ONCOLOGY

Prostatic Specific Antigen in Prostatic Cancer and Benign Hyperplasia

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Total and free prostatic specific antigens were measured using heterogeneous two-step streptavidin enzyme immunoassay. Serum concentrations of both antigens were significantly increased in patients with prostatic cancer in comparison with those with benign hyperplasia and controls. The levels of prostatic specific antigen in patients with benign prostatic hyperplasia varied within the normal range (75%), while in 92.2% of patients with cancer these values were higher than the threshold value of 4.0 ng/ml. A statistically significant difference between the levels of total and free prostatic specific antigens reflects the difference in the expression of bound and free forms of the antigen in malignant and benign processes. Analysis of the concentrations of total antigen and the ratio of free to total prostatic specific antigen permits an accurate differentiation between cancer and benign hyperplasia of the prostate at total antigen concentrations of up to 10.0 ng/ml.

Key Words: *prostatic cancer; benign hyperplasia of the prostate; prostatic specific antigen*

Prostatic cancer is an important problem of modern oncology because of constantly increasing morbidity and mortality of elderly and senile men and difficulties in early diagnosis and inefficiency of treatment.

Prostate specific antigen (PSA) is a universally acknowledged tumor marker widely used for the diagnosis and monitoring of prostatic cancer [1,2,8]. Utilization of PSA for screening and early diagnosis of the disease is hampered by its low diagnostic specificity, which is explained by increased serum concentration of this marker in patients with benign diseases of the prostate and in healthy elderly and senile men [3,7]. The problem of differential diagnosis of benign hyperplasia (BH) and cancer of the prostate could not be solved without improving the specificity of the method for assaying PSA as a tumor marker. This became possible after discovery of heterogeneity of the antigen circulating in the blood [4-6].

PSA is a glycoprotein with a molecular weight of 30-33 kD and proteolytic activity; it is produced by epitheliocytes of normal, hyperplastic, and malignant tissues of the prostate [1,4,10]. When released into the blood, PSA binds circulating proteinase inhibitors, after which it is detected in the serum within different bound molecular forms possessing no specific enzymatic activity, mainly in complex with α_1-antichymotrypsin and α_2-macroglobulin. Since the first identification of the antigen in 1979 [10] the attention of scientists was drawn to the immunoactive form of PSA: α_1-antichymotrypsin (about 90 kD). It became possible to measure this form virtually immediately after the antigen had been isolated, in contrast to the macromolecular complex of PSA with α_2-macroglobulin (about 720 kD); it was impossible to obtain monoclonal antibodies to it. Further studies showed that, despite an excess of proteinase inhibitors, a free form of the antigen not

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bound to inhibitors always circulates in the serum. In addition, it was revealed that the concentrations of different forms of PSA and their percent ratios vary in different prostatic diseases. Several years ago the free form of PSA (free PSA) was measured by the double-antibody enzyme immunoassay [5,9]. Recent publications indicate that the use of free to total PSA ratio markedly improves the specificity of PSA as a tumor marker in differential diagnosis of prostatic cancer and BH [4,5].

We assessed the clinical significance of serum concentrations of total and free PSA in prostatic cancer and BH.

MATERIALS AND METHODS

Sixty-eight patients with prostatic cancer aged 46-76 years and 24 with prostatic BH aged 36-78 years, in whom the disease was diagnosed for the first time, were examined. The patients were not treated before PSA measurements. Control group consisted of 14 healthy men and patients with prostatic inflammations aged over 40 years. The concentrations of total and free PSA in the serum were measured by heterogeneous two-stem enzyme immunoassay (Enzymun-Test PSA, Boehringer Mannheim) based on the streptavidin technology. The concentrations of two PSA fractions were measured with a Boehringer Mannheim ES300 automated enzyme immunoassay analyzer. In accordance with the recommendations of the manufacturer, PSA concentration of 4.0 ng/ml was taken as the threshold level for total PSA and 0.15 as the threshold value for the free to total PSA ratio.

RESULTS

Comparison of serum PSA levels in patients with prostatic cancer showed statistically significant increase in both total and free PSA compared with BH patients and controls (Table 1). The mean values of total PSA in cancer patients were 113 times higher than in controls and 21.4 times higher than in patients with BH. The mean PSA level in patients with prostatic BH was 5.3 times higher than in the control, but this difference was insignificant ($p>0.05$) because of high variability of the parameter (0.30-51.0 ng/ml). In the controls variability was lower (0.42-5.16 ng/ml). In only one patient the concentration of total PSA surpassed the threshold value of 4 ng/ml. The maximum antigen concentrations (51.0 and 5.16 ng/ml, respectively) were observed both in patients with BH and in controls aged over 70 years; these high levels may reflect the severity of hyperplastic processes and age-specific changes in the prostate.

