Ratios of Free to Total Prostate-Specific Antigen and Total Prostate-Specific Antigen to Protein Concentrations in Saliva and Serum of Healthy Men

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Introduction: We evaluated the ratio of free to total prostate-specific antigen (PSA) and PSA to protein concentrations in saliva and serum of healthy men.
Materials and Methods: Concentrations of protein, free PSA, and total PSA in serum and saliva were measured in 30 healthy men aged 42 to 73 years, and their ratios were compared between the two fluids.
Results: There was a significant direct correlation between serum free-total PSA ratios of serum and saliva ($P = .04$) and between total PSA-protein ratios of serum and saliva ($P = .02$). Also, there were significant correlations between total and free PSA levels in saliva ($P = .05$) and between those in serum ($P < .001$). Significant inverse and direct correlations were detected between the body mass index and serum values of total PSA-protein ($P = .04$) and free-total PSA ($P = .01$), respectively.
Conclusion: We can use saliva sample instead of serum sample for estimation of free-total PSA and total PSA-protein levels in men without prostate diseases. There is, however, a pressing need for much additional research in this area before the true clinical value of saliva as a diagnostic fluid can be determined.

Keywords: saliva, serum, prostate-specific antigen

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INTRODUCTION

Saliva has been considered as a diagnostic medium during the past 15 years. Thanks to the current developments in technology, the use of saliva as a diagnostic fluid is now possible in practice. For example, the ability of measuring a wide spectrum of molecular components in saliva and comparing them with serum has made it possible to study chemicals and immunologic markers.[1] The major advantages of using saliva instead of blood are its ease of access and noninvasive collection method.[1-4]

One of the markers that can be measured in saliva is prostate-specific antigen (PSA). Given its high specificity, PSA is known as a useful tumor marker for prostate cancer.[5] It is a 33-kd glycoprotein secreted by the prostate.[6] Values of the total PSA, free PSA, and PSA-alpha-1-antichymotrypsin (PSA-ACT) are independent prognostic factors of survival in patients with prostate cancer.[6] However, several reports have described elevation in serum PSA levels in a variety of nonprostatic malignancies.[7] Moreover, it has recently become widely accepted that PSA is also present in many nonprostatic sources, casting doubts about the specificity of its tissues expression.[8]

Immunoreactivity for PSA in salivary gland neoplasms has also been reported.[9] In addition, a consistently positive reaction for PSA and prostate acid phosphatase, independent of sex, in the ductal cells of normal salivary glands has been demonstrated using monoclonal antibody assay.[10] It can be concluded that PSA might also be secreted by salivary glands, and therefore, it is important to assess extraprostatic sources of PSA to avoid errors in diagnosis and management.[11] To emphasize this issue, it should be noted that PSA has also been found in human endometrium and amniotic fluid albeit its significance is unclear.[12,13]

Based on these findings, we evaluated free and total PSA levels and the free-total ratio of PSA in the fasting saliva and serum of the healthy men. Also, we investigated the ratios of serum total PSA to serum protein and salivary total PSA to salivary protein in healthy individuals using radioimmunoassay technology.

MATERIALS AND METHODS

Serum and saliva concentrations of free and total PSA were measured in 30 men aged between 42 and 73 years who presented for routine check-up. All of the participants underwent physical examination, chest radiography, electrocardiography, routine biochemical analyses of blood, urine analysis, and urine culture, and they had no evidence of any diseases. All of them were confirmed to have a normal prostate by digital rectal exam and ultrasonographic evaluations performed 1 month earlier in their prostate examination. The participants were informed of the purpose of the study and provided written consent. The study was approved by the medical ethics committee of Mashhad University of Medical Sciences.

One month after recruitment of the volunteers, we obtained serum and saliva samples after 12 hours of fasting between 8 AM and 9 AM. Each participant rinsed his mouth with water for 5 minutes and then 5 mL of saliva was collected. Samples of blood and saliva were centrifuged at 2000 g for 10 minutes. Bubbles in the samples were removed prior to analysis.

Free and total PSA concentrations in serum and saliva samples were measured on the same day at our laboratory with radioimmunoassay technique (Kavoshyar, Tehran, Iran). Interassay and intra-assay coefficients of variation for serum total PSA were 5.6% and 4.1%, respectively. The PSA levels were shown as ng/mL and the lowest measurable concentration that could be distinguished was 0.01 ng/mL for free PSA and 0.02 ng/mL for total PSA. The precision of measurement method for total PSA in saliva was determined according to the following protocol: 10 samples of saliva were assayed in 2 replicates at 2 separate times per day for 20 days and interassay and intra-assay coefficients of variation were 6.2% and 6.5%, respectively.

Serum and saliva protein levels were measured by Biuret method (Boehringer Mannheim GmbH, Diagnostica, Germany) using Technicon RA-1000 auto-analyzer and Pyrogallol red method (Chem Enzyme, Iran) using spectrophotometric assay, respectively.

Data analyses were done using the paired sample *t* test to investigate the differences in serum and saliva PSA levels and Pearson correlation test to evaluate correlations. A *P* value less than .05 was considered significant.

