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Spontaneous Circadian Fluctuations of Prostate Specific Antigen and Prostatic Acid Phosphatase Serum Activities in Patients with Prostatic Cancer*

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Summary. Spontaneous circadian variations of prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), determined simultaneously by radioimmunoassay (RIA), were investigated by multiple sampling, over a 24-hour period, in 32 patients with prostatic cancer. In 29/32 patients (91%), the coefficient of variation of 24-hour values, for either marker, was greater than that of the RIA method at the same range of values; stage D patients showed the greatest spontaneous variability. Fluctuations around the mean of 24-hour values ranged from −65% to +85% for PAP, from −72% to +190% for PSA, occurring random and independently for each marker. Variability was about 20% greater for PSA than for PAP. The existence of spontaneous fluctuations should be considered in multiple marker evaluation of prostatic cancer patients.

Key words: Prostate specific antigen – Prostatic acid phosphatase – Circadian fluctuations – Spontaneous variability – Prostatic cancer

Introduction

Prostatic acid phosphatase (PAP) and prostate specific antigen (PSA) are two tissue-specific proteins with different biochemical, immunological and functional properties [7, 13, 14]. Recently, their association has been claimed to improve the accuracy of single-marker determination in the diagnosis and follow-up of prostatic cancer patients [7, 10].

It is well known that false-positive elevations of serum PAP are possible in patients with benign prostatic hypertrophy (BPH), due to urinary retention and/or various manipulations of the prostate gland [5, 12].

In a previous paper, we have reported about similar false-positive elevations of serum PSA [1].

Moreover, in patients with prostatic carcinoma, the relationship existing between clinical response to treatment and PAP serum activity is not always clear [4].

It has also been shown that serum levels of prostatic phosphatase have spontaneous circadian fluctuations, both in normal individuals and in patients with prostatic cancer [2, 6, 8, 9].

There were no published reports about spontaneous fluctuation in serum activity of PSA. In this study we describe the spontaneous variation of both markers, observed over a 24-hour period, which are important in the evaluation of patients with prostatic cancer.

Materials and Methods

Patients and Controls

32 patients with prostatic cancer were studied (age range: 55–81, mean 72). They were classified according to Whitmore [15]: 2 were stage A, 1 stage B, 16 stage C and 13 stage D. Tumors were graded according to Broders [3]: they were adenocarcinomas, well differentiated in 6 cases (G2), poorly differentiated in 26 (G3-G4). 12 patients were under treatment with androgen suppression during this study: 7 were clinically stable or in remission, 5 had progressive disease. All patients underwent determination of serum levels of testosterone and LH. As controls we evaluated 6 age-matched patients with BPH, and 3 male volunteers under 40 with no signs of prostatic disease. 13/32 cancer patients, and none of the controls, had an indwelling urethral catheter at the moment of the study.

Sample Collection and Timing

After the informed consent from the patients, blood samples were obtained by direct venipuncture or using an indwelling heparin-locked plastic catheter, depending of the number of samples to be collected. In every instance, 6 cc of blood were drawn (3 cc to ensure clearance of the heparinized solution from the tubing, plus 3

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Fig. 1. Multiple sampling for PAP and PSA determination in 13 subjects: 12–18 samples were obtained in a 24-hour interval (mean: 14)

Table 1. Intra-assay variability of the RIA method (Pooled sera, 10 determinations)

PAP		CV	PSA	CV
1)	0.7 ng/ml	16%	1.7 ng/ml	12.5%
2)	5.2 ng/ml	11%	11.4 ng/nl	10%
3)	18.7 ng/ml	13.5%	26 ng/ml	16.3%

Table 2. Intra-assay variability of th RIA method (Commercial sera, 24 consecutive sessions)

PAP		CV	PSA	CV
1)	1.92 ng/ml	14.5%	1.5 ng/ml	19.6%
2)	4.02 ng/ml	16.3%	3.3 ng/ml	12.3%
3)	11.9 ng/ml	14.1%	11.4 ng/ml	14%

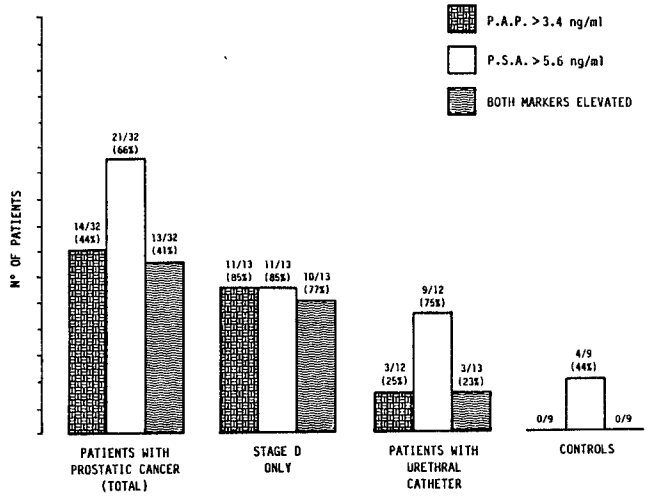

Fig. 2. Basal values at 8 a.m.

cc for determination). Serum was immediately separated from clot and stored at −20 °C until PAP and PSA were measured. Samples were collected at 8 a.m., 4 p.m., midnight and 8 a.m. in 28 subjects. 13 subjects (11 with cancer and 2 with PBH) underwent multiple sampling over the 24-hour period, at predetermined time intervals (Fig. 1): 12–18 samples were obtained (mean: 14). One patient, was studied incompletely (8 a.m- noon).

