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Research Paper

Analysis of results related to the percent free prostate specific antigen among men without prostate diseases in Xi'an area

Peng Zhang[a], Ziming Wang[a], Tie Chong[a], Lihua Zhao[b]

[a]*Department of Urology, the Second Affiliated Hospital of Medical College of Xi'an JiaoTong University, Xi'an 710004, China*
[b]*Department of clinical laboratory, the Second Affiliated Hospital of Medical College of Xi'an JiaoTong University, Xi'an 710004, China*

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Abstract

Objective: To measure the percent of free prostate specific antigen (fPSA) among men without prostate diseases in Xi'an area, and to study the relationship of percent fPSA with age and pathological grade, clinical stage of prostate cancer (PCa) with percent fPSA, and to analyze the difference between the data in China and the overseas data to determine appropriate reference range for Chinese male. **Methods**: A total of 713 participants were enrolled into the study, with PSA, fPSA in serum measured and the percent fPSA calculated. Out of 713 cases, 679 without prostate diseases were divided into 5 groups by age, and then the relationships of PSA, fPSA and percent fPSA with age were studied, respectively. The relationship of pathological grade and clinical stage with percent fPSA of the 34 participants with PCa was also studied. With the help of the related data of men without prostate disease, the appropriate reference range for Chinese male was established. **Results**: The increases in PSA or fPSA were correlated with age, while there was no significant correlation between age and percent fPSA. The percent fPSA was also correlated with pathological grade and clinical stage of PCa. The percent fPSA of men without prostate disease in Xi'an area was significantly lower than that in the related overseas data. The reference range of percent fPSA for Chinese male was ≥15%. **Conclusion**: Percent fPSA might be more useful than PSA in the detection of prostate cancer. As the percent fPSA is decreased, the pathological grade is decreased, and the clinical stage is increased, the malignant degree is increased. The reference range of ≥ 15% is more appropriate for Chinese male.

Keywords: percentage free prostate specific antigen; prostate cancer; Age; relativity; reference range; Xi'an area

INTRODUCTION

Prostate specific antigen (PSA) is one of the most valuable tumor markers available for the diagnosis of prostate cancer (Pca). However, it lacks sufficient sensitivity and specificity due to age and volume of prostate. The conception of percent free PSA(fPSA) was put forward in recent years and has became more prevalent in research. Because the morbidity of PCa in our country is rather different from that in other countries, we studied the percent fPSA in 679 participants without prostate disease and 34 with PCa. The difference between our data and oversea data was analyzed. We also explored the effect of age on the percent fPSA among men without prostate diseases. We tried to establish the reference range which was appropriate for Chinese.

MATERIALS AND METHODS

Clinical materials

The medical data of 713 men were collected in Xi'an area from September 2001 to September 2005. Among them, 679 participants presented without prostate diseases, with mean age 67.7 ± 7.6 years (range 30-86 years). We divided them into 5 groups by age(30-39, 40-49, 50-59, 60-69, ≥ 70 years), and the number of participants for each group was 73, 58, 144, 252, 152 respectively. Individuals with any prostate diseases according to the international prostate symptom score (I-PSS), digital rectal examination (DRE) and ultrasound were excluded.

34 patients suffered from PCa, with mean age 69.5± 4.1 years(range 62-76 years). All of them were confirmed as adenocarcinoma by multiple spot needle biopsy or biopsy guided by DRE. The pathological stage for each patient was graded by the standard of Gleason score (1992). Among them, 6 presented with well differentiated tumor, 4 with moderately differentiated tumor and 24 with poorly differentiated tumor. The clinical stage was categorized by Jewett-Whitmore-Prout system, and among them, 3 was in A stage, 15 in B stage, 12 in C stage and 4 in D stage.