RESULTS

The free and total PSA values in serum and saliva samples are shown in the Table. There was a significant difference between serum and saliva total PSA levels (0.75 ± 0.46 ng/mL versus 0.11 ± 0.06 ng/mL; $P = .03$). Also, such a difference was noted between serum and saliva free PSA levels (0.16 ± 0.10 ng/mL versus 0.03 ± 0.02 ng/mL; $P = .04$). No correlation was found between serum and salivary

Demographic Data and Values of Prostate-Specific Antigen (PSA) and Protein in Serum and Saliva of Healthy Men

Parameter	Mean (Range)
Age, y	56.6 ± 9.1 (42 to 73)
Body mass index, kg/m^2	25.70 ± 4.62 (19.7 to 41.4)
Total PSA, ng/mL	
Serum	0.75 ± 0.46 (0.4 to 2.5)
Salivary	0.11 ± 0.06 (0.05 to 0.30)
Free PSA, ng/mL	
Serum	0.16 ± 0.10 (0.02 to 0.50)
Salivary	0.03 ± 0.02 (0.01 to 0.10)
Protein, g/dL	
Serum	6.90 ± 0.35 (6.20 to 7.70)
Salivary	0.08 ± 0.01 (0.06 to 0.11)

levels of the total or free PSA in the participants.

Not surprisingly, there is a direct correlation between total PSA and free PSA levels in saliva, and also between total PSA and free PSA levels in serum (r = 0.45, *P* = .04 and r = 0.56, *P* < .001, respectively). Furthermore, a significant correlation was found between free-total PSA ratios in serum and saliva (r = 0.26, *P* = .04) and also between serum total PSA-protein ratios in serum and salivary (r = 0.30, *P* = .02). Finally, body mass index (BMI) inversely correlated with serum total PSA-protein ratio (r = -0.38, *P* = .04) and serum free-total PSA (r = 0.45, *P* = .01).

DISCUSSION

Prostate-specific antigen, a single-chain 33-kd glycoprotein serine protease, is widely used as a clinical marker of prostate cancer.[6] The gene responsible for its expression is a member of the human kallikrein (*HK*) gene family on the long arm of chromosome 19. Serum PSA exists in different molecular forms and mainly binds to antitrypsin and α-2- macroglobulin in a smaller proportion. Approximately, 10% to 30% of total PSA is not bound to protein and is called free PSA.[5]

Although the function of PSA in the salivary glands is not clear, it can be involved in regulating insulin-like growth factor binding protein and insulin-like growth factors. Also, it may play a role in proteolysis or digestion-related function.[10] The concentration of salivary PSA is very low (less than 0.03 ng/mL to 0.34 ng/mL).[12,14] In a study by Mannello and colleagues on a group of 40 female volunteers, half of whom were taking contraceptives, the group taking the contraceptive exhibited a higher concentration of PSA in saliva (mean, 0.099 ± 0.016 ng/mL; range, 0.04 ng/mL to 0.34 ng/mL). In the control group, the mean value was 0.048 ± 0.007 ng/mL (range, 0.02 ng/mL to 0.15 ng/mL). However, PSA concentration in serum did not differ between the two groups.[14] Aksoy and associates determined changes in the PSA concentration in serum and saliva over the menstrual cycle. The highest values were found on day 9 (follicular phase) and day 14 (midcycle) with values of 0.024 ± 0.011 ng/mL and 0.029 ± 0.013 ng/mL, respectively. No cases of PSA concentration higher than 0.06 ng/mL was found in this study.[8]

A study by Breul and coworkers did not show the low PSA concentration in saliva.[15] In their study, extremely high concentrations of PSA were found in the saliva of 165 patients (20 women, 39 patients with benign prostatic hyperplasia, 24 with localized prostate carcinoma, 17 with metastasizing prostate carcinoma, 14 with a history of transurethral manipulation, and 51 with other urological diseases) with the values ranging between 129 ng/mL and 688 ng/mL. These values did not correlate with the serum concentrations, and so far, we cannot explain what caused these high values. It may be due to the hybritech assay showing a cross-reactivity with another protein in saliva. However, it is felt that these high values are somehow doubtful.

In our study, we selected the healthy men, just for evaluation of correlations between free and total PSA levels and free-total PSA ratio between serum and saliva in their normal ranges as a pilot study. Although a positive correlation was found between serum and saliva PSA in one study,[8] Turan and colleagues found that there was neither a correlation between serum and salivary PSA levels nor between the patient's age and level of PSA in saliva.[11] In our study, there was a significant difference between serum PSA and salivary PSA, or beween their free portion in the fluids. We also found that there was no correlation between serum and salivary total PSA or free PSA in healthy men, but a significant correlation was found in the ratio of free to total PSA between serum and saliva.

In order to eliminate the effect of protein concentrations in serum and saliva, we used the total PSA-protein ratio in these two fluids. To our best knowledge, our experience is the first report in the literature that includes the ratio of PSA to protein concentration in saliva. A significant correlation was found between serum total PSA-protein and salivary total PSA-protein ratios (*P* = .02). It can be concluded that free-total PSA or total PSA-protein ratios in saliva may be used to predict the same ratios in serum of healthy people, but due to another significant inverse correlation found between BMI and serum total PSA-protein ratio and a positive correlation between BMI and serum free-total ratio, the discussed correlation between free-total PSA ratios in serum and saliva should be analyzed with attention to BMI.

CONCLUSION

We concluded that fasting salivary ratio of free to total PSA levels detected by radioimmunoassay technology may be useful for predicting the same ratio in serum of healthy men. Also, there was a significant correlation between serum total PSA-protein ratios in saliva and serum of men with a normal prostate. Further studies are needed to draw final conclusion.

CONFLICT OF INTEREST

None declared.

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