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·Original Article·

Measurement of serum zinc improves prostate cancer detection efficiency in patients with PSA levels between 4 ng/mL and 10 ng/mL

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Abstract

Aim: To investigate whether the measurement of serum zinc may improve the detection of prostate cancer (PCa) in men who had total prostate-specific antigen (PSA) levels higher than 4.1 ng/mL. **Methods:** A mass screening for Pca of 3940 men over 50 years old was undertaken using total serum PSA. Of the 190 men (4.8 %) with elevated PSA, 143 (3.6 %) underwent a transrectal ultrasonography (TRUS)-guided biopsy of the prostate, and 42 men (1 % of total and 29.3 % of men undergoing biopsy) were found to have cancer. The areas under the receiver operating characteristic curves (ROC-AUC) were used to compare the diagnostic power of cancer detection by means of serum zinc, and free PSA/total PSA ratio (f/t). **Results:** The men with levels of serum zinc that ranged from 40 ng/mL–60 ng/mL, had an age-adjusted odds ratios(OR) of 5.0. A cutoff value of 100 μg/mL for serum zinc concentration provided a sensitivity of 90.5 % and a specificity of 32.7 % in elevated PSA range, and a sensitivity of 93.3 % and specificity of 27.1 % in gray zone, respectively. In the gray zone ranges of 4.1 ng/mL–10.0 ng/mL, the ROC-AUC for zinc was 73.0 % higher than 62.7 % of f/t PSA ratio and 56.7 % of total PSA. **Conclusion:** Pca displays a lower serum zinc concentration. The measurement of zinc levels improves Pca detection in the gray zone compared with the f/t PSA ratio and total PSA. *(Asian J Androl 2005 Sep; 7: 323–328)*

Keywords: prostate cancer; prostate-specific antigen; zinc; gray zone

1 Introduction

Although the measurement of prostate-specific antigen (PSA) has improved the ability to detect prostate cancer (PCa), this test remains limited by lack of specificity [1]. The use of variations in PSA measurement such as PSA velocity, PSA density or age-specific PSA levels has failed to improve significantly the accuracy of Pca detection compared with the measurement of total PSA alone [2].

Zinc is a component of numerous metalloenzymes and is important for cell growth and replication. The total zinc levels in the prostate are ten times higher than those in other soft tissues [3]. Under the physiologic

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condition of testosterone and prolactin levels, prostate epithelial cells can rapidly uptake zinc, which is possibly facilitated by a cell membrane transporter. In contrast, the adenocarcinoma cells in prostate tumors lose their ability to amass zinc [4]. Some case-control studies have demonstrated that the concentrations of zinc in plasma/serum or total prostate tissue in men with Pca are lower than those in men without prostate disease or with benign prostate hyperplasia (BPH) [5–11], although other small case-control studies showed no differences [12, 13]. All these studies were conducted using small samples. In the present study, the serum concentrations of zinc were determined using larger samples to analyze the role of serum zinc as an adjuvant index in enhancement for detection of PCa, especially for those with elevated PSA range.

2 Materials and methods

2.1 Subjects

Between April 1998 and December 2000, a mass screening project of prostate cancer was conducted using serum PSA in the urban area of Changchun, China. A total of 3940 men over 50 years old were screened. One hundred and ninety men were found to have an elevated PSA of more than 4.0 ng/mL. They were subsequently advised to have a digital rectal examination and transrectal ultrasonography (TRUS)-guided needle biopsy of the prostate. None of the 3940 men had previously received PSA measurement or undergone digital rectal examination. Of the 3940 men, none had a family history of Pca. Of the 190 men (4.8 %, 190/3940), only 143 (75 %, 143/190) underwent a TRUS-guided biopsy of the prostate and 42 men (1 % of total and 29.3 % of men undergoing biopsy) were diagnosed as having PCa; the other 47 (25 %, 47/190) men rejected TRUS-guided needle biopsy.