Measurement of serum PSA and fPSA

The samples of venous blood were collected before prostate operation or one week after operation, then the serum was separated. The concentrations of serum PSA and fPSA were measured by two-site "sandwich"enzyme linked immunosorbent assay. First, the samples (patients or standard) and either the dilutions (when fPSA was measured) or the buffers (when PSA was measured) were put into a tiny finestra plank, warmed for 1h and the plank was washed. Secondly, the antibody marked by enzyme was mixed in, again warmed for 1h and the plank was washed. Then, the TMB solution was mixed in, and later it was put in the terminate solution , and the data of absorbency(data A) was measured at the site of 450nm, when the color completely became yellow.

The linear regression equation of standard curve with absorbency and concentration of standard sample were calculated. The concentrations of PSA and fPSA were measured respectively. Both PSA and fPSA test kits were produced by Bioseed Company, U.S.A. The normal reference range of PSA was 0-4 mg/L, and the percent fPSA was calculated by dividing PSA by fPSA.

Statistical analysis

The results were expressed as mean ± standard deviation ($\bar{x} \pm s$). The mean value of PSA, fPSA and percent fPSA among groups were compared by *t* test and q test, The relationship was analyzed by linear regression model. Categorical data were analyzed by chi-square test and Fisher's exact test.

RESULTS

Percent fPSA in men without prostate disease vs. PCa patients

The mean percent fPSA of men without prostate disease was 20.5% ± 6.3%, compared with 7.3% ± 5.3% in men with PCa. The difference was significant($P < 0.005$).

PSA, fPSA and percent fPSA of each age group for men without prostate disease

PSA was a ssociated with age in men without prostate disease, and there were significant differences between any of each two groups ($P < 0.05$-0.001). fPSA was also associated with age, but just the group of age 30-39 was significantly different from the group of 60-69 and the group of over than 70 as to fPSA; and so was the group of 40-49; the group of 50-59 was significantly different from the group of over than 70 ($P < 0.01$). There were no significant differences of the percent fPSA among 5 age groups ($P > 0.05$) (See ***Table 1***). The correlation coefficient (r) between age and PSA, between age and fPSA, between age and percent fPSA was 0.608, 0.534 and -0.044 respectively. The data of serum PSA, fPSA increased as the age increased, but the data of percent fPSA did not change as the age changed.

Table 1 **Serum PSA fPSA and percent fPSA in different age**

Age	*n*	PSA(ng/ml)	fPSA(ng/ml)	Percent fPSA(%)
30–39	73	0.86 ± 0.81	0.18 ± 0.30	21.2 ± 7.3
40–49	58	1.18 ± 0.52	0.25 ± 0.23	20.8 ± 3.2
50–59	144	1.62 ± 0.63	0.31 ± 0.17	19.4 ± 6.0
60–69	252	2.12 ± 0.84	0.43 ± 0.22	20.3 ± 7.0
⩾ 70	152	2.76 ± 0.91	0.54 ± 0.25	19.6 ± 6.0

Relationship between percent fPSA and pathological grade, clinical stage of PCa

There was significant difference of the percent fPSA between different pathological grades and different clinical stages($P < 0.05$). The *r* between percent fPSA and pathological grade, between percent fPSA and clinical stage was 0.427 and–0.332 respectively ($P < 0.05$). As the percent fPSA was decreased, the malignant degree became worse while the pathological grade and the clinical stage became worse (***Table 2***).

Table 2 **The relationship of pathological grade, clinical stage of PCa with percent fPSA**

	Pathological grade			Clinical stage			
	Well	Moderate	Poor	A	B	C	D
n	6	4	24	3	15	12	4
PSA(ng/ml)	28.4 ± 4.5	33.1 ± 7.6	39.7 ± 8.1	21.7 ± 3.4	30.2 ± 6.1	39.6 ± 4.9	48.8 ± 7.3
fPSA(ng/ml)	4.3 ± 1.6	3.3 ± 2.0	2.0 ± 1.7	4.8 ± 2.2	3.7 ± 3.0	3.1 ± 1.8	2.8 ± 1.6
Percent fPSA(%)	15.1 ± 5.2	9.7 ± 3.3	4.8 ± 2.3	20.1 ± 3.4	11.3 ± 4.2	7.9 ± 3.0	4.8 ± 1.5