Rectal examination, endoscopic procedures and other possible sources of error were avoided, 48 hours before and during the period of study.

The impact of the urethral catheter (when present) on 24-hour variability was evaluated separately.

Radioimmunoassay (RIA)

PAP and PSA were determined simultaneously by liquid-phase radioimmunoassay, with overnight incubation at room temperature, utilizing two commercially available kits (PAP-Ter, Biodata and PSA-Double Antibody, Diagnostic Products Corporation). Minimal detectable values were, respectively, 0.4 ng/ml (PAP) and 0.6 ng/ml (PSA). As the upper limits of the normal range we used the mean +3 standard deviations (SD) of the values observed at 8 a.m. in 51 healthy males under 40, yielding 3.4 ng/ml for PAP and 5.6 ng/ml for PSA. Intra- and interassay variabilities of the methods are shown in Tables 1 and 2.

Criteria employed to verify the accuracy of the methods have been described in detail elsewhere [11].

Statistical methods

Statistical analysis included: calculation of the mean, range, SD, coefficient of variation (CV) and variance of the observed 24-hour values for each patient, and, for the group sampled at 8-hour intervals, a comparison of the longitudinal distribution of values determined at different times. Analysis of variance within the latter group was evaluated by non-parametric tests (Kruskal-Wallis test and Mann-Whitney test). Coefficients of variation observed were compared with intra- and inter-assay variabilities of the two RIA methods at the same ranges of values.

Results

Basal Values (8 a.m.)

Figure 2 summarizes basal values observed at 8 a.m. in our series of patients and controls. 23/32 patients with cancer (72%) and 2/6 controls (33%) had PAP or PSA (controls: PSA only) above the upper normal limits. The only statistically significant result shown by the analysis of variance was the greater dispersion of PAP values in metastatic versus non-metastatic patients ($0.01 < P < 0.05$).

Although 66% of patients with carcinoma had abnormal PSA levels, the distribution of values showed no significant difference between stage D and non-metastatic subjects.

24-Hour Variability

Figure 3 summarizes the variability of values over a 24-hour time interval. In 29/32 cancer patients (91%) the coefficient of variation of 24-hour values was greater than CV of the RIA method, at that range of serum levels, for either marker. Controls showed similar results, but values of PAP varied within the range of normality in all cases. Mean CV of both markers are shown in Table 3. No significant difference in circadian variability was seen, between previously castrated patients and patients with intact testes, whose serum levels of testosterone and LH were within the range of normality in all cases.

In some instances, we observed false-negative and false-positive results which could be attributed to spontaneous

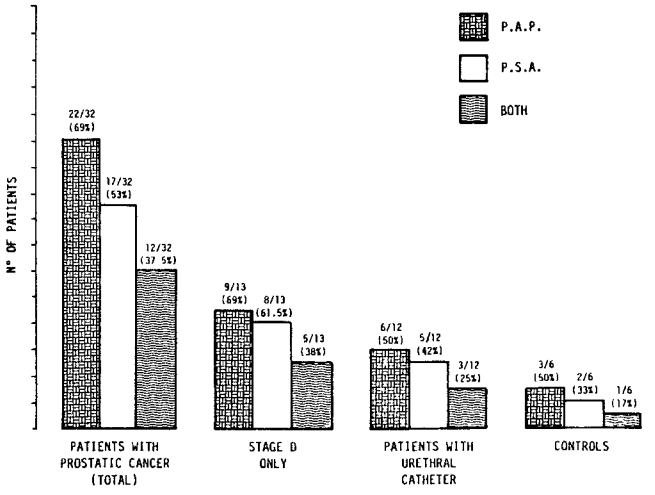

Fig. 3. Spontaneous variability of PAP and PSA serum activities: figures in the diagram refer to patients showing CV of the 24-hour values greater than CV of the RIA methods at the same range of values

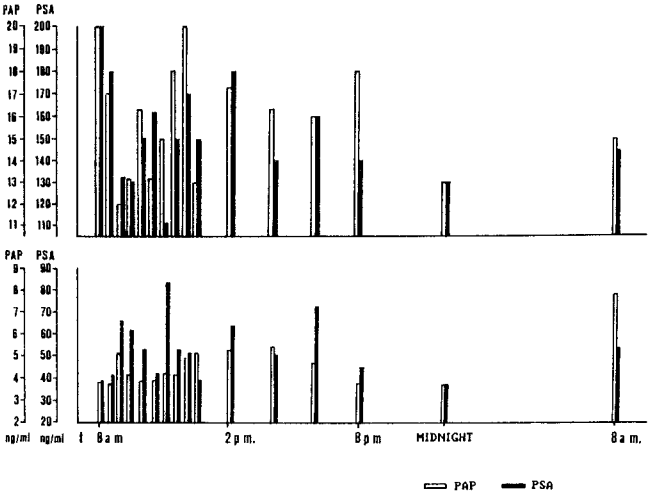

Fig. 5. Different 24-hour fluctuations in two patients with poorly differentiated stage D2 prostatic cancer