Analysis of serum concentrations of free PSA in comparison with total antigen showed that the increase in free PSA in cancer patients was smaller (51.9 and 16.6 times, respectively) in comparison with the controls and BH patients, which probably reflects the difference in the expression of free and bound antigens in malignant and benign processes. This is confirmed by statistically significant correlation between total and free PSA. The correlation coefficients in cancer and BH were 0.79 ($p<0.05$) and 0.89 ($p<0.01$), respectively. In the controls this correlation was weak ($r=0.61$, $p<0.05$), which was apparently due to the virtually independent expression of free and bound PSA in health. On the other hand, strong correlation in patients with prostatic tumors confirms that the different forms of the antigen are related to each other. This relationship was expressed as the predominant binding of PSA to proteinase inhibitors in prostatic cancer (up to 88% of the total concentration), while in patients with BH free antigen circulated in the sera in relatively high concentrations (up to 50%, Table 1).

Table 1 shows the high variability of PSA concentration in cancer and BH. Analysis of PSA distribution in accordance with the international criteria of the increase in the total PSA concentrations (Table 2) shows a characteristic regularity: serum PSA levels in patients with prostatic BH are mainly (75%) within the normal range, whereas in prostatic cancer they are higher than the threshold value (92.9%). At present, the so-called "indefinite" zone of total PSA concentrations (4-10 ng/ml) is the most difficult for differential diagnosis of prostatic cancer and BH. It was reported [2,3,7,8] that 15-55% of patients with

TABLE 1. Concentrations of Total and Free PSA in Prostatic Cancer and BH ($X \pm m$)

Parameter		Control (n=14)	Prostatic cancer (n=68)	Prostatic BH (n=24)
PSA, ng/ml				
	total	1.44±0.50 (0.42—5.16)*	162.91±34.48 (1.54—789.0)	7.62±5.44 (0.30—51.0)*
	free	0.31±0.04 (0.15—0.46)*	16.08±3.65 (0.39—96.90)	0.97±0.45 (0.11—2.49)*
	free/total	0.31±0.05 (0.08—0.54)*	0.12±0.01 (0.02—0.36)	0.30±0.08 (0.05—0.53)*

Note. Range of concentrations and ratios of free to total PSA are given in parentheses; *$p<0.001$ in comparison with prostatic cancer patients.

TABLE 2. Distribution of Serum Concentrations of Total PSA in Patients with Prostatic Cancer and BH and Controls

Groups	Number of cases	PSA concentrations, ng/ml				
		0-4	4.1-10	10.1-20	20.1-40	>40.1
Control	14	13 (92.9)	1 (7.1)	0	0	0
Prostatic BH	24	18 (75.0)	3 (12.5)	1 (4.2)	0	2 (8.3)
Prostatic cancer	68	4 (5.9)	6 (8.8)	10 (14.7)	6 (8.0)	42 (61.8)

Note. Percentage of cases is given in parentheses.

prostatic BH are in this zone. It was shown that 12.5% of such patients had total PSA levels of 4-10 ng/ml, which is abnormal. In addition, in 4 out of 68 examined patients with prostatic cancer the levels of total PSA were normal and in 8.8% of observations were 4-10 ng/ml. The mean total PSA values in this range virtually did not differ in the patients with prostatic cancer and BH (6.1 and 5.2 ng/ml, respectively). Analysis of the ratio of free to total PSA in these groups revealed significant differences. In cancer, the mean ratio (free to total PSA) was 0.13, which is significantly lower ($p<0.05$) than that in BH (0.29). Since the threshold value of this parameter is 0.15, it is obvious that measurements of total and free antigen followed by analysis of the concentration of total PSA and the free to total PSA ratio allows the use of this method for differential diagnosis of prostatic cancer and BH with a high accuracy, provided total PSA concentrations do not surpass 10 ng/ml.