These subjects were divided into two groups: the cancer group (42 men) and the control group (101 men). The serum zinc and total PSA levels of these 143 men were measured. There were 143 men with an elevated PSA of more than 4.0ng/mL and 85 of them were in the gray zone with a total PSA ranging from 4.1 ng/mL to 10.0 ng/mL.

2.2 Transrectal six-point puncture biopsy of prostate under ultrasound guidance

With the consent of patients, transrectal six-point puncture biopsies of the prostate were performed under ultrasound guidance. A Toshiba ultrasound machine, PVM-740RT (Toshiba, Kawasaki, Japan), was used. A transrectal ultrasound detector head, a prostate biopsy gun (Promag, Las Vegas, USA) and an 18-gauge biopsy needle (2.2 Biopsy Needle, MD Tech, USA) were designed specifically for prostate biopsy. The puncture biopsy was distributed in six areas with higher Pca incidence.

2.3 Pathological examination of prostate biopsy tissues

Biopsy prostate tissues were fixed with 10 % neutral buffered formalin, embedded in paraffin, sectioned following standard protocols and stained with haematoxylin and eosin (HE). The sections were observed with a microscope with multi-view attachments.

2.4 Clinical staging of PCa patients

Pathological diagnosis of each patient was based on the clinical data and current international clinical staging methods. PCa patients were staged using ABCD stagings [14].

2.5 Serum zinc and total and free PSA levels

The blood samples were taken before prostate examination. After centrifugation, the serum was obtained and kept at –80 ºC until use.

Serum trace element zinc was determined by the deproteinization method using a Perkin-Elmer 503 atomic absorption spectrophotometer [15].

Serum total and free PSA concentrations were measured using an automated, polyclonal-monoclonal immunochemiluminometric assay kit (CanAg, Gothenburg, Sweden).

2.6 Statistics

Data were expressed as mean ± SE. Inter-group difference was studied using Student's *t*-test. $P < 0.05$ was considered significant.

The odds ratios and their corresponding 95 % confidence intervals were calculated by multiple logistic regressions including crude or adjusted for age. *P*-values to test the linear trend were calculated. All *P*-values were two-tailed. The receiver operating characteristic (ROC) curves for PCa detection were generated for serum zinc and the free PSA/total PSA ratio (f/t), plotting sensitivity versus 1–specificity. The area under the curve (AUC) was calculated and used to compare the performance of

the two assays. All statistic analyses were performed using SPSS 10.0 software (SPSS Inc., Chicago, USA).

3 Results

3.1 Cancer groups

Fifteen men of 42 men in the cancer group had abnormal digital rectal exam (DRE). Clinical staging revealed nine men (21.4 %) were of stage A, 12 men (28.6 %) stage B, 10 men (23.8 %) stage C and 11 men (26.2 %) stage D. Three men (7.14 %) had a Gleason score of 2–4, 30 men (71.43 %) of 5–7 and nine men (21.43 %) of 8–10.

3.2 Total PSA(t-PSA), free PSA(f-PSA) and serum trace element zinc in cancer and control groups

Table 1 summarizes the age, total PSA and serum trace element zinc levels in the cancer and control groups. The mean age of the cancer group show no statistic differences compared with the control. The free PSA was higher in the cancer group than that in control ($P < 0.01$). Serum zinc levels in the cancer group were significantly lower than those in control ($P < 0.05$).

3.3 PCa risk in various range of serum zinc in the two groups

Table 2 shows the regression analysis of PCa risk in various range of serum zinc in the two groups. Men with serum zinc 40 ng/mL–60 ng/mL had an age-adjusted odds ratio (OR) of 5.0 (95 % confidence interval [CI]: 5.441–56.698) ($P = 0.001$).

3.4 The sensitivity, specificity, positive and negative predictive value in diagnosis of PCa at different serum zinc levels.