Appropriate reference range of percent fPSA

The percent fPSA values in the 5 th, 25 th, 50 th, 75 th and 95 th percentiles were 13.6%, 16.8%, 19.2%, 24.5% and 30.9% respectively. The threshold data of percent fPSA were unilateral threshold data. The data of 13.6% (the 5 th percentile) were selected as the reference threshold data of percent fPSA for the further study. According to the reference range of the percent fPSA given by the test kit and reported by the previous medical references, the data of 10%, 15% and 20% were selected as the threshold data of percent fPSA to distinguish PCa and men without prostate disease. The sensitivity of diagnosing PCa was 70.6% and the specificity was 97.4% as the reference range was more than 10%; when the reference range was more than 15%, the sensitivity was 94.1% and the specificity was 89.7%; when the reference range was more than 20%, the sensitivity was 94.1% and the specificity was 62.8%. Finally 15% was selected as the threshold data of the percent fPSA. The percent fPSA of PCa was defined as less than 15%.

DISCUSSION

Our results exhibited that the average serum percent fPSA of men without prostate disease was significantly higher than that of Pca patients. The tumor cells in most of PCa patients have the protein for α-chymotrypsin (ACT) transfer and expression. ACT produced by tumor cells is easy to combine with PSA and enter the blood circulation. The barrier of normal people to PSA is more effective than that of PCa patients, and it can keep the PSA-ACT from entering the blood circulation. Thus, people without prostate diseases may have more percent fPSA rather than PSA-ACT. The above can explain why the percent fPSA of men without prostate disease was higher than that of PCa patients.

Can percent fPSA be affected by age? Scholars have different opinions on it [1-6]. We found that PSA increased with age, there was positive correlation between PSA and age. fPSA also increased with age. There was positive correlation between fPSA and age. The percent fPSA was not significantly different among 5 age groups, and there was no correlation between percent fPSA and age. PSA is influenced by age, which may cause the increase in PSA of some men without prostate diseases aged 50 or older. PSA's clinical value was limited because of this[7], but percent fPSA was not affected by age, and its clinical value can be better than PSA.

Our study showed that there was positive correlation between percent fPSA and pathological grade of PCa, and negative correlation between percent fPSA and clinical stage of PCa. It was consistent with the results reported abroad [4]. The percent fPSA can be used as a predictive marker of pathological grade and clinical stage of PCa.

In our study, the percent fPSA value at each percentile was much lower than the data reported by the Oesterling [1]. It may increase the rate of misdiagnosed PCa and make unnecessary biopsy if we apply their reference range of percent fPSA on us.

There was no optimal threshold data of the percent fPSA for the diagnosis of PCa and men without prostate disease presently. It is difficult to use it clinically because of its great discrepancies in different countries [8-15]. To establish a good indicator for diagnosis and staging PCa, we should consider not only the sensitivity but also the specificity. We tried to get the ideal values of both when we established the threshold data of percent fPSA. Some scholars[1] abroad used the 5th percentile of the men without prostate disease as the cutoff point of percent fPSA and other scholars [9,12] used the Receiver Operating Characteristic Curve (ROC Curve). They made a graph with curves of positive ratio and false-positive ratio first, then selected the point of intersection of two curves, and both the sensitivity and the specificity of percent fPSA were the best at this point theoretically. In clinical application, we selected the data of percent fPSA closest to this point as its threshold data. We found that the incidence rate of PCa increased significantly when the data of percent fPSA decreased and the possibility of getting PCa

was very low when the percent fPSA increased. Our results showed that reference range of more than 15 % could help diagnose most of PCa, and both the sensitivity and the specificity were higher than those of other reference range. Finally 15% was selected as the threshold data of percent fPSA, a man with percent fPSA less than 15% would be diagnosed as PCa.