Table 3. Mean coefficients of variation of the 24-hour values of serum PAP and PSA

Mean CV PAP (32 ca.)	=	20.1%
Mean CV PSA (32 ca.)	=	21.5%
Mean CV PAP (stage D only)	=	18.1%
Mean CV PSA (stage D only)	=	26.1%

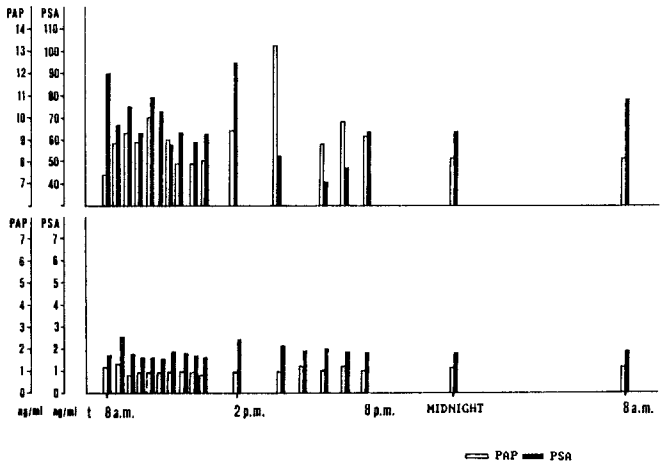

Fig. 4. Different 24-hour behavior of PAP and PSA serum activities in two patients with surgical stage D1, G3, prostatic cancer. Note the different scales of values, and the independent variations of the two markers

fluctuations: among cancer patients with normal basal PAP, 1/8 (12.5%) showed at least one abnormal value over the 24-hour interval (1/9, 11%, for PSA). Similarly, in 2/6 patients with BPH (33%) serum values of PSA were above the upper cut-off limit at 8 a.m., being normal throughout the subsequent 24-hour interval. On the other hand, 3/14 with elevated basal PAP (21%) and 3/21 with elevated PSA (14%) showed at least one normal value over the same period. Fluctuations around the mean of 24-hour determinations ranged from –65% to +85% for PAP, from –72% to +190% for PSA: they appeared to be random, without any predictable behavior, and occurred independently for each marker.

Figs. 4 and 5 show the 24-hour variations of PAP and PSA in two couples of patients with tumors comparable by stage and grade.

Discussion

Doe and Mellinger [6] first showed, in 1964, that prostatic phosphatase levels may vary spontaneously over a 24-hour interval. In 5 cancer patients, they found the highest values between 9 a.m. and 3 p.m. and the lowest from 9 p.m. to 3 a.m.

A subsequent study by Brenckman et al. [2] of 5 patients with prostatic carcinoma confirmed the existence of spontaneous fluctuations, but did not show any circadian rythm of PAP serum levels, which were determined by an enzymatic method.

Nissenkorn et al. [9] and Maatman et al. [8], utilizing RIA methods for determination of PAP in 10 patients with prostatic cancer, came to similar conclusions.

Although these studies included a limited number of cases, they pointed out the inaccuracy of single determinations of serum PAP as a parameter of response to treatment in these patients.

Since multiple marker evaluation of prostatic cancer is made possible by the existence of prostate specific antigen, it was important to discover whether serum activity of this marker showed similar spontaneous fluctuations.

In this study we observed, for both markers, spontaneous 24-hour variations of serum values that could not be explained by the variability of the radioimmunoassays employed. As could be expected, variations were greatest in stage D patients, who most often showed elevated basal values. On an average, spontaneous variability of PSA (as evaluated by CV) was about 20% greater than variability of PAP, although their behavior in the individual patient were unpredictable and never coincidental. The latter finding is not surprising, since PAP and PSA probably enter the blood stream independently, and it confirms that elevations observed were not due to factors interfering from outside, this is further emphasized by the fact that the presence of an indwelling urethral catheter (which may, theoretically, cause false elevations by compressive and/or inflammatory stimuli) was not related to significantly greater 24-hour variability in this series of patients.

Obviously, the significance of spontaneous fluctuation depends on the level of values observed: it is limited when the mean values are close to the lowest detectable level, and when values are all within the normal range, or when they are extremely high. However, fluctuations around the upper limits of normality do occur, and reach, in our experience, a maximum peak of +190%: these fluctuations must be kept in mind after or during treatment of patients with prostatic carcinoma, when attempting correlations between clinical response and PAP or PSA serum activity, particularly if the latter is evaluated by single (usually: 8 a.m.) determinations.

From this point of view, multiple marker evaluation of prostatic cancer can be misleading unless great caution is used in interpreting single variations of each marker during follow-up, especially if they are not in agreement with the clinical findings.

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