The performance characteristics with respect to sensitivity, specificity, positive and negative predictive value and diagnostic overall accuracy targetting to PSA gray zone at various serum zinc concentrations are shown in Table 3. Using the standard cutoff of 100 μg/mL, a sensitivity of 90.5 % and specificity of 32.7 % in all men with elevated PSA and a sensitivity of 93.3 % and specificity of 27.1 % in gray zone were observed, respectively.

3.5 Comparison of the diagnostic efficiency of serum zinc and f/t PSA ratio and total PSA in PCa

In the elevated PSA range more than 4.1 ng/mL, the ROC-AUC for zinc was 69.9 % lower than 71.8 % of f/t PSA ratio and 81.1 % of total PSA. Whereas in the gray zone ranges of 4 ng/mL–10 ng/mL, the ROC-AUC for zinc was 73.0 % higher than 62.7 % of f/t PSA ratio and 56.7 % of total PSA (Figure 1, Table 4).

4 Discussion

PSA remains one of the most useful markers currently available for PCa detection. However, the use of PSA is limited by a significant lack of specificity. The search for improvement in specificity led to the evaluation of multiple PSA variants, such as PSA velocity, PSA density and PSA age reference range [2]. However, none of these parameters has been shown to significantly improve the detection efficiency of PCa.

Zinc is an essential heavy metal and is more abundant in the human prostate than in other tissues. The concentration of zinc in whole prostate tissue appears to increase with the increasing distance from the bladder, with the highest concentrations in the lateral lobe of the peripheral zone and the lowest levels in the central zone [4]. Zinc is an essential trace element and is a normal component of many enzyme systems such as dehydrogenases, phosphatases, 5 alpha-reductases, carboxypeptidases and carbonic anhydrase [5].

Table 1. The mean ± SE values of age, total PSA (ng/mL), free PSA (ng/mL) and serum trace element zinc (μg/mL) in the cancer group and the control group.

	Cancer group	Control group	*P*
Age	70.10 ± 1.32	67.80 ± 0.85	0.145
PSA (ng/mL)	29.43 ± 4.60	8.45 ± 0.58	0.0001
f/t PSA (ng/mL)	0.17 ± 0.03	0.22 ± 0.02	0.120
Zinc (μg/mL)	83.00 ± 2.13	97.80 ± 2.57	0.001

Table 2. Regression analysis of PCa risk in various range of serum zinc between the cancer group and the control group. OR: compared with the cancer group and the control group with an elevated PSA adjusted by age.

Zinc concentration (μg/mL)	Cancer group (*n*)	Control group (*n*)	OR	95% CI
>100	4	33	0.217	0.071-0.659
80-100	22	55	0.920	0.447-1.892
60-80	14	12	3.708	1.538-8.943
40-60	2	1	5	5.441-56.698
P for trend				0.001

Table 3. Calculations of sensitivity, specificity, positive and negative predictive value, diagnostic overall accuracy for PSA gray zone using various concentrations of serum zinc. Sn: sensitivity; Sp: specificity; +PV: positive predictive value; -PV: negative predictive value.

	Men with elevated PSA (n = 116)				Gray zone (n = 82)			
	Sn (%)	Sp (%)	+PV (%)	-PV (%)	Sn (%)	Sp (%)	+PV (%)	-PV (%)
<60	4.3	99.0	66.7	71.4	6.7	100	100	83.3
<70	16.7	99.0	87.5	74.1	13.3	100	100	84.3
<80	38.1	87.1	55.2	77.2	26.7	84.3	26.7	84.3
<90	66.7	64.4	43.8	82.3	66.7	60.0	26.3	89.4
<100	90.5	32.7	35.8	89.2	93.3	27.1	21.5	95.0
<110	95.2	18.8	32.8	90.5	93.3	17.1	19.4	92.3
<120	100	10.9	31.8	100	100	10.0	19.2	100

Table 4. Area under the receiver operating characteristic (ROC) curves with respect to the efficiency of differentiating cancer and noncancer for elevated PSA or gray zone using zinc, f/t PSA ratio and total PSA.