References

[1] Oesterling JE, Jacobsen SJ, Klee GG. Free, complexed and total serum prostate specific antigen the establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* 1998;159:1090.

[2] Ornstein DK, Smith DS, Humphrey PA,Catulona WJ. The effect of prostate volume, age, total prostate specific antigen level and acute inflammation on the percentage of free serum prostate specific antigen levels in men without clinically detectable prostate cancer. *J Urol*, 1998;159:1234-7.

[3] Catalona WJ,Southwick PC, Slawin KM,Partin AW,Brawer MK,Flanigan RC,*et al*. Comparison of percent free PSA, PSA density, and age-specific PSA cutoffs for prostate cancer detection and staging. *Urology* 2000; 56:255-60.

[4] Minardi D,Galosi AB,Recchioni A,Giammarco L,Polito M, Muzzonigro G. Diagnostic accuracy of percent free prostate-specific antigen in prostatic pathology and its usefulness in monitoring prostatic cancer patients. *Urol Int* 2001;67:272-82.

[5] Gelmann EP, Chia D, Pinsky PF,Andriole GL,Crauford ED, Reding D, *et al*. Relationship of demographic and clinical factors to free and total prostate-specific antigen. *Urology* 2001; 58:561-6.

[6] Battikhi MN. Age-specific reference ranges for prostate-specific antigen (PSA) in Jordanian patients. *Prostate Cancer Prostatic Dis* 2003; 6:256-60.

[7] Wang ZM, Liu DP, Zhao LH. Significance of using prostate specific antigen for the diagnosis of prostate cancer. *J Xi'an Med Univ*, 1996;17 (3):336-7.

[8] Weinrich MC, Jacobsen SJ, Weinrich SP,Moul JW,Oesterling JE,Jacobson D, *et al*. Reference ranges for serum prostate-specific antigen in black and white men without cancer. *Urology* 1998; 52(6):967-73.

[9] Herranz Amo F, Verdu Tartajo F, Diez Cordero JM,Leal Hernandez F,Bielsa Carrillo A,Garcia Burgos J, *et al*. Usefulness of free PSA/total PSA ratio in the diagnosis of prostatic cancer in symptomatic patients with PSA levels ranging from 2.5 to 20 ng/ml. *Actas Urol Esp* 2000; 24:24-30.

[10] Catalona WJ, Partin AW, Slawin KM,Naughton CK,Brawer MK,Flanigan RC, *et al*. Percentage of free PSA in black versus white men for detection and staging of prostate cancer: a prospective multicenter clinical trial. *Urology* 2000; 55:372-6.

[11] Vessella RL, Lange PH, Partin AW,Chan DW,Sokou LJ,Sasse EA, *et al*. Probability of prostate cancer detection based on results of a multicenter study using the AxSYM free PSA and total PSA assays. *Urology* 2000; 55:909-14.

[12] Wians FH Jr, Cheli CD, Balko JA,Bruzek DJ,Chan DW, Sokoll LJ. Evaluation of the clinical performance of equimolar- and skewed-response total prostate-specific antigen assays versus complexed and free PSA assays and their ratios in discriminating between benign prostatic hyperplasia and prostate cancer. *Clin Chim Acta* 2002; 326:81-95.

[13] Gray MA, Delahunt B, Fowles JR,Weinstein P,Cooke RR, Nacey JN. Assessment of ethnic variation in serum levels of total, complexed and free prostate specific antigen. Comparison of Maori, Pacific Island and New Zealand European populations. *Pathology* 2003; 35(6):480-3.

[14] Ito K, Yamamoto T, Ohi M,Kurokawa K,Suzuki K,Yamanaka H. Free/total PSA ratio is a powerful predictor of future prostate cancer morbidity in men with initial PSA levels of 4.1 to 10.0 ng/mL. *Urology* 2003; 61:760-4.

[15] Zhang L, Ji GY, Li XM. Early diagnosis of prostate cancer using free/total prostate-specific antigen ratio with population-based screening data. *Natl J Androl* 2004;10(8):582-5.