86±1.08	0.16±0.22	<0.01	2.51±2.65	0.92±0.59	<0.01	0.39±0.22	0.17±0.07	<0.01
PC (n = 16)	5.72±5.01	0.11±0.07	<0.01	50.6±50.2	0.91±0.81	<0.01	0.12±0.04	0.15±0.06	<0.05

PC = Prostate cancer.
[a] Wilcoxon signed-rank test.

Discussion

PSA, a single-chain 33-kD glycoprotein serine protease, is widely used as a clinical marker of prostate cancer [19]. The gene responsible for PSA expression is a member of the human kallikrein (hK) gene family mapped to the long arm of chromosome 19. Serum PSA exists in different molecular forms. Serum PSA is predominantly bound to α_1-antichymotrypsin and also binds to α_1-antitrypsin and α_2-macroglobulin in a smaller proportion. Approximately 10–30% of tPSA is not bound to proteins and is called fPSA [20].

Serum PSA levels are routinely used to monitor patients with prostate cancer and facilitate the diagnosis. However, several recent reports have described elevation in serum PSA in a variety of nonprostatic malignancies. These include adenocarcinoma of Skene's (paraurethral) gland [6], small cell carcinoma of unknown primary [7], renal cell carcinoma [8], adenocarcinoma of the lung [9] and salivary duct carcinoma [17]. The immunohistochemical studies also demonstrated a consistently positive reaction for PSA, independent of patient sex, in ductal cells of normal salivary glands and salivary gland tumors using a polyclonal and monoclonal assay [11, 18].

Biochemical detection of PSA in other body fluids such as urine [4, 5, 21] and in selective blood samples [22] has provided much more information about the metabolism and dissociation of PSA. These investigations concluded that PSA is not secreted in the urine, is not dialyzable, is also produced by the periurethral glands, and the liver has a significant role in the elimination of tPSA, fPSA and complexed PSA, and the kidneys have a significant role only in the elimination of fPSA. Recently it was shown that PSA production by the periurethral glands is under hormonal control [23].

Although PSA was initially undetectable in the saliva [24], Breul et al. [25] recently reported a high concentration in the saliva independent of the clinical condition and serum level using a commercial monoclonal antibody-based immunoassay. Although the function of PSA in salivary glands is not clear, it can be involved in regulating insulin-like growth factor binding proteins, and insulin-like growth factor, and in proteolysis or digestion-related functions [18, 26]. The latter case may explain the reported high concentration in saliva independent of serum level [25]. Also the pH level of the sample is important and it is well known that higher urinary pH gave higher PSA results [27].

Recently, expression of PSA and α-fetoprotein (AFP) genes in several tissues and detection of mRNAs in normal circulating blood by reverse transcriptase-polymerase chain reaction (RT-PCR) were investigated by Ishikawa et al. [28]. In their study PSA was expressed in prostate, salivary gland, pancreas and uterus. As a conclusion they stated that neither PSA nor AFP showed tissue-specific expression, and PSA mRNA was detected in several diseased and nondiseased tissues and normal circulating blood by RT-PCR.

In our study, there was a significant correlation between fPSA and tPSA levels in saliva ($r = 0.44$, $p < 0.001$) for the total of 76 men (fig. 1) and no significant difference was determined between the groups for salivary fPSA and tPSA levels and salivary f/t ratios (table 1). Also, the differences between mean serum and salivary levels of fPSA and tPSA and f/t ratios in each group were statistically significant except mean serum and salivary f/t ratios in normal subjects (table 2). These findings conclude that salivary fPSA and tPSA are not affected by the high serum levels and are present in the saliva in an ordered level. We also found no correlation between patient age and salivary PSA levels.

One can make a hypothesis that salivary PSA levels may be used to detect salivary gland tumors which can produce PSA and increase the serum levels of PSA [17] and for the differential diagnosis of elevated serum PSA levels of extraprostatic origin. However, further studies are needed to prove this hypothesis, including patients with benign and malignant salivary gland tumors which are very rare.

We found the f/t ratio in the saliva as being 0.16 in normal subjects, 0.17 in the BPH and 0.15 in the prostate cancer group, and the differences were not statistically significant. These ratios are also very close to the serum f/t ratio of the normal population defined as 0.18 ± 0.06 in our study (table 2).

Conclusion

We showed that fPSA and tPSA are detectable in the saliva and the f/t ratio of saliva was very close to the serum ratio of normal subjects. According to our study, fasting salivary PSA levels detected by microparticle enzyme immunoassay technology are not affected by high serum levels of prostatic origin. Determination of fPSA and tPSA in saliva to improve and simplify the differentiation between prostate cancer and BPH is not suitable for use as alternative measurement of serum.

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