Analysis of the distribution of PSA levels in prostatic cancer of different dissemination showed a significant relationship between serum PSA concentration and stage of tumor process (TNM classification of tumors, WHO, 1987). Table 3 shows that the levels of total and free PSA are significantly higher in patients with stage IV cancer than in those with locally disseminated forms (stages I-II) and stage III. The concentrations of PSA are presented in Table 3; the highest values of the marker are typical of patients with prostatic cancer metastases to remote organs. An increase in the PSA level in comparison with the threshold PSA value varied in these patients from 1.6 to 197 times. There were no significant differences between the mean values of total and free PSA in locally disseminated process and stage III cancer. The following distribution of the lowest values of total PSA is characteristic of patients with stages I-II prostatic cancer in comparison with stage III patients: decreased concentrations of the marker below threshold (4 ng/ml) in patients with the initial stages of malignant process in the prostate and its increased concentrations in all patients with stages III and IV. It is noteworthy that at the IV stage of cancer, relatively low PSA concentrations were observed in 3 cases, whereas in other 47 patients the levels of the marker were at least 15 ng/ml. In 19 (38%) patients of this group PSA levels surpassed 70 ng/ml and were rather high. Such levels were not observed in any patient with stage III, suggesting that dissemination of malignant process can be assessed by serum PSA level with a high degree of reliability; specifically, metastases of prostatic cancer to remote organs can be thus detected. Analysis of the levels of free PSA in complex with total PSA did not show any additional advantages of this parameter for assessing the dissemination of prostatic cancer. This is due to a small number of observations in analysis of the marker relative to the disease stage. There are no published reports about the use of free PSA for assessing the stage of prostatic cancer.

Our data indicate that differential diagnosis of prostatic cancer and BH can be markedly improved by measuring both total and free PSA levels. Our findings agree with published reports [4-6] and demonstrate that enzyme immunoassay of total and free PSA is an indispensable tool for the early diagnosis of prostatic cancer. Moreover, our results show the possibility of using serum PSA concentrations for defining the stage of tumor process in prostatic cancer.

TABLE 3. Concentrations of Total and Free PSA in Different Stages of Prostatic Cancer ($X \pm m$)

Parameter	Stages I-II (n=8)	Stage III (n=10)	Stage IV (n=50)
PSA, ng/ml			
total	20.57±8.66 (1.54—43.80)*	26.38±9.30 (4.60—54.10)*	213.03±42.72 (6.20—789.0)
free	2.03±1.14 (0.93—4.93)**	2.52±1.08 (1.20—4.74)*	20.33±4.36 (0.90—96.90)

Note. Range of PSA concentrations is given in parentheses; *$p<0.001$, **$p<0.01$ in comparison with stage IV.

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Relationship between Tobacco Smoke and Uterotropic Effect of Estrogen

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Female rats aged 3-3.5 months were daily exposed for 2 h to 2.25% tobacco smoke in a 20-liter chamber for 3 weeks. A week after the beginning of the experiments, all rats were subjected to bilateral oophorectomy. Some of them were injected with estradiol in a daily dose of 2 μg or diethylstilbestrol in a daily dose of 1 μg for 10 days. Tobacco smoke exposure led to an increase in the uterus weight in oophorectomized rats, and combined exposure to tobacco smoke and estrogens resulted in an increase in the proportion of intact (twisted) DNA in the uterus. These results can be explained by a relatively short term of tobacco smoke exposure (stimulation stage).

Key Words: *estrogens; uterus; DNA; tobacco smoke*

Tobacco smoke is an environmental factor which affects both the endocrine system and increases the incidence of some malignant tumors. In addition to the risk of cancer, females with a many-year history of tobacco smoking earlier develop the menopause and intense postmenopausal osteoporosis; the incidence of endometriosis, fibromyoma, and cancer of the corpus uteri is decreased in smoking women [1,3]. These signs of estrogen deficiency are not supported by data on blood estrogen levels, which are, as a rule, normal in the female tobacco smokers [6]. There are reports about increased 2-hydroxylation of estrogens in intense tobacco smoking [9]. On the other hand, accumulation of 2-hydroxyestrogens prevents inactivation (methoxylation) of 4-hydroxyestradiol and 4-hydroxyestrone, which increases the total genotoxic (DNA-damaging) potential of estrogens [8]. Clinical manifestations of estrogen insufficiency in smoking women, the spectrum of tumors they develop, and the important role of estrogen genotoxicity in the induction of neoplastic transformation [8] led us to a hypothesis that tobacco smoke attenuates the specific hormonal effects of estrogens and increases the estrogen capacity to damage the DNA [1,4]. In this study we tested this hypothesis, which is important for analysis of the mechanisms of hormonal carcinogenesis, and other estrogen-dependent processes [1,4].

MATERIALS AND METHODS

Experiments were carried out on 3-3.5-month female rats from the *Rappolovo* breeding center. At the be-

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