	Zinc			free PSA/total PSA			Total PSA		
	AUC	95 % CI	*P*-value	AUC	95 % CI	*P*-value	AUC	95 % CI	*P*-value
Elevated PSA (n = 143)	0.699	0.596–0.802	0.0004	0.718	0.617–0.820	0.0001	0.811	0.721–0.902	0.0001
Gray zone (n = 85)	0.73	0.587–0.873	0.003	0.627	0.493–0.760	0.101	0.567	0.404–0.730	0.383

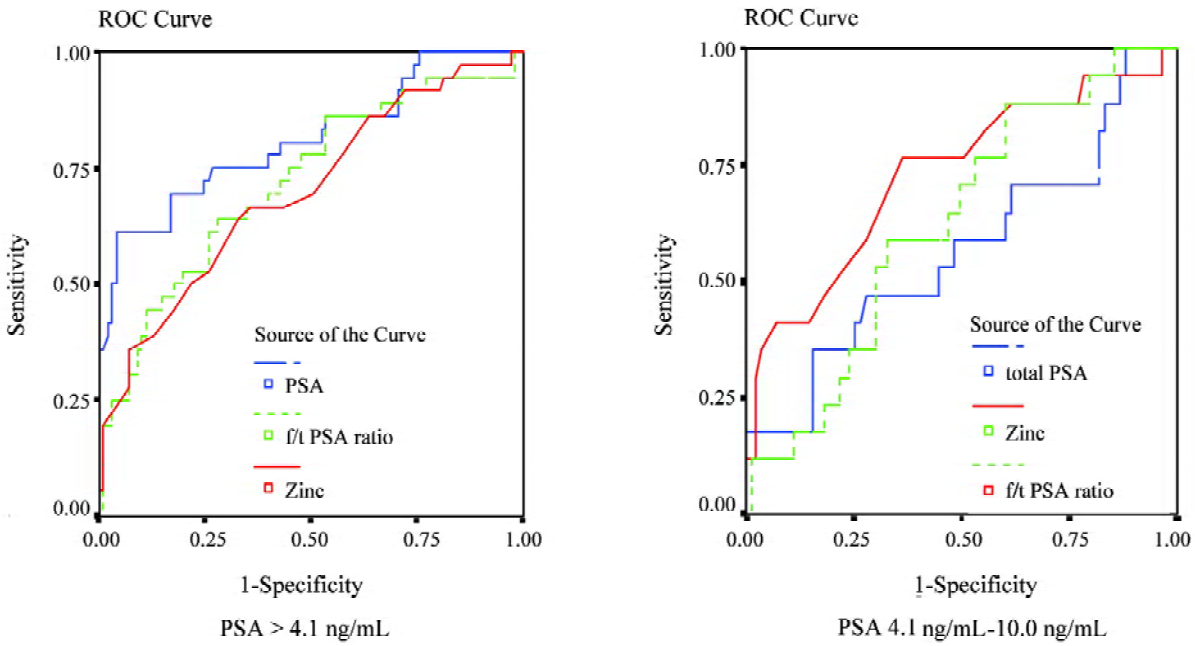

Figure 1. Receiver operating characteristic (ROC) curves with respect to the efficiency of differentiating cancer using zinc, f/t PSA ratio in elevated PSA group (up) in gray zone (down).

In this study, we found that serum zinc levels in the PCa group were significantly lower than those in in the controls. The cancer group showed the lowest zinc level, whereas the control group consisting mostly of BPH showed the highest zinc level; lower serum zinc levels were shown to be related to PCa adjusted for age. Whelan *et al.* [6] measured the serum zinc levels of 27 patients with BPH and 19 patients with carcinoma of the prostate. A significantly lower ($P < 0.05$) level of serum zinc (105 ± 19) μg/mL was found in the cancer group compared to the BPH group (122 ± 28) μg/mL [6]. Lekili *et al.* [7] found the zinc plasma levels significantly different between Pca and BPH patients. They also found remarkable differences in the plasma concentrations of zinc in patients with prostate carcinoma before and after therapy [7]. Feustel *et al.* [15] used the flame atomic absorption spectrometry (FAAS) to observe the serum zinc of patients with prostate carcinoma and men without PCa. The serum zinc level in patients with prostate carcinoma having metastases was decreased in comparison to other groups. No significant differences were found between the groups with prostate carcinoma without metastases [15]. Ogunlewe and Osegbe [8] found the mean plasma zinc concentration of healthy men was (96.8 ± 0.5) μg/mL (Mean ± SEM), whereas those with BPH and malignant glands were (107.2 ± 0.6) μg/mL and (71.5 ± 0.7) μg/mL, respectively. The mean prostate tissue zinc concentration in normal glands was (78.6 ± 0.8) μg/mL. It was (116.3 ± 0.6) μg/mL for BPH glands and (18.8 ± 0.4) μg/mL for cancer glands [8]. Other researchers also found that the level of zinc in tissues from PCa was significantly lower than that in BPH or normal tissues [9, 10]. Zaichick *et al.* [11] found that zinc concentration in human prostate fluid decreased more quickly. Prostate neoplasm resulted in a significant decrease of zinc secretion, with the concentration averaging (34.7 ± 9.6) μg/mL, $P < 0.01$, and those for normal levels being (455 ± 60) μg/mL (Mean ± SE) and (540 ± 50) μg/mL (Mean ± SE), respectively [11].

We found that to avoid unnecessary prostate biopsies and to elevate detection specificity in the gray zone where the PSA level ranged from 4.1 ng/mL to 10.0 ng/mL, serum zinc is a better marker than f/t PSA ratio and total PSA. In our study, where the standard cutoff was 100 ng/mL, a sensitivity of 90.5 % and a specificity of 32.7 %, respectively, were observed in all men with elevated PSA; the sensitivity and specificity in the gray zone were 93.3 % and 27.1 %, respectively. So in this study, with a serum zinc test with a 100 ng/mL cutoff value set for those men in the PSA gray zone, the ROC-AUC values of total PSA and f/t PSA ratio in the diagnostic gray zone are similar to those reported in previous papers [16, 17].

Many laboratory studies have shown that the loss of a unique capability to retain high levels of zinc is an important factor in the development and progression of malignant prostate cells. Very high levels of zinc are found in the mitochondria of prostate epithelial cells where zinc inhibits mitochondrial aconitase, resulting in decreased citrate oxidation. Interestingly, malignant prostate cells have a higher rate of citrate oxidation than normal prostate cells although it is not currently believed that altered citrate oxidation contributes to prostate carcinogenesis [18]. According to Liang's studies [19], incubation of prostate carcinoma cell lines with physiological levels of zinc resulted in a marked inhibition of cell growth. The zinc-induced G_2/M phase arrest and apoptosis were accompanied by increased mRNA expression levels of p21 in two prostate cancer cell lines (LNCaP and PC-3) [19]. Our study revealed that the serum level in PCa is decreased. This is, however, in contrast to the findings of Nemoto *et al.* [20]. They demonstrated that an enhancement of telomerase activity in the human renal cell carcinoma (NRC-12) and PCa cell lines (DU145) that is induced by zinc is responsible for the unlimited proliferation of cancer cells. Ishii *et al.* [21] regarded that highly expressed aminopeptidase (AP-N) in human cancerous prostate may play an important role in the invasion and metastasis of PCa cells. The suppression of PC-3 cell invasion by zinc is attributable to inactivation of AP-N by zinc [21].

In conclusion, the measurement of zinc offers improved PCa detection in the gray zone (PSA range 4 ng/mL–10 ng/mL), compared with either total PSA or f/t PSA ratio. Further investigations are warranted and we will continue this prospective study if enough cases of Pca are enrolled